

**PROBIOTICS**  
*Little Bugs. Big Impact.*

REVODERM PHARMA



Probiotics were defined by a group of experts convened by the Food and Agriculture Organization of the United Nations (FAO) as “live microorganisms administered in adequate amounts which confer a beneficial health effect on the host”. Most probiotics are bacteria, which are small, single-celled organisms.

Most probiotic products contain bacteria from the genera Lactobacillus or Bifidobacterium, although other genera, including Escherichia, Enterococcus, Bacillus, Propionibacterium and Saccharomyces (a yeast) have been developed as probiotics.

The microbes present in the gastrointestinal (GI) tract have the potential to act in a positive, negative or neutral manner. Due to unfavourable conditions, microbes are not very prevalent in the stomach or upper small intestine. However, toward the lower small intestine, they begin to attain higher populations (10<sup>6</sup>-10<sup>8</sup>/gram of small intestinal contents) and in the colon they constitute about 0.11-10<sup>12</sup>/gram of colon contents (a very large number).

## DEFINING THE TERMS

### PROBIOTICS: THE GOOD BACTERIA

The term probiotic is derived from the Latin preposition “pro,” which means “for” and the Greek word “biotic” meaning “bios” or “life”. The concept that the gut flora can be modified and harmful microbes replaced with beneficial ones was first introduced in 1907 by a Russian scientist called Elie Metchnikoff. He proposed that putrefactive or proteolytic bacteria generate toxins in the large bowel that cause “intestinal auto-intoxication,” which contributes to the aging process.

### PREBIOTICS: FIBRE FOOD FOR BACTERIA

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of one or a limited number of bacterial species in the colon, such as Bifidobacteria and Lactobacilli, which have the potential to improve host health.” Prebiotics are, simply speaking, the “food” for beneficial bacteria. Common prebiotics are inulin and carbohydrate fibres called oligosaccharides and are not digestible by humans.

### SYNBIOTICS: COMBINING PROBIOTICS AND PREBIOTICS

A synbiotic is a supplement that contains both probiotics and prebiotics.

### FLORA

“Flora” generally means all the microbes (the bacteria, fungi (including yeasts), protists, archaea and even viruses) that live in and on our bodies. Many scientists prefer to call it “microbiota.”

### CFUS

This stands for “colony-forming unit” and is the way probiotics are measured. A colony-forming unit is a bacteria or yeast that is capable of living and reproducing to form a group of the same bacteria or yeasts. Microbiologists use CFU to describe the number of active, live organisms instead of the number of all the bacteria – dead, inactive and alive – in a laboratory sample. Only the viable organisms are considered to be probiotics. “Viable” means that the microbes are capable of living under the proper circumstances.

### DYSBIOSIS

This is the medical term for when the healthy and unhealthy bacteria in the body get out of balance. Taking probiotics and prebiotics can help correct dysbiosis.

## GENUS, SPECIES AND STRAIN

Bacteria are categorized by scientists with genus, species and strain names. Each named species of living things is always referred to by its genus and species name, never the species name alone.

The genus is the first word in a bacterium's name; it's the large group to which the bacteria belongs. The species is the type of individual bacteria. Some bacteria have several strains or differentiations of the species, and is identified by the last part of the name. Here are some examples:

- For the bacterium *Lactobacillus rhamnosus* GG, the genus is *Lactobacillus*, the species is *Rhamnosus* and the strain is GG. Sometimes companies also develop a marketing name for probiotic strains. For this probiotic strain, the commercial name is "LGG".
- For *Lactobacillus acidophilus* DDS-1, the genus is *Lactobacillus*, the species is *Acidophilus* and the strain is DDS-1.

## GENERAL BENEFITS OF LACTOBACILLI

### GENERAL CHARACTERISTICS

*Lactobacillus* is the name of a genus of Gram-positive, non-sporulating, rod-shaped bacteria. Most of them are facultative anaerobes or microaerophilic. *Lactobacilli* are some of the most well-known probiotics. The genus *Lactobacillus* currently consists of over 180 species and encompasses a wide variety of organisms. These microbes, like *Bifidobacterium*, are lactic-acid producing bacteria (LAB) and, in fact, the two genera share a few common genes.

### LOCATION

In humans, they are found in the GI tract, from the mouth, the sinuses, the throat, the esophagus, the stomach, the small intestine and the colon. They are also found in the female vagina and urogenital tract.

