Prebiotics, probiotics and synbiotics in human health, clinical applications – an update

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Abstract

Probiotics have become well-known and widely used, especially as supplements or add-on therapy, in the prevention and management of antibiotic-associated diarrhoea. However, their mechanisms, strain-specific effects and appropriate clinical use remain poorly understood by both pharmacists and patients. Furthermore, recent reports of an *Alkalihalobacillus clausii* outbreak emphasised the importance of appropriate clinical use of probiotics, particularly in severely immunocompromised patients. This article provides an overview of prebiotics, probiotics and synbiotics, including their mechanisms of action, clinical applications and available products on the local market.

Keywords: prebiotics, probiotics, synbiotics, *Lactobacillaceae* or lactic acid bacteria, gut–brain axis or microbiota

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Introduction

Scientific interest in probiotics dates back to the early 20th century when lactic acid bacteria (*Streptococcus thermophiles* and *Lactobacillus delbruekii* subspecies of *bulgaricus*) in fermented milk products were linked to improved gastrointestinal health.

During a dysentery outbreak among soldiers in World War I, Nissle isolated a non-pathogenic *Escherichia coli* strain from an unaffected individual and later developed it as a therapeutic probiotic *(E. coli)*.[1] The family *Enterobacteriaceae* (Gram-negative rods) were found to be associated with plant material, as well as soil and water.[1] This might be due to the difficult circumstances and lack of hygiene.[1] Soldiers were severely affected by Shigellosis, however, the German Professor Alfred Nissle noticed that one of the soldiers was not affected during this severe outbreak.[1] Through careful research, he isolated a non-pathogenic strain of *E. coli* from the faeces of that soldier. All indications suggested that this non-pathogenic strain had prevented the soldier from acquiring Shigellosis.[1] This was subsequently interpreted to be a so-called probiotic. The strain isolated by Nissle in 1917 is an example of a non-lactic acid bacteria probiotic.[1]

The growth of favourable organisms can be stimulated by microbial factors, and in 1965 Lilly and Stilwell introduced the term "probiotics".[1,2] The beneficial effect from probiotics on the host was emphasised by Roy Fuller in 1989.[1,2] Relevant and commonly used terminology is described in Table I.

What are probiotics?

Probiotics are currently defined as 'live' micro-organisms which, when administered in adequate amounts, confer a health benefit on the host.[4] The host may benefit from probiotics when living micro-organisms are administered in an adequate amount to restore microflora symbiosis in the gastrointestinal (GI) tract.

Most clinically used probiotics belong to genera historically classified as *Lactobacillus, Bifidobacterium, Enterococcus, Lactococcus and Streptococcus*.[5] These Gram-positive bacteria and strains are also found in hair, skin, the mouth, respiratory tract, intestinal tract and other parts of the human body. Favourable strains, such as *Lactobacilllus acidophilus, Lacticaseibacillus rhamnosus, Lactobacillus bulgaricus, Lacticaseibacillus casei, Lactiplantibacillus plantarum*, *Ligilactobacillus salivarius*, *Lactobacillus sporogenes*, and *Bifidobacterium bifidus*, *Bifidobacterium bifidum*, *Bifidobacterium infantis* and *Bifidobacterium longum*, are commonly used in a probiotic formula.[6]

Probiotics that are most frequently encountered contain the predominant and subdominant organisms of the GI microbiota

Table I: General probiotic-related terms and definitions[1,3]

Lactic acid bacteria (LAB)	Gram-positive bacteria which are fermentative, non-pathogenic and non-toxigenic. They produce lactic acid from carbohydrates, which makes them valuable for food fermentation.[1] These species include: *Lactobacillus*, *Lactococcus*, and *Streptococcus thermophiles*[1]
Fermentation	Micro-organisms produce lactic acid, ethanol, and other metabolic end-products to convert food into other products[1]
Species	A group of related bacteria which are highly similar by phenotype but differ in specific characteristics[3]
Bacteriocins	A protein that is produced by some bacteria that constrains or kills closely related species[3]
Mucin	Glycoproteins that contain a high molecular weight and found in the secretion of mucous membranes[3]

and added to different types of food. The yeast species, *Saccharomyces boulardii*, has also been shown to have a beneficial effect on health status. Food industry personnel are interested in these organisms, because of the beneficial effects that they have on health, as well as the history of the safe use of fermented milk products.[7]

