White Paper: Probiotics

PROBIOTICS OVERVIEW

The human microbiome considered our "last organ",[2] or "second human genome",[102] is a complex ecosystem of commensal, symbiotic and pathogenic microorganisms (bacteria, archaea, viruses, fungi, and parasites) that resides in the intestines, skin, vagina and oral cavity, and significantly influences gastrointestinal and systemic health.[2-5, 102]

Genesis of the human microbiome is thought to begin in utero with marginal transfer of the mother's microbiota to the developing foetus recently being found to occur when evidence of microbes existing in meconium was discovered.[102] The majority of inoculation occurs during birth as the baby comes into contact with the birth canal, fecal flora transferred by the mother and environmental exposure.[1] Ongoing evolution of the microbiome occurs over the next three years as a result of diet, degree of hygiene, the use of medication and other environmental and or genetic influences.[1, 103]

The resulting composition and diversity of the adult human *intestinal* microbiome has been estimated to number up to one hundred trillion microbes from around 5000 different species, weighing around 2kg[102] and is relatively stable in composition and function from around three years of age.[103] However, it may be subject to significant intra- and inter- individual variability consequent to many continuous influencing factors including dietary patterns, medical interventions (medications or surgery), infection, age, stress, body composition, physical activity and hormonal cycles.[3-6] Diet has the most influence over the growth and activity of the microbiome.[102]

The micro-organisms that make up the microbiota can be supplemented to positively affect the composition, diversity and/or volume of the microbiome. Micro-organisms in supplemental form are called "probiotics" (a Greek derived word meaning "for life").[1] Humans have been consuming fermented food products rich in probiotics for hundreds if not thousands of years for their effects on health. The early 1900's saw the popularisation of the consumption of fermented food products for their specific ability to support the health of the intestinal microflora. Scientists of the time believing that many diseases can be traced back to altered bacterial fermentation in the gut.[1] These days, probiotic supplements come in the form of encapsulated or powdered medicinal products, with modified or natural foodstuffs still being popular options to enrich or support the body's microbiome.

In order for supplementation or ingestion of probiotics to positively affect the microbiome, the micro-organisms need to have particular characteristics or qualities. These qualities will enable them to survive the harsh conditions of the upper gastrointestinal system, and then be able to overcome resistance to colonisation from the pre-existing symbiotic bacteria.[1, 103] In summary, they must be able to pass through the harsh environments of the upper gastrointestinal system which will otherwise render them non-viable. These sites include the mouth, the stomach and the duodenum, they must be able to adhere to the intestinal epithelium and they must be able to colonise the gastro-intestinal tract.[1, 103] Table one provides more information on the characteristics necessary for probiotics to exert maximal therapeutic effects.

A healthy microbiome will:[1, 102, 106, 107]

- Prevent colonisation and overgrowth of pathogenic micro-organisms in the GIT
- Deconjugate and dehydroxylate bile acids
- Assist in the conversion bilirubin to urobilinogen
- Stimulate SCFA production in the large intestine
- Ensure healthy cholesterol metabolism (convert cholesterol to coprostanol)
- Modulate the immune system
- Stimulate GIT motility
- Support gut barrier function (stimulate mucin production)
- Support digestion and nutrient absorption
- Metabolise xenobiotics
- Produce vitamins (K, B1, B2, B6 and B12)
- Produce neurotransmitters (for example: serotonin)
- Support optimal energy production from food
- Support healthy mood, cognition, stress response and vitality

QUALIFYING CHARACTERISTICS OF PROBIOTIC MICROORGANISMS[8,11,12]

> Proper strain characterisation (established named genus, species and subspecies).
> Viable (survives gastric and intestinal transition through acidic pH, enzymes, bile salts).
> Adheres to intestinal epithelium.
> Safe for intended use and human consumption.
> Clinical therapeutic efficacy demonstrated by human studies (at least 1).
> Strains active and at a therapeutic concentration for whole shelf life.