### MECHANISM

- *Lactobacilli* take in carbohydrates and produce lactic acid. It prefers different kinds of carbohydrates depending on their species.
- Some of them can use lactose, which is beneficial for those suffering with lactose intolerance.
- Some of them also produce alcohol, gases, vitamins, short-chain fatty acids, hydrogen peroxide, bacteriocins (antibacterials) and antibiotic chemicals, depending on their living conditions.
- They produce the following:
  - Lactic acid
  - Hydrogen peroxide
  - Acetic acid
  - Benzoic acid
- Of the more than 180 *Lactobacillus* species, the following are commonly used probiotics:

|                       |                      |                     |                      |                     |
|-----------------------|----------------------|---------------------|----------------------|---------------------|
| <i>L. acidophilus</i> | <i>L. bulgaricus</i> | <i>L. casei</i>     | <i>L. helveticus</i> | <i>L. paracasei</i> |
| <i>L. plantarum</i>   | <i>L. reuteri</i>    | <i>L. rhamnosus</i> | <i>L. salivarius</i> | <i>L. lactis</i>    |

### CLINIC PRACTICE

- They enhance the immune system.
- They prevent overgrowth of disease-causing microbes such as *Candida* species, *E. coli*, *H. pylori* and *Salmonella*.
- They help prevent vaginal and urinary tract infections.
- They prevent and treat antibiotic-associated diarrhea.
- They synthesize and enhance the bioavailability of nutrients and improve nutrient absorption.

- They maintain the integrity of intestinal tract and protect against macromolecules entering the bloodstream and causing antigenic response.
- They lessen intestinal stress from food poisoning.
- They reduce symptoms of lactose intolerance and decrease prevalence of allergy in susceptible individuals.

## GENERAL BENEFITS OF BIFIDOBACTERIA

### GENERAL CHARACTERISTICS

Bifidobacterium is a genus of gram-positive, nonmotile, often branched anaerobic bacteria. They make up 25% of adult fecal bacteria and 80% in infants. Before the 1960s, Bifidobacterium species were collectively referred to as "Lactobacillus bifidus". Bifidus is a genus or group of species, with 32 species currently known in this group. These microflora contribute to the degradation of undigested polysaccharides in the human colon and ferment carbohydrates into short chain fatty acids (SCFA's) for use a primary energy source for intestinal cells. The Bifidobacterium were originally identified in the feces of a breast-fed infant. They are the primary bacteria in infants and they help infants grow. These good bacteria are some of the first microbes to take up residence in the sterile GI tract of newborns and they dominate the intestines in breast-fed babies.

### LOCATION

They are ubiquitous inhabitants of the GI tract, vagina and mouth of mammals, including humans.

### MECHANISM

- They also produce vitamins, bacteriocins (antibacterial chemicals) and antibiotic-like substances.
- Unlike many Lactobacillus, Bifidobacterium also produce acetic acid, a short-chain fatty acid SCFA. The significance of this is that acetic acid is more effective at reducing the growth of yeasts and molds than is lactic acid. Acetic acid can also be used as energy by the human body. Producing both lactic and acetic acids and other beneficial compounds makes Bifidobacterium the probiotic most qualified to be in the vagina and urogenital tract, where yeasts and opportunistic bacteria can cause distress, and also in the colon, where the opportunity for disease to flourish is greater because fecal transit time slows. Bifidobacterium support the immune system by competing with unwanted or harmful bacteria for nutrients and colonization space.
- Bifidobacteria produce:
  - Short-chain fatty acids
  - Acetic acid
  - Propionic acid
  - Butyric acid
  - Lactic acid
  - Formic acid
- Of the more than 30 Bifidobacterium, the following five are frequently used as probiotics:

|                   |                 |                    |                  |                  |
|-------------------|-----------------|--------------------|------------------|------------------|
| <i>B. bifidum</i> | <i>B. breve</i> | <i>B. infantis</i> | <i>B. lactis</i> | <i>B. longum</i> |
|-------------------|-----------------|--------------------|------------------|------------------|

### CLINIC PRACTICE

- They prevent colonization of the intestine by pathogenic bacteria and yeasts by protecting the integrity of the intestinal lining. They inhibit growth of bacteria that produce nitrates in the bowel. These nitrates are toxic and can cause cancer.
- They decrease the side-effects of antibiotic therapy. They prevent and treat antibiotic-associated diarrhea.
- They help prevent production and absorption of toxins produced by disease-causing bacteria, which reduces the toxic load on the liver.