Safety is of paramount importance, therefore, probiotics should adhere to specific standards, including their tolerance of gastric acid and bile in the GI tract, ability to adhere to the GI mucosa, their competitive exclusion of pathogens, production of lactic acid and shorter generation time.[8] Additionally, probiotics need to be non-invasive and non-carcinogenic to form normal balanced flora.[8]

The survival of probiotics through the GI tract can be influenced by the acidity of the stomach, the concentration and length of exposure to the acid and bile salt, and the level of bile salt hydrolase activity. Therefore, it is important that probiotics can survive gastric and bile acid when administered so that they can reach the GI tract. They must also be able to colonise the host epithelium and demonstrate a beneficiary effect.[7] It has been shown that non-spore-forming lactobacilli-type probiotics are inactive in the low gastric pH and the bile. Probiotics can be found in food and dietary supplements, such as tablets, capsules and powder. It has been stated that the bacteria may have already been present or added during the preparation of probiotic food. These probiotics should be stored in acceptable conditions to ensure that they have long-term activity and feasibility for use in the general population.[6]

Mechanism of action of probiotics

Probiotics exert multiple, strain-specific effects that include enhancement of epithelial barrier integrity, competitive exclusion of pathogens, production of antimicrobial compounds and metabolites, and modulation of host immune and neuroendocrine signalling pathways.[7] Furthermore, probiotics can produce neurotransmitters in the gut through the gut-brain axis. Specific probiotic stains can modulate the serotonin, gamma-aminobutyric acid (GABA), and dopamine levels, affecting mood, behaviour, gut motility, and stress-related pathways.[9] Within the established brain-gut axis, strains can influence neuromodulator production, vagal signalling and stress-response pathways, although human evidence remains heterogeneous and appears to be condition- and strain-specific.[5,10]

While largely established during the prenatal period and early childhood, these interactions can be modified throughout life by factors such as diet, medication use, and stress.[10] Emerging human evidence suggests that dysregulation of this axis may play a role in brain-gut disorders.[10]

Probiotic bacteria can stimulate the host defence mechanisms by enhancing the immune system, which acts on both humeral and cellular responses. Probiotics can also ease digestion by stabilising the microflora, as well as preventing hypersensitivity-reactions to food antigens.[11] In stimulating the synthesis of immunoglobulins and cytokines, the effects of general probiotics are also associated with modulation of the immune response. *Lactobacillus* spp. shows macrophage activation, as well as an increase of phagocytosis, as confirmed by various clinical studies. Organisms such as bacteria, fungi and viruses are responsible for activation of the inflammatory cascade. Probiotics reduce the inflammatory reaction and simultaneously enhance the immune response. The duration of acute infections, like diarrhoea in children, traveller's diarrhoea and diarrhoea caused by *Clostridium difficile* infection, is effectively reduced by numerous probiotic strains, including *Limosilactobacillus reuteri, L rhamnosus* and *L casei*.[2] Meta-analyses indicate that specific probiotics, particularly *L rhamnosus,* GG and *S. boulardii* CNCM I-745, can reduce the incidence of antibiotic-associated diarrhoea by approximately 40–50% in adults and children, although effect sizes vary by strain, dose and population.[12]

Multiple factors are prominent regarding the beneficial effects of probiotics, although the mechanisms are not yet fully understood. Mechanism of action is achieved as:[13]

Table II: An overview of human intestinal microbiota[2,3,15,16]