(Plaza-Diaz 2019, Binda 2020, Maldonado Galdeano 2019)

Table 1: Qualifying Characteristics of Probiotics[1, 103]

Necessary Characteristic	The Challenge	Why is it necessary
Gastric acid and bile salt stability	• Probiotics are vulnerable to Immunological compounds in the saliva such as IgG, IgA and IgM • Probiotics are highly vulnerable to gastric acid, pepsin and mechanical agitation in the stomach • Bile salts and digestive enzymes can impact probiotic viability by damaging membranes and destroying DNA	Probiotics need to be able to survive these harsh environments so they can get to the colon or functional site lower in the GIT where they exert most of their therapeutic effects.
Adherence to intestinal mucosa	• Pre-existing commensal bacteria can exert "colonisation resistance" to a probiotic taken in supplemental form. Probiotics need to compete with pre-existing commensal bacteria for adhesion sites and nutrients in order to colonise effectively.	Adherence is the first step in the colonisation process. This characteristic is also vital for immune signalling and also for competitive inhibition of pathogenic organisms.
Colonisation of the intestinal tract	• Again, due to the phenomenon of "colonisation resistance", supplemented probiotics need to compete with pre-existing commensal bacteria for adhesion sites and nutrients in order to proliferate and exert a therapeutic effect	Colonisation is required in order for an adequate interaction with the host to occur. It is also required for immune cell signalling, crowding out pathogenic micro-organisms (competitive inhibition).
Human origin* * with the exception of the spore-based probiotics	• Bacteria of non-human origin may not survive the human gastrointestinal tract as well as human derived bacteria	Provides species specific benefits to health
Documented safety	• Supplementation with any medicine may produce adverse health outcomes	Medicine safety is vitally important to ensure that the therapeutic effects outweigh the risks associated with the consumption of a medicine
Production of anti-microbial compounds	• Pathogenic organisms may flourish if not challenged	Necessary to suppress the growth of pathogenic organisms and maintain the normal microbiome
Antagonism of pathogenic organisms	• Pathogenic organism may also adhere to and colonise the GIT if left unchallenged	Required to prevent the adherence of pathogens and subsequent toxin production
Clinically documented and validated health effects	• Probiotic may be ineffectual	Clinically studied strains with documented effectiveness instil confidence in the prescriber. A validated dose and effect will achieve therapeutic results
Documented shelf-life and stability	• Probiotic may become non-viable during both the manufacturing process and during product storage	Adequate stability/viability data ensures that all of the above characteristics are maintained for a known length of time

Much research has been done on different species of probiotic bacteria and their health effects. Several thousand studies have been published in the last hundred years or so.[1] Probiotics are now defined as live microorganisms that induce a beneficial medicinal effect when used in therapeutic concentrations.[7-10] Probiotic nomenclature requires specification of the genus, species and subspecies followed by the specific strain identification/designation.[11] This increasing body of research emphasises the importance of strain specificity for probiotic identification, mechanisms of action and efficacy in relation to clinical outcomes for many symptoms, disorders and pathologies.[11,13]

Several meta-analyses have found that while some probiotic mechanisms are exhibited by the genus group (e.g. *lactobacillus, bifidobacterium*), others are strain-specific.[11,13,14] This combination of genus-specific and strain-specific mechanisms exhibited by probiotic bacteria enables a therapeutic benefit for a range of symptoms, disorders and pathologies. See Figure 1 and Table 2.

Figure 1: Probiotic mechanisms of action.

Diverse mechanisms are likely to drive probiotic benefits to host health. In some cases, such as production of antimicrobial products and cross- feeding other resident microorganisms, these mechanisms are driven directly by interactions with the resident microbiota. In other cases, such as direct interaction with immune cells, their effects might be direct via interaction with host cells. Overall, clinical benefits delivered by probiotics could result from the combined action of several mechanisms.