- They manufacture B-complex vitamins.
- They help regulate peristalsis and bowel movements.
- Reside primarily in the colon (lactobacillus strains are found in the small intestine as well), bifidobacterium only products can be ideal for patients suffering from SIBO.
- Bifidobacterium infantis, Bifidobacterium longum and Lactobacillus plantarum may be beneficial for histamine intolerance because they degrade histamines, whereas some gut bacteria produce histamine so should be avoided.
- For some people, the formation of excess D-lactic acid in the gut (usually in combination with overgrowth of one or more D-lactate-producing bacteria species such as Lactobacillus acidophilus and Lactobacillus plantarum) causes problems.
- Our bifidobacteria only formulas contain none of these species and thus would be safe for those who have D-lactate sensitivity.

## SOIL-BASED ORGANISMS (SBOS)

- The term SBO stands for “soil-based organism” and is used to refer to a new class of probiotic supplements based on a greater understanding of the incredible diversity of the human gut, and a deeper appreciation for how humans and their commensal “helper” bacteria work together to produce a healthy system.
- Soil-based probiotics are probiotics that are often found in soil and that confer a health benefit to humans.
- Soil-based bacteria, also known as spore-forming bacteria, have the ability to “seed” the digestive tract with bacteria which will flourish and support a balanced microbiome.
- Spores being heat-stable have a number of advantages over other non-spore-formers, such as Lactobacillus spp. that can be stored at room temperature in a desiccated form without any deleterious effect on viability.
- A second advantage is that the spore is capable of surviving the low pH of the gastric barrier which is not the case for all species of Lactobacillus, so in principle spores can be stored indefinitely without refrigeration and the entire dose of ingested bacteria will reach the small intestine intact.
- When isolated from the human GI tract, bacilli have long been thought to be transient bacteria, however current research shows that Bacillus species are present in the GI tract in numbers higher than can be explained by ingestion alone.
- Germination of Bacillus spores within the human small intestine and transient colonization is now thought to be part of the normal life cycle of human-associated Bacillus species providing specific stimuli for healthy GI and immune system development and function.
- They are in a ‘dormant’ state until ingested by the body and activated by saliva, juice or water. These bacteria come to life amidst warm, moist surroundings.
- These probiotics have a seed-like structure that protects them from heat, oxygen and even passage through the stomach and small intestine.
- Soil-based probiotics are well-adapted to the environment of the gut and have been shown to remain in the digestive tract where they can provide long term benefit.
- They have an affinity for a more acidic environment and will not be affected by hydrochloric acid in the stomach.
- They are therefore enabled to reach the lower GI tract where they will multiply into billions.
- SBOs have also been found to be beneficial for autoimmune conditions.
- Sensitive individuals often tolerate SBOs better than traditional probiotics and this is especially true for conditions such as histamine intolerance or SIBO (Small Intestinal Bacterial Overgrowth).
- SBOs have also been shown to colonize the gut more effectively than lactic-acid bacteria.
- SBOs are indicated for the following situations:
  - Patients who are not experiencing success with traditional lactic acid based probiotics
  - Patients who have taken a course of antibiotics
  - Patients with tough digestive issues

- Patients who travel frequently and require room temperature stable probiotics
- Patients who have a history of high stomach acid
- Patients who do not want to keep their probiotics in the refrigerator
- Unlike lactic acid bacteria (e.g. Lactobacillus), the many Bacillus species have their own cycle of spore-proliferation and spore-release in the gut.
- This activity continues long after the spore-forming bacteria are ingested – creating a truly unique symbiotic relationship with the human host.

### ACTIONS OF SBOS

- |   |  |
|---|--|
| <ul style="list-style-type: none"> <li>• Support for balancing gut microbiota</li> <li>• Improve gastrointestinal health</li> <li>• Promote and normalize bowel function</li> <li>• Improve diarrhea, abdominal pain, bloating and stool consistency Improve IBS severity</li> <li>• Decrease leaky gut and heal damaged mucosa</li> <li>• Decrease inflammation, promote intestinal regeneration</li> <li>• Balance colon pH Assist in digestion and the absorption of nutrients</li> <li>• Enhance metabolism in the gut</li> <li>• Produce beneficial by-products such as B vitamins, Vitamin K2, carotenoid antioxidants and enzymes such as Nattokinase</li> </ul> | <ul style="list-style-type: none"> <li>• Support overall health and well being</li> <li>• Improve immune system function</li> <li>• Increase secretory IgA</li> <li>• Decrease respiratory tract infection</li> <li>• Support the development of Gut-associated Lymphoid Tissue (GALT)</li> <li>• Decrease adverse effects from antibiotics</li> <li>• Produce higher levels of lactic acid than conventional probiotics</li> <li>• Prevent gut colonization by harmful bacteria and fungi</li> <li>• Produce natural bacteriocins to reduce opportunistic and harmful bacteria</li> <li>• Secrete antimicrobial peptides</li> </ul> |
|---|--|