Oral cavity (saliva)	*Bacteroides*, *Bifidobacterium*, *Corynebacterium*, *Fusobacterium*, *Lactobacillus*, *Neisseria*, *Staphylococcus*, *Streptococcus*, *Veillonella*[3,15]
Stomach (pH 1-2) Duodenum (pH 6-7)[3]	Only a small number of micro-organisms, because of the low pH[15] < 10^3 bacterial cells per gram of stomach contents[2] Mainly *Enterococcus*, *Helicobacter*, *Lactobacillus*, *Streptococcus*[1,15] Acidic, pancreatic secretions as well as bile secretions cause an unfavourable environment for microbes[1] Stomach: *Bacteroidetes*, *Bifidobacterium*, *Enterobacteriaceae*, *Lactobacillus*, *Streptococcus*, yeasts[3] Duodenum: *Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, *Lactobacillus*, *Streptococcus*, *Veillonella*, yeasts[3]
Jejunum and ileum (pH 6-7)[3]	There is a progressive increase in the number and diversity of the bacteria[1] Ileum: *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterobacteriaceae*, *Lactobacillus*, *Streptococcus*, yeasts[3]
Colon (pH 5-7)[3]	The large intestine contains a high population of anaerobes[1] The colon contains the majority of gastrointestinal microbes[15] Colon: *Bacteriodetes* such as *Bacteriodaceae*, *Prevotellaceae* and *Rikenellaceae,*[16] *Bifidobacterium*, *Clostridium*, *Coprococcus*, *Enterobacteriaceae*, *Eubacterium*, *Lactobacillus*, *Peptostreptococcus*, *Ruminococcus*, *Streptococcus*[15]
Faeces[3]	Faeces: *Bacteroides, Bifidobacterium, Clostridium, Coprococcus, Enterobacteriaceae, Eubacterium, Lactobacillus, Peptostreptococcus, Ruminococcus, Streptococcus, Veillonella*, yeasts[3]

- The gastrointestinal epithelial barrier function is enhanced
- Pathogen adhesion is inhibited, owing to concomitant probiotic adhesion to the intestinal mucosa
- Pathogenic micro-organisms are excluded through competition with the probiotics
- Anti-microorganism substances are produced
- The immune system is modulated

Immune modulation is achieved through the interaction of the probiotics and the host cells.[14] The target is predominantly gastrointestinal epithelial- and gastrointestinal-associated immune cells in this process.[14] An overview of naturally occurring human intestinal microbiota is provided in Table II.

The non-immune mechanisms and the mucosal immune mechanisms show a positive reaction when stimulated by probiotics, affecting the intestinal ecosystem. This is achieved through antagonism and competition with potential pathogens. Probiotics are mostly recognised for the decrease in the incidence of diarrhoea, as well as the severity of the disorder. Excellent results have been obtained in certain animal models in decreasing colon cancer, probably due to the suppressing activity of certain bacterial enzymes which may have raised the levels of the procarcinogens. Unfortunately, this has not yet been achieved in human models. Probiotics have numerous benefits, which can be classified as either beneficial or non-immunological.[1] Probiotics have the following immunological benefits:[1]

- Increased antigen presentation of B lymphocytes and increased secretory immunoglobulin A production is activated by the local macrophages, and affect the system both locally and systemically
- The cytokine profiles are modulated
- Hypo-responsiveness to food antigens is established

Non-immunological benefits include:[1]

- Food digestion is improved, and increased competition with pathogens for the nutrients is achieved
- The local pH is adjusted to create an unfavourable local environment for pathogens
- Pathogens are inhibited by the production of bacteriocins
- Superoxide radicals are removed
- The epithelial mucin production is stimulated
- The intestinal barrier function is enhanced
- There is competition for adhesion with the pathogens
- The pathogen-derived toxins are modified

Excellent results have been reported in various human studies and animal models regarding the clinical potential of probiotics against many diseases. Probiotics have been reported to:[11]

- Suppress diarrhoea
- Alleviate postoperative complications and lactose intolerance
- Exhibit anti-colorectal cancer and antimicrobial activity
- Reduce irritable bowel symptoms
- Prevent inflammatory bowel disease

To summarise, it can be stated that probiotics are confirmed to be safe and should adhere to certain conditions. For example, probiotics should:[4]

- Not lose their properties during storage
- Normally be present in the human intestines
- Survive in the gastrointestinal tract and colonise the intestinal cells
- Have beneficial effects on human health
- Display antagonism against pathogenic micro-organisms
- Not demonstrate any noticeable side-effects

The potential health benefits of probiotics tend to be strain-specific and generalisations of probiotic benefits do not attribute to different strains within one species.[4] Consequently, caution should be exercised when consuming or prescribing probiotics. This is illustrated by a recent outbreak reported by the National Institute for Communicable Diseases (NICD), which was linked to the use of the Enterogermina probiotic in private hospitals in South Africa.[17] The product contained *Alkalihalobacillus clausii*, and whole-genome sequencing identified Enterogermina as the likely source of *A. clausii bacteraemia*. Although *A. clausii* is generally considered a non-pathogenic bacillus, these findings highlight the need for caution when administering probiotics, specifically to severely immunocompromised patients.