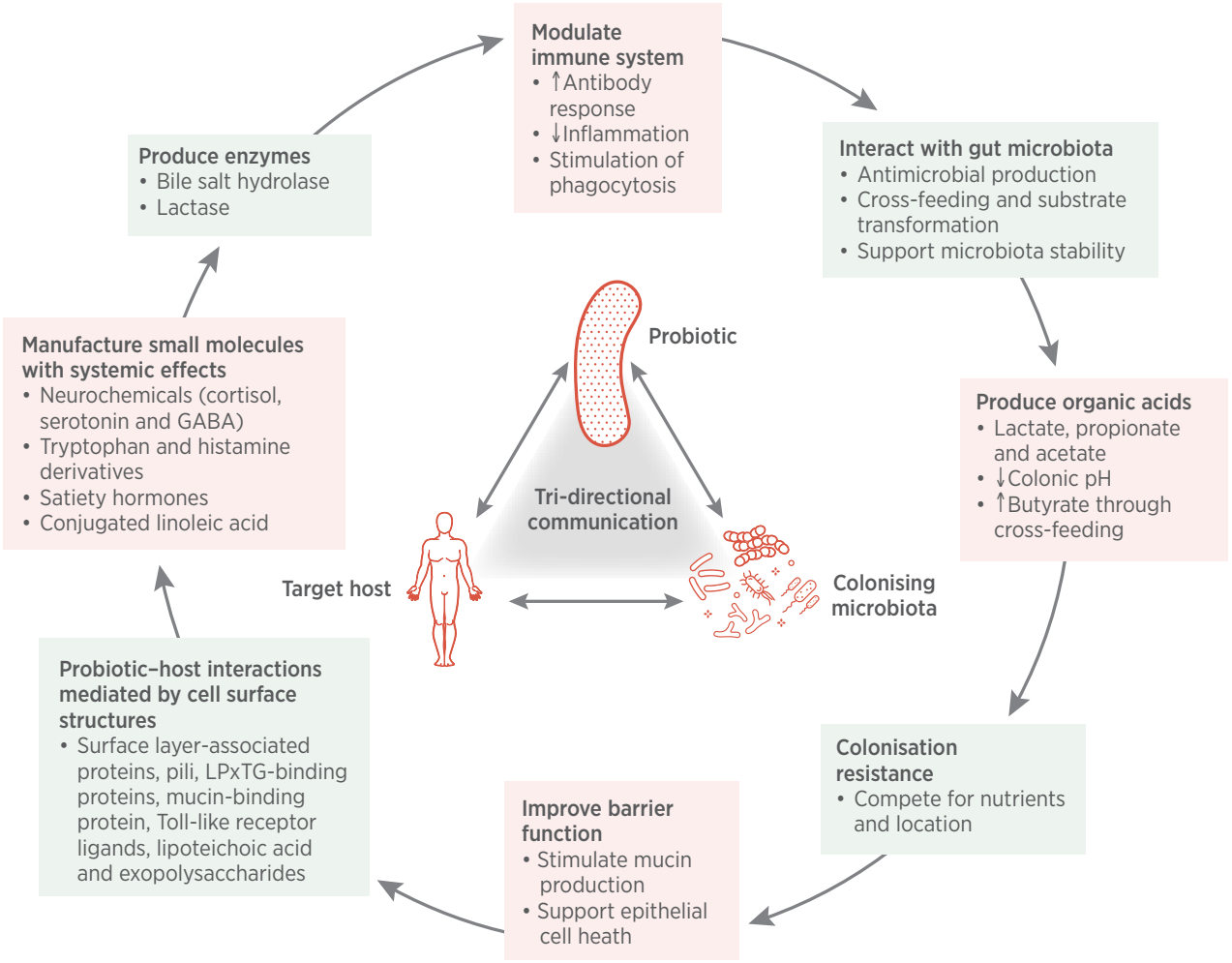
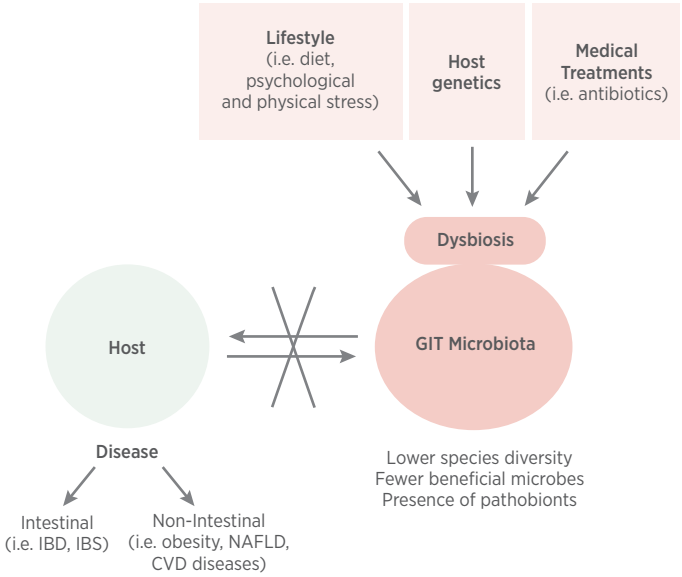