### ACTIONS OF LACTIC ACID BACTERIA

- Competitive inhibition
- Generation of non-conductive acidic environment
- Production of antibiotic-like substances (bacteriocins)

### HOW SBOS DIFFER FROM REGULAR PROBIOTICS

- They are much more likely to make it to the small intestine intact; thus fewer CFUs are necessary to deliver benefit.
- They colonize the gut more effectively, thus fewer CFUs are necessary to deliver a therapeutic benefit.
- They are capable of surviving the low pH of the gastric barrier.
- Remain stable at room temperature and do not require refrigeration.

## SIBO PROBIOTIC FORMULAS

- It seems somewhat counterintuitive to treat SIBO, small intestine bacterial overgrowth, with additional bacteria, however recent research has seen success using probiotics as part of a comprehensive SIBO protocol.
- The problem with probiotics is not necessarily the bacterial species but the prebiotics present in them.
- The importance of probiotics in treating SIBO cannot be ignored just because some of them are not ideal.
- They can be effective in correcting dysbiosis particularly after taking antibiotics or herbal treatments for SIBO.

- Here are a few reasons why probiotics are helpful in treating SIBO and fix up the dysbiosis:
  - Probiotics strengthen the mucosal barrier of gut, which prevents pathologic bacteria from attaching to GI tract walls.
  - Probiotics control immune system of the gut.
  - They produce anti-microbial compounds which help to reduce pathogens.
  - They also help to keep the bad bacteria growth under control and prevent dysbiosis.
- It is suggested to start slow to see how the patient reacts to new probiotics.
- At the initial stages of treatment, some patients may not tolerate any probiotics. It is recommended to take them after a course of antibiotics to restore the gut flora or after herbal antimicrobials.
- Prebiotics are fermentable food for bacteria that can exacerbate symptoms during active SIBO and encourage bacterial growth post SIBO.
- Common prebiotics found in probiotic supplements include FOS (fructooligosaccharide), inulin, arabinogalactan, MOS (mannose-oligosaccharide) and GOS (galactooligosaccharide).
- Our single species probiotics are all made with rice maltodextrin and not FOS.
- These formulas were formulated based on research of other formulas on the market, with emphasis on two criteria:
  - D-lactate free status and species that either reduce histamine or prevent increases in histamine in the body.
  - There is conflicting information surrounding these topics so we encourage each practitioner to perform their own research on these topics before selecting any formulas.

## CHARACTERISTICS OF AN EFFECTIVE PROBIOTIC

- Should be able to maintain good viability.
- Should be able to survive the passage through the digestive system.
- Must not deconjugate bile salts.
- Should be able to attach to the intestinal epithelia and colonize.
- Should carry no antibiotic resistance genes that can be transferred to pathogens.
- Should be able to utilize the nutrients and substrates in a normal diet.
- Should be capable of exerting a beneficial effect on the host.
- Should produce antimicrobial compounds such as:
  - Organic acids
  - Hydrogen peroxide
  - Carbon dioxide
  - Diacetyl
  - Acetaldehyde
  - Bacteriocins
- Should be safe, noninvasive, non-carcinogenic and non-pathogenic.
- Should co-aggregate to form a normal balanced flora.
- Must not induce an immune reaction in the host.
- Should be stable and capable of remaining viable for long periods of time.

## MECHANISM OF ACTION

- Competition for dietary ingredients as growth substrates.
- Bioconversion of, for example, sugars into fermentation products.

- Production of growth substrates, for example vitamins, for other bacteria.
- Direct antagonism by bacteriocins.
- Competitive exclusion for binding sites.
- Improved barrier function.
- Reduction of inflammation, thus altering intestinal properties.
- Stimulation of innate immune response (by unknown mechanisms).