The use of probiotics and prebiotics

Evidence has been demonstrated in some studies, suggesting that probiotics have various clinical applications (see Table III).[2]

Prebiotics

The term 'prebiotic' was first introduced in 1995 and was recently redefined as a beneficial substrate that is selectively used by host micro-organisms.[16] They enhance the growth and activity of specific intestinal strains, and can therefore effect a favourable change in the balance of the intestinal microflora.[2] Prebiotics are mainly dietary fibre, particularly soluble fibre, and are also known as 'colonic food', consisting of specific carbohydrates.[2] Established prebiotics such as inulin, fructo-oligosaccharides and galacto-oligosaccharides are selectively fermented by components of the gut microbiota, leading to increased production of short-chain fatty acids that can modulate luminal pH, epithelial barrier integrity, immune responses and host energy metabolism.[19] The mechanism of action of prebiotics constitutes their effects on the intestinal bacteria through their ability to enhance the amount of beneficial anaerobic bacteria, and to decrease the pathogenic micro-organism population in number.[1,2]

Table III: The clinical applications of probiotics[2]

Probiotic/prebiotic	Recommended dose
Acute diarrhoea in adults	
Enterococcus faecium	10^8 cfu, three times daily
Lacticaseibacillus paracasei or *Lacticaseibacillus rhamnosus*	10^9 cfu twice daily
Saccharomyces boulardii, a strain of *Saccharomyces cerevisiae*	10^9 cfu per capsule of 250 mg, 2–6 capsules per day
Acute infectious diarrhoea	
Lacticaseibacillus rhamnosus	10^{10}-10^{11} cfu, twice daily
Saccharomyces boulardii, a strain of *Saccharomyces cerevisiae*	200 mg, three times daily
Antibiotic associated diarrhoea	
Saccharomyces boulardii, a strain of *Saccharomyces cerevisiae*	250 mg, twice daily
Lacticaseibacillus rhamnosus	10^{10} cfu, once or twice daily, or even 2×10^{10} cfu, twice daily
Enterococcus faecium	10^8 cfu, twice daily
Lacticaseibacillus casei in fermented milk	10^{10} cfu, twice daily
Bacillus clausii (Enterogermina strains)	2×10^9 spores, three times daily
Lactobacillus acidophilus + *Lacticaseibacillus casei*	5×10^{10} cfu, once or twice daily
Clostridium difficile diarrhoea in adults	
Lacticaseibacillus casei in fermented milk	10^{10} cfu, twice daily
Lactobacillus acidophilus + *Bifidobacterium bifidum* (Cultech strains)	2×10^{10} cfu each strain, once daily
Oligofructose	4 g, three times per day
Lacticaseibacillus rhamnosus + *Lactobacillus* acidophilus	10^9 cfu each, once daily
Heliobacter pylori eradication	
Lacticaseibacillus casei in fermented milk	10^{10}-10^{12} cfu daily, for 14 days
Lacticaseibacillus rhamnosus GG	6×10^9 cfu, twice daily
Bacillus clausii (Enterogermina strains)	2×10^9 spores, three times daily
Saccharomyces boulardii, a strain of *Saccharomyces cerevisiae*	500 mg to 1 g or $2\text{-}4 \times 10^9$ cfu per day
Kefir	250 mL twice daily
Limosilactobacillus reuteri	10^8 cfu/day
Nosocomial diarrhoea	
Lacticaseibacillus rhamnosus	10^{10}- 10^{11} cfu, twice daily
Bifidobacterium lactis + *Streptococcus thermophiles*	$10^8 + 10^7$ cfu/g of formula
Prevention of respiratory tract infections in athletes	
Lacticaseibacillus casei (Shirota strain in fermented milk)	10^{10} cfu, once daily
Remission in ulcerative colitis	
Escherichia coli	5×10^{10} viable bac, twice daily
Symptoms of irritable bowel syndrome	
Bifidobacterium infantis	10^8 cfu, once daily
Bifidobacterium animalis in fermented milk	10^{10} cfu, twice daily
Lactobacillus acidophilus	10^{10} cfu per day
Treatment of constipation	
Lactulose	20–40 g/day
Oligofructose	> 20 g/day
Treatment of hepatic encephalopathy	
Lactulose	45–90 g/day
Treatment of mildly active ulcerative colitis or pouchitis	
Mixture of eight strains (one *Streptococcus thermophilus*, four *Lactobacillus*, three *Bifidobacterium*)	2×10^{11} cfu, twice daily