Table 2: Strain-specific therapeutic actions

Strain	Strain-Specific Therapeutic Action
Bifidobacterium animalis ssp. lactis (BS-01)	• Increases gastrointestinal bifidobacteria colonisation.[35] • Improves bowel movement frequency and stool consistency.[36]
Lactobacillus plantarum (Lp-115)	• Reduces intestinal inflammation (myeloperoxidase levels) when used adjunctively with sulfasalazine.[37]
Lactobacillus acidophilus (La-14)	• Enhances immunoglobulin G (IgG) responses following oral vaccination.[38] • Antimicrobial.[39]
Bifidobacterium breve (Bb-18)	• Ameliorates intestinal dysbiosis in a multi-probiotic formulation.[40]
Lactobacillus salivarius ssp. salivarius (Ls-33)	• Beneficially modulates gastrointestinal microbiota.[41] • Promotes intestinal immune activity.[42] • Ameliorates intestinal inflammation.[43]
Lactobacillus paracasei (LPC-37)	• Increases SCFA levels (acetate, propionate, butyrate) and total bifidobacterial counts.[6]
Lactobacillus rhamnosus (HN001)	• Modulates gastrointestinal microbiota composition by reducing pathogenic and increasing beneficial bacteria.[44-46] • Beneficial for management of intestinal inflammation.[8] • Significantly protects against eczema development and atopic sensitisation in children when taken from 35-weeks' gestation to 6-months postpartum.[33,34]
Bifidobacterium bifidum (Bb-06)	• Ameliorates intestinal dysbiosis in a multi-probiotic formulation (including Bb-06 and Bl-05).[40]
Bifidobacterium longum (Bl-05)	• Ameliorates intestinal dysbiosis in a multi-probiotic formulation (including Bb-06 and Bl-05).[40]
Bacillus coagulans (Unique IS-2™)	• Assists digestive processes by directly producing digestive enzymes, supporting the health of mucous linings of the GIT and assisting excretion processes[98, 99] • Effectively reduces abdominal pain associated with medical diagnosed irritable bowel syndrome[100, 101]
Bifidobacterium longum (1714)	• "Psychobiotoc" that can impact stress-related behaviours, mood and cognitive performance[107, 110] • Reduces mental fatigue and activates brain coping centres to counter-regulate negative emotions[106]

Perturbation of intestinal microbiota composition (dysbiosis) is associated with impaired/disordered health and increased disease susceptibility.[4-6] Improving the incidence, severity and long-term impact of such clinical symptoms and disorders requires a multifaceted therapeutic approach that includes the beneficial modulation of the intestinal microbiome using a combination of lifestyle strategies with researched, strain-specific probiotics.

Figure 2: Causes of gastrointestinal tract microbiota dysbiosis and effect on host health.
GIT: Gastrointestinal tract; NAFLD: Non-alcoholic fatty liver disease; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome; CVD: Cardiovascular disease.



GENERAL PROBIOTIC ACTIVITY

Intestinal health

The gastrointestinal microbiome fundamentally influences intestinal health by regulating many functional aspects including stool frequency and consistency, intestinal pH, mucosal barrier integrity, immune response and function, antimicrobial peptide synthesis and macro- and micronutrient absorption.[6, 15-21]

GIT transit time (GTT) – GTT can be influenced by a number of different probiotic strains. Transit time is generally increased with supplementation; however, probiotics are often employed in the alleviation of acute diarrhoea as well.