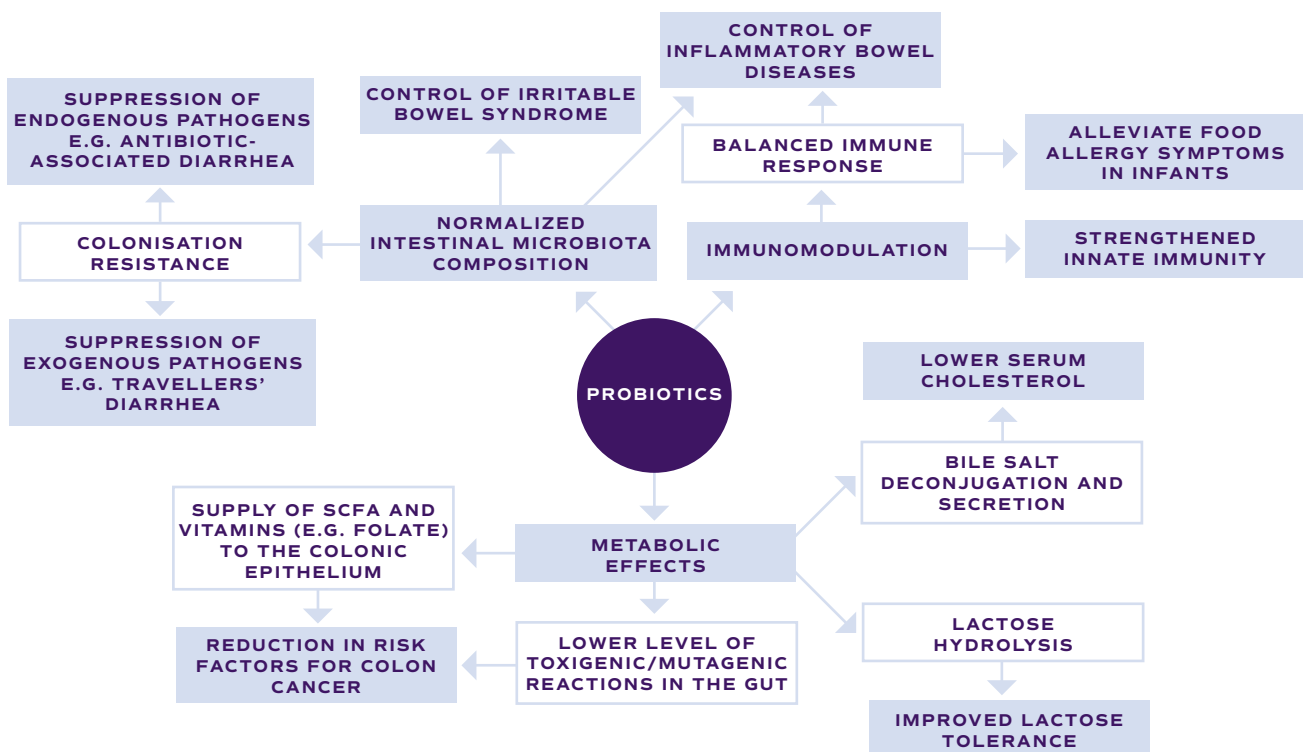
## DRUG INTERACTIONS

- Concurrent administration of antibiotics could kill a large number of the organisms, reducing the efficacy of the Lactobacillus and Bifidobacterium species.
- Patients should be instructed to separate administration of antibiotics from these probiotics by at least two hours.
- S. boulardii might interact with antifungals, reducing the efficacy of this probiotic.

## PRECAUTIONS AND CONTRAINDICATIONS

- In critically ill or severely immunocompromised patients, probiotic strains of Lactobacillus have been reported to cause bacteremia, endocarditis, and meningitis.
- Short-bowel syndrome can have bacteremia, possibly due to altered gut integrity.
- Lactobacillus preparations are contraindicated in persons with a hypersensitivity to lactose or milk.
- S. boulardii is contraindicated in patients with a yeast allergy.
- No contraindications are listed for bifidobacteria, since most species are considered nonpathogenic and nontoxicogenic.

## BENEFITS OF PROBIOTICS





## LACTOBACILLUS SPECIES

### LACTOBACILLUS ACIDOPHILUS

#### Characteristics

- Lactobacillus acidophilus (New Latin 'acid-loving milk-bacillus') is a species of gram positive rod-shaped bacteria in the genus Lactobacillus. It is a homofermentative, microaerophilic species, fermenting sugars into lactic acid, and grows readily at relatively low pH values (below pH 5.0) and has an optimum growth temperature of around 37°C (99°F).

#### Location

- L. acidophilus occurs naturally in the human and animal GI tract and mouth, as well as the vagina and urinary tract.