Taxonomic nomenclature follows the 2020 reclassification of the genus *Lactobacillus*[18]

Prebiotics are present in numerous edible plants, such as asparagus, bananas, chicory, garlic, leeks, oats, onions, soybeans and wheat. Raw vegetable matter is also a key component of a high percentage of commercial prebiotics. Production is achieved via an enzymatic method, through the trans-glycosylation of monosaccharides or disaccharides, or the hydrolysis of complex polysaccharides.[2]

Synbiotics

A synbiotic is a nutritional supplement containing both probiotics and prebiotics.[20] Synbiotics can be defined as "a mixture comprising live micro-organisms and substrate(s) selectively utilised by host micro-organisms that confers a health benefit on the host", with complementary and synergistic subtypes distinguished based on how the substrate and micro-organisms interact.[20]

This mixture of probiotics and prebiotics works together to ensure that bacterial microflora in the GI tract remain healthy. Synbiotic products include fermented milk products, such as yoghurt and kefir. This may be regarded as functional food, because it restores the normal bacterial microflora and supplies the necessary food for the normal microflora to proliferate. *Bifidobacteria* and fructo-oligosaccharides, *Lactobacillus* GG, inulin, as well as *Bifidobacteria* and *Lactobacilli* with fructo-oligosaccharides or inulin, are the best combinations of available synbiotics.[20]

An overview of commercially available probiotic, prebiotic and synbiotic products is provided in Table IV.

Conclusion

Probiotics are live non-pathogenic micro-organisms, which have a beneficial effect on the health of the host. They are present in the GI tract without causing any side-effects. Probiotics can be used for several conditions, e.g. antibiotic-induced diarrhoea, irritable bowel syndrome and inflammatory bowel disease. Prebiotics are known to be a non-digestible food ingredient. They exert a favourable change in the balance of intestinal microflora by enhancing the growth and activity of some intestinal strains. Synbiotics, a combination of probiotics and prebiotics, are a nutritional supplement.

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Table IV: Examples of commercially available probiotic, prebiotic and synbiotic products in South Africa[21]

Probiotics*	Organism	Detected using DGGE
BioPro Reuteri® straws	*Limosilactobacillus reuteri*	*Limosilactobacillus reuteri*
BioPro Reuteri® tablets	*Limosilactobacillus reuteri*	*Limosilactobacillus reuteri*
Combiforte® capsules	*Lactobacillus acidophilus* *Bifidobacterium bifidus* *Bifidobacterium longum*	*Lactobacillus acidophilus* *Bifidobacterium infantis*
Infantiforte® capsules	*Bifidobacterium infantis*	*Bifidobacterium infantis*
QuantroFlora®	*Bifidobacterium, Lactobacillus acidophilus, Lactobacillus bulgaricus, and Streptococcus thermophilus*	
PREBIOTICS		
Asparagus, bananas, chicory, garlic, leeks, oats, onions, soybeans and wheat[1]		
SYNBIOTICS		
Yogurt and kefir[3]		

Other products include: ProbiFlora®, Probitec®, Reuterina®, Viral Guard®, Duphalac®
*DGGE = denaturing gradient gel electrophoresis

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