In the case of increasing GTT, the exact mechanism has not yet been fully elucidated however, various metabolites produced by beneficial bacteria such as SFCA'S, lactic acid have been found to reduce luminal pH and influence the tone of the sigmoid colon thereby enhancing peristalsis.[1, 99]

As mentioned above, Probiotic supplements are also often employed during episodes of acute diarrhoea and often include species from the *Bacillus, Saccharomyces, Streptococcus, Lactobacillus* and *Bifidobacterium* genera.[104]

Acute diarrhoeas can result from infection, antibiotic use, diet and lifestyle factors and other causes. A recent meta-analysis has found that *Bacillus coagulans* is the most effective probiotic in the relief of anti-biotic associated diarrhoea.[104] Potential mechanisms behind the efficacy of some bacterial genera for reducing diarrhoea include:

- remodelling the gut microbiome and correcting dysbiosis,
- affecting immunomodulation,
- reducing visceral hypersensitivity, and
- modulating tight junction proteins to support the epithelial barrier.[1, 104]

Digestive function – probiotics have been found to support and even enhance general digestive function by their ability to produce enzymes to assist food breakdown and nutrient absorption; produce metabolites such as short chain fatty acids, butyrate and lactic acid which can reduce colonic pH and be used as energy sources by colonocytes; reduce the production of harmful substances such as amines helping to reduce toxin build up in the bowel and generally improve the intestinal metabolic environment.[1, 98]

I**ntestinal barrier function –** probiotics have been found to increase mucin production in the gut, thereby providing a protective barrier over the epithelial lining. They are also able to influence zonulin and occludin location, preserve enterocytes and modify tight junction proteins.[1]

Given the pleiotropic nature of the microbiota in relation to gastrointestinal health, consequences of microbiome disruption are broad including intestinal symptoms (bloating, constipation, excessive gas, food intolerances), dysbiosis, pathogenic overgrowth, altered immunity, local and systemic inflammation, impaired nutrient absorption and functional or structural intestinal disorders.[6,16,17,21-23]

Competitive Inhibition

Probiotics compete with pathogenic bacteria for physical space and nutrients in the host tissue. The probiotic is thought to be able to block pathogenic bacteria from finding a spot on the host tissue to bind and grow.[47]

When taken orally, *lactobacilli* pass through the gut and attach to the intestinal mucosa where they can persist for at least a week. Once the *lactobacilli* latch on to and colonize the intestinal or urogenital tract, epithelial attachment by pathogenic bacteria is reduced.[48] *Lactobacilli* are thought to do this in part by regulating the host symbiotic microbiota,[98] increasing epithelial mucus production and competing with pathogens for mucosal binding sites.[49]

Some probiotics are also able to produce metabolic products that have antibacterial effects against some other organisms. Some probiotics excrete bactericidal proteins called bacteriocins, hydrogen peroxide, lactic acid, or acetic acid. Each of these chemicals can block colonization by pathogenic organisms.[47] For example, *lactobacilli* probiotics produce lactic acid, and many can also produce hydrogen peroxide. In the vagina, the production of lactic acid secretions from probiotics reduces vaginal pH, which prevents pathogen growth. The hydrogen peroxide

produced by *lactobacilli* is bactericidal against many vaginal pathogens, such as *Gardnerella vaginalis*.[50]

Immunomodulating effects

Some probiotics seem to modulate non-specific cellular and humoral immunity, possibly by stimulating lymphocyte and macrophage activity.[51] and modulating cytokine production by mononuclear cells. Probiotics have also been shown to decrease markers of hypersensitivity reactions and intestinal inflammation, such as tumour necrosis factor (TNF), and alpha-1-antitrypsin. Some probiotics also appear to enhance the synthesis of antibodies in response to microbial pathogens, particularly secretory immunoglobulin A.[51]