#### Mechanism

- Lactobacillus acidophilus produces natural antibiotics in the gut (including acidolin, acidophilin, lactocidin, and bacteriocin), which can inhibit the growth and toxin producing capabilities of some 23 known disease-causing pathogens (including Campylobacter, Listeria, Staphylococci, Salmonella, Shigella, S. faecalis and E. coli).
- It stimulates the immune system, increasing levels of interleukin-1 alpha (IL-1 alpha) and tumor necrosis factor alpha (TNF-alpha) which suppress cancerous tumor growth.
- Lactobacillus acidophilus has strong adhesion properties to intestinal tissue and mucus cells.
- Lactic acid produced by Lactobacillus acidophilus lowers the pH in the body parts where it lives, making the living conditions too acidic for many pathogenic microbes.
- L. acidophilus also secretes chemicals that assist in intestinal electrolyte absorption.
- It has the ability to prevent the invasion and adhesion of pathogenic microbes.
- It can increase levels of interleukin-1 alpha (IL-1 alpha) and tumor necrosis factor alpha (TNF-alpha) which suppress cancerous tumor growth.

#### Clinical Practice

- It is used orally for general digestion problems such as IBS, IBD, Crohn's disease, UC, relapsing Clostridium difficile colitis, bacterial overgrowth in short bowel syndrome, H. pylori infection.
- It alleviates dermatitis and other skin conditions (like atopic allergy), lactose intolerance, UTIs, vaginal and Candida-related (yeast) infections, hyperlipidemia, Lyme disease, hives, canker sores and adolescent acne.
- It has been shown to stimulate the immune system.

### LACTOBACILLUS BREVIS

#### Characteristics

- Lactobacillus brevis, also known as L. brevis, is discovered as far back as 1921, like other Lactobacillus, it is a Gram-positive, rod-shaped bacterium which cannot move about on its own and does not form spores. It is an obligate heterofermentative bacterium. This bacterium occurs naturally in the human body and is even present in breast milk. It is one of the bacteria most commonly associated with the fermentation of vegetation, dairy, cheeses, sauerkraut, olives and even sourdough.

#### Location

- Vagina, small intestine.

#### Mechanism

- Lactobacillus brevis is a probiotic with some strains having unusual herpes simplex virus 2 (HSV2) anti-virus capabilities, as well as other beneficial, probiotic capabilities.
- Digestion of these beneficial bacteria by test animals resulted in a marked suppression of colonic tumor formation and reduction of DNA damage.
- Lactobacillus brevis provides anti-inflammatory benefits to the human digestive system.
- L. brevis helps boost immune system function by increasing natural killer cells.

**Clinical Practice**

- Benefits range from help with constipation to inflammatory bowel disease to even colon cancer.
- Some research indicates its ability to combat ulcers.
- It also has anti-microbial facets, can help the health and condition of the gums, and boost the effectiveness of antibiotics.

**LACTOBACILLUS BULGARICUS****Characteristics**

- Lactobacillus delbrueckii subsp. bulgaricus (until 2014 known as Lactobacillus bulgaricus) is one of several bacteria used for the production of yogurt. It is also found in other naturally fermented products. It is a gram positive rod that may appear long and filamentous. It is non-motile and does not form spores. It is regarded as aciduric or acidophilic, since it requires a low pH (around 5.4-4.6) to grow effectively. It feeds on lactose to produce lactic acid and has a symbiotic relationship with *S. thermophilus*. *L. bulgaricus* is called a symbiotic bacterium; it lives in harmony with the other helpful bacteria that normally live in the GI tract. *L. bulgaricus* supplements have been compared to a spore, in that it can be stored like a seed but once digested, blooms and grows into the appropriate function within the human GI tract. This species is a particularly rugged strain of microbial flora and is able to survive stomach acid, bile salts and pancreatic enzymes by adapting their existence until better conditions arrive. They reproduce rapidly in mid-range temperatures.
- The organism grows when it's needed and diminishes when it's not. It can shrink or multiply within the intestinal mucosa.

**Location**

- Naturally occurring in the vagina and GI tract.

**Mechanism**

- It is able to use simple sugars from the diet and produce exopolysaccharides (EPS), complex branched carbohydrate-like molecules that slow down the absorption of simple sugars into the bloodstream, which can be helpful in normalizing blood sugar levels.
- It produces interferon, which can improve the body's natural response to infection and disease.
- Lactobacillus bulgaricus excretes natural antibiotics, which can have a broad spectrum of immune-boosting functions. It helps the micro-organism to neutralize toxins and kill harmful bacteria such as pseudomonas, *E. coli*, *S. aureus*, Salmonella, *H. pylori* and Shigella by producing its own natural antibiotics. It also suppresses inflammatory immune reactions in the intestinal wall thus preventing tissue damage.

**Clinical Practice**

- It boosts immune function.
- It suppresses inflammatory immune reactions in the intestinal wall thus preventing tissue damage.
- Other uses include: indigestion, heartburn, acid reflux, GERD, relief from over-eating, protein digestion, enterocolitis, constipation, acute diarrhea.

**LACTOBACILLUS CASEI****Characteristics**

- This particular species of Lactobacillus is documented to have a wide pH and temperature range, and complements the growth of *L. acidophilus*, a producer of the enzyme amylaseme. It is well suited for intestinal survival and functionality, and highly tolerant of acid and bile.
- The organism grows when it's needed and diminishes when it's not. It can shrink or multiply within the intestinal mucosa.

**Location**

- It is naturally occurring in the mouth and digestive tract, primarily in the small intestine.

**Mechanism**

- It breaks down carbohydrates and inhibits the growth of pathogenic and putrefactive bacteria in the small intestine; it has the most potent protective activity against Listeria bacteria.
- It is critical for the proper differentiation of immune dendritic cells, increases the number of intestinal cells secreting IgA, and contains enzymes that facilitate the breakdown of polypeptides including gluten.

**Clinical Practice**

- L. casei can counteract the pro-inflammatory effects of E. coli on Crohn's disease.
- It is helpful in treating diarrhea caused by antibiotics and viruses, inhibits the growth of the peptic ulcer causing bacteria H. pylori, helpful for constipation and urinary tract infections.

**LACTOBACILLUS FERMENTUM****Characteristics**

- Lactobacillus fermentum is a Gram-positive species of bacterium in the genus Lactobacillus. It is associated with active dental caries lesions. It is also commonly found in fermenting animal and plant material. It has been found in sourdough but it is also a normal inhabitant of the human intestinal tract. Being a resilient form of bacteria, Lactobacillus fermentum is able to survive in low pH environments of the stomach and has good bile tolerance.

**Mechanism**

- Studies have shown that L. fermentum has antibiotic resistances.
- It produces lysozyme, gas from glucose and NH<sub>3</sub> from arginine.

**Clinical Practice**

- This species of bacteria is known to reduce cholesterol levels better than other bacteria species.
- Some strains of this probiotic offer antioxidant and antimicrobial properties.
- This species supports optimal digestion, reduces respiratory illnesses and can treat urogenital infections within females.

**LACTOBACILLUS GASSERI****Characteristics**

- Lactobacillus gasseri is a species in the genus Lactobacillus. L. gasseri produces gassericin A, a bacteriocin. This strain can be obtained from fermented foods like kefir, sauerkraut, kimchi, lassi, natto, miso and tempeh. Research has shown that L. gasseri can protect the body against harmful organisms.

**Location**

- Lactobacillus gasseri is a normal inhabitant of the lower reproductive tract in healthy women.

**Mechanism**

- L. gasseri may help reduce abdominal fat and speed up metabolism.

**Clinical Practice**

- It supports weight loss.
- L. gasseri may help lessen menstrual pain in women with endometriosis.
- L. gasseri may suppress the airway inflammation associated with allergic asthma.

**LACTOBACILLUS HELVETICUS****Characteristics**

- Lactobacillus helveticus is a lactic-acid producing, rod-shaped bacterium of the genus Lactobacillus.

**Mechanism**

- L. helveticus consists mainly of cell envelope proteinases which initially cleave caseins to large peptides, intracellular peptidases further degrading peptides to small peptides and amino acids, and specific transport proteins which transfer amino acids and peptides across the cytoplasmic membrane.



- Peptides with physiological functions, such as immunostimulating peptides, antimicrobial peptides, opioid peptides, mineral binding peptides and antihypertensive peptides, can be isolated from products fermented with *L. helveticus*.
- *L. helveticus* possesses many commonly recognized probiotic features, such as the ability to survive gastrointestinal transit, adhere to epithelial cells and antagonize pathogens.
- *L. helveticus* is also able to prevent gastrointestinal infections, enhance protection against pathogens, modulate host immune responses and affect the composition of the intestinal microbiota.
- *L. helveticus* can enhance the bioavailability of nutrients and removal allergens and other undesired molecules from food.

#### **Clinical Practice**

- This species has anti-mutagenic, anti-tumorigenic, anti-hypertensive and immunomodulatory activity.
- It has proved to have significant effects on bone density and in preventing trabecular bone loss when compared to other milk products that did not contain the organism.
- It prevents and reduces the duration of diarrhea and possibly reduces cholesterol levels.
- Shown to have potential anxiolytic, anti-depressive, and anti-stress activities in humans.

## **LACTOBACILLUS PARACASEI**

#### **Characteristics**

- This species is found naturally in fermented products, yogurt and natural dairy products like raw milk.