The way probiotics affect immune function may differ depending on the health status of the person taking it. In people with immune system hypersensitivity, probiotics seem to down-regulate immune function. In healthy people without immune system hypersensitivity, probiotics seem to stimulate the immune system. Due to these immunomodulating effects, some researchers hypothesize that probiotics might not only fight intestinal and urogenital pathogens but might also be helpful for several immune-based indications, such as inflammatory bowel disease, pouchitis and food allergy.[52]

Nervous system

Due to the discovery that dysbiosis can affect the gut-brain axis (GBA), there is an increasing amount of interest in how probiotic therapy affects the nervous system. The microbiota is able to impact interactions that occur between the emotional and cognitive centres of the brain and gastrointestinal function.[105]

The intestinal microbiota has a local and peripheral interactive relationship with the central, enteric and autonomic nervous systems as well as the HPA axis through immune, neuroendocrine, microbial and metabolic signalling pathways.[105, 106, 111] Bacteria can signal through these pathways impacting behaviour and brain activity and affect changes in central nervous system matrices involved in emotional or cognitive responses, neuroinflammation, neurodevelopment and neuroendocrine stress responses.[108, 111] Evidence of the association of dysbiosis with disorders of the central nervous system including autism, anxiety, depression and functional disorders of the GIT that are associated with mood disorders such as irritable bowel syndrome has been discovered.[105, 113]

It is thought that disordered GBA functioning results from altered motility and toxin secretion, visceral hypersensitivity and alterations in the functioning of the entero-endocrine and immune system. The fact that the microbiome is involved in the maintenance of these aspects of health, and also that specific dysbiotic patterns have been found in some conditions (e.g. autism)[105] suggests a potentially unique contributing role of probiotic therapy, and research is progressing into specific strain therapy. Collectively, these probiotic strains have been named "Psychobiotics", and are defined as "live micro-organisms that convey a benefit upon the host's mental health when consumed in adequate quantities".[107]

PROBIOTICS SPECIES

Lactobacillus acidophilus: a potent immunomodulatory probiotic strain shown to enhance immune activity by increasing regulatory T cells, inducing chemokine and cytokine response, stimulating dendritic cells to promote Th1/ Th2/Th3 immunity, and improving IgA response.[53-57]

Studies show *L. acidophilus* may help to reduce the incidence and duration of cold and flu symptoms, improves colitis, and enables immune maturation in foetal enterocytes.[54-56, 58, 59] It has also been shown to improve microbiome diversity following antibiotic therapy and is effective against *C. difficile*, candidiasis, and SIBO, while reducing constipation and increasing bowel frequency.[60-71]

Lactobacillus plantarum: significantly inhibits the invasion of pathogenic *E. coli*, especially when combined with other probiotic strains, and effectively reduces disturbance of the microbiome resulting from antibiotic therapy.[72, 73] Studies show it reduces abdominal pain, bloating and other gastrointestinal symptoms associated with IBS and colitis.7[4, 75] As an immunomodulatory agent, *L. plantarum* enhances the IgG response and improves the body's response to influenza in elderly individuals, especially.[76, 77]

Bifidobacterium animalis sp. lactis: has been present in human food for decades and is broadly recognised for its key role in the human intestinal microflora throughout life. Its anti-inflammatory properties are useful in attenuating the symptoms of colitis, while supporting the body against allergies and allergic rhinitis.[75, 78] It protects and restores the microbiome following antibiotic therapy and boosts the body's IgG response.[61, 78]

Lactobacillus casei: improves systemic and mucosal immune responses, reducing the occurrence of infections in the elderly, especially.[79, 80] Its anti-inflammatory properties are noted as it lowers hsCRP, reduces the occurrence of necrotising enterocolitis, modifies the expression of toll-like receptor in ulcerative colitis, and repairs aspirin-induced bowel injury.[81-84] *L. casei* also improves insulin sensitivity, thus, playing a role in helping to prevent diabetes mellitus.[85]

Bifidobacterium breve: is a normal commensal microorganism that prevents and improves constipation, abdominal bloating, anal itch, burn, pain, and other symptoms of ulcerative colitis and necrotising enterocolitis.[84, 86, 87] It also maintains fasting glucose, decreases hsCRP, and increases plasma glutathione,[88] as well as helping to improve fasting insulin sensitivity and reduce body weight in overweight youths.[113]

Lactobacillus paracasei: can inhibit pathogenic salmonella, *S. aureus, E. coli*, and listeria, while protecting and restoring the microbiome following antibiotic therapy.[61, 62] As an immunomodulatory agent, it induces IL-10, (TNF)-α, (IFN)-γ, and IL-12, and enhances the IgG and IgM response.[76]

Additionally, *Lactobacillus paracasei* strain LPC-37 has been studied as a potential psychobiotic in stress affected adults and shown improvements in perceived stress among the general population. Treatment subgroups experiencing both high and low chronic stress also showed improved

responses to acute stress including a reduction in heart rate, perceived exhaustion and evening cortisol levels.[111]

Lactobacillus salivarius sp. salivarius: mitigates inflammatory symptoms and modulates cytokine production and the cellular response to pathogenic challenges while restoring a disrupted microbiome.[61, 89] It also improves oral health by reducing gum bleeding and physiologic halitosis while increasing resistance to caries.[90, 91] It may also be protective against mastitis in breastfeeding women with one study showing that *L. salivarius* supplementation was superior to antibiotic therapy in decreasing levels of pathogenic bacteria in breast milk and reducing breast pain.[1]

Lactobacillus rhamnosus: is a potent immunomodulatory strain that increases interleukin and cytokine production, phagocytosis and NK-cell activity, sIgA secretion, foetal immunity, and immunomodulatory components of breastmilk.[64] It is effective against *C. difficile*, *E. coli* O157:H7, and *S. typhimurium*.[92]

Bifidobacterium bifidum: improves functional constipation and symptoms of IBS, including abdominal pain, bloating, belching, flatulence, and diarrhoea.[93] Upper gastrointestinal symptoms associated with *H. pylori* infections also benefit from *B. bifidum*.[94]

Bifidobacterium longum: improves the composition and metabolic activities of colonic bacterial communities and immune parameters, helping the symptomatic effects of celiac disease, IBS, and functional constipation. Studies show *B. longum* significantly reduces TNF-alpha, CRP, serum AST, insulin resistance, serum endotoxin, and steatosis in patients with non-alcoholic steatohepatitis.[95, 96]

As mentioned above, there is surging interest in the possibility that positively manipulating the gut microbiome may beneficially impact the brain and nervous system. As a result, some innovative research on strain specific probiotics has come about. One of the most impressive psychobiotic strains studied so far is *Bifidobacterium longum* strain 1714.

Preclinical and clinical studies have highlighted benefits to cognitive performance, mood, memory and stress. It has been shown to decrease stress-related behaviours, improve the stress response in both the short and long term, promote cognitive function in stressful situations and create a sense of vitality in the resting state.[106, 107, 109, 110]

Mechanisms behind the effects of *B. longum* 1714 include modulation of neural responses by increasing theta band power in parts of the frontal and cingulate cortexes and decreasing beta-2 band power in the fusiform gyrus, bilateral hippocampus, parts of the bilateral middle temporal cortex and the left cerebellum.[106]

Bacillus coagulans: Originally named *Lactobacillus sporogenes*, *Bacillus coagulans* is a transient, gram positive, lactic acid producing bacteria that is protected from stomach acid by a protein-based spore coating – a characteristic that gets around the necessity for other probiotics to be of human origin as discussed earlier. Instead of dying off as a result of coming into contact with stomach acid, the spore coating expands and *B. coagulans* begins to germinate as it moves out of the stomach and into the duodenum where the environment is more amenable[97, 98] *B. coagulans* affects the microbial colony in that it helps to regulate beneficial flora and inhibit the proliferation of opportunistic bacteria via the production of "bacteriocin-like substances" and short chain fatty acids (SCFAs). These compounds support the health of the GI lining and provide direct antagonism to microbial pathogens.[97]

B. coagulans is also able to produce several enzymes that assist digestive processes such as nutrient breakdown and absorption. Some of these enzymes include lipase, protease, α-amylase and both α- & β-galactosidase and assist with protein and carbohydrate digestion.[98] Absorption is also facilitated as *B. coagulans* can reduce inflammation of the gut lining and promote the health of the permeable areas of the micro villi.[98]

As well as digestion, *B. coagulans* (Unique IS-2™) has also been found to assist excretion and is useful in cases of functional constipation. It produces metabolites such as diacetyl, SCFAs and vitamins, stimulates peristalsis, reduces amine production, reduces bowel transit time, and improves the overall health of the bowel. This in turn helps to improve bowel regularity and reduce toxin build up.[98, 99]

B. coagulans (Unique IS-2™) is also able to effectively reduce abdominal pain associated with medical diagnosed irritable bowel syndrome.[100, 101]

INTERACTIONS WITH DRUGS

Antibiotics: as probiotics are bacteria, there is some concern that the concomitant administration of antibiotics might decrease the effectiveness of these probiotics. Since these preparations usually contain live and active organisms, simultaneously taking antibiotics might kill a significant number of sensitive organisms. Recommend taking probiotics 2 hours away from antibiotics.

Immunosuppressants: theoretically, taking probiotics could increase the risk of infection in patients taking medications that suppress the immune system. Additionally, probiotics might stimulate the immune system, potentially decreasing the effectiveness of immunosuppressant drugs. These include cyclosporine, tacrolimus, azathioprine, and cancer chemotherapeutic agents like cyclophosphamide and cisplatin.

Table 3: Study Summary

Reference	Strain and dose studied	Outcomes/findings
Patterson, E. et. al. 2020[111]	*Lactobacillus paracasei* (LPC-37); 17.5 billion cfu daily	Significant reduction in perceived stress among the treatment group. Parameters including exhaustion, perceived productivity, heart rate, 8pm cortisol levels and sleep were improved in some treatment subgroups
Wickens, K. et. al. 2018[33]	*Lactobacillus rhamnosus* (HN001); 6 billion taken by the mother from 35 weeks gestation to 6 months post-partum	Significant protection against eczema development from birth to years in infants
Wickens, K. et. al. 2013[34]	*Lactobacillus rhamnosus* (HN001); 6 billion taken by the mother from 35 weeks gestation to 6 months post-partum	Cumulative presence of eczema halved at 2 and 4 years of age
Sheih, Y. H. et. al. 2001[112]	*Lactobacillus rhamnosus* (HN001); 10 billion cfu twice daily in low fat or lactose free milk daily	Support systemic cellular immune responses
Lombardi, F. et. al. 2020[40]	*Bifidobacterium bifidum* (BB-06) & *Bifidobacterium longum* (BB-05); 4 billion cu daily as part of a multi-strain probiotic	Ameliorated intestinal dysbiosis in patients with Nickel allergy syndrome
Wang, H. et. al. 2019[106]	*Bifidobacterium longum* (1714); 1 billion cfu daily	Modulation of neural activity correlating with enhanced vitality, improved stress induced coping mechanisms
Allen, A. P. et. al. 2016[107]	*Bifidobacterium longum* (1714); 1 billion cfu daily	Reduction in daily reported stress, improved memory performance
McFarland, L. V. et. al 2021[101]	*Bacillus coagulans* (Unique IS-2™); 2 billion cfu daily	Significant reduction in abdominal pain scores in IBS patients
Madempudi, R. S. et. al. 2019[100]	*Bacillus coagulans* (Unique IS-2™); 2 billion cfu daily	Significant reduction in abdominal pain and increased number of complete spontaneous bowel movements in IBS patients
Madempudi, R. S. et. al. 2019[99]	*Bacillus coagulans* (Unique IS-2™); 2 billion cfu daily	Increased number of bowel movements, improvements in stool consistency, relief of symptoms of incomplete evacuation, painful defecation and abdominal pain associated with functional constipation

References supplied on request.