#### **Mechanism**

- It antagonizes pathogens such as rotavirus, staphylococcus aureus and clostridium difficile.

#### **Clinical Practice**

- *Lactobacillus paracasei* has been safely used to treat diarrhea in infants.
- It improves immune function, helps to calm digestive upsets, assists other strains of bacterium, as well as improves the absorption of nutrients and lipids in the gut.

## **LACTOBACILLUS PLANTARUM**

#### **Characteristics**

- *Lactobacillus plantarum* is found in plant material and the GI tract of animals, including humans. It is used in the fermentation of foods like sauerkraut, kimchi, pickles and sourdough bread. It is a temperature and extreme pH resistant strain. *Lactobacillus plantarum* is able to adapt to stressors better than some other lactic acid bacteria, so although it replicates best in anaerobic conditions (no oxygen), it is able to adapt to some oxygen, various levels of pH and other conditions that would kill other bacteria. It produces bacteriocins (antimicrobial products) called "plantaracins". Some companies use cultures with *L. plantarum* as antimicrobial agents in a variety of foods but not in infant formula or infant foods.

#### **Location**

- *Lactobacillus plantarum* is found frequently in the digestive tract, from the mouth to the intestines, as well as the vagina.

#### **Mechanism**

- As the most prevalent species in most naturally fermented foods, it is being studied for its efficacy against allergies, cancer and irritable bowel syndrome, and is able to break down bile acids and lower cholesterol.
- It has an extremely high adherence potential for epithelial tissue and seems to favour colonizing the same areas of the intestinal tract that *E. coli* prefers – serving to crowd *E. coli* out of the body. Meanwhile, it secretes the naturally occurring antibiotic lactolin.
- It is capable of digesting semi-digestible fibres such as those found in onions, garlic, wheat, oats, rye and yeast; therefore, may help with digestive problems like gas and bloating.

- *L. plantarum* creates a healthy barrier in your colon to keep dangerous bacteria from penetrating the lining of your intestines and entering your blood stream. As well as it may be helpful in reducing bacterial translocation, a benefit dependent on bacterial adhesion to the intestinal wall. It enhances gut immune function, is anti-inflammatory and is able to reduce the ability of pathogens to prosper. It may also reduce gastrointestinal side effects commonly associated with antibiotics.
- It produces lactic acid and short-chain fatty acids. It has also shown the rare ability to produce *L. lysine*, a beneficial amino acid.

#### **Clinical Practice**

- It has been shown to be highly effective in preventing soy-related allergies.
- This species is of benefit to pregnant women, newborns, children with ADD and ADHD and ASD.
- It has been shown to be an effective treatment for IBS, Crohn's disease and colitis. It has the ability to destroy pathogens and to preserve critical nutrients, vitamins and antioxidants.

## **LACTOBACILLUS REUTERI**

### **Characteristics**

- *Lactobacillus reuteri* (*L. reuteri*) is a species of *Lactobacillus* bacteria. It is also commonly found in some cultured vegetables, in the intestinal tracts of animals, in meat and dairy products, and in the natural environment. Scientists have been aware of *L. reuteri* since early in the 20th century, when it was mistakenly classified as a member of *Lactobacillus fermentum*.

### **Location**

- *L. reuteri* naturally inhabits the digestive tract, the female vaginal and urinary tracts, and breast milk.

### **Mechanism**

- As a species, it is best known for its ability to fight pathogenic microbes. Some strains of this bacteria produce a broad-spectrum antimicrobial substance, reuterin. It also produces carbon dioxide, ethanol, acetic acid and lactic acid from glucose.
- It is also able to survive in low-to-no oxygen environments and to produce hydrogen peroxide under the right conditions. It has shown to withstand stomach acid and bile acids in the digestive tract.
- It is able to convert lactose to lactic acid which may help with lactose intolerance.

### **Clinical Practice**

- *L. reuteri* has been shown to be an effective in infantile colic, acute diarrhea, rotavirus, *gardnerella vaginalis*, eczema, UTIs and *H. pylori*.
- *L. reuteri* destroys *streptococcus mutans*, which hastens tooth decay.

## **LACTOBACILLUS RHAMNOSUS**

### **Characteristics**

- *L. rhamnosus* is Gram-positive, non-motile, non-sporulating rod-shaped facultative anaerobic lactic acid bacterium. *Lactobacillus rhamnosus* is one of the most widely studied probiotics, noted and valued for its ability to survive and even thrive in the harsh conditions of the digestive and urinary tracts. It has a very high resistance to bile salts and harsh stomach acid and therefore grows prolifically (very strong species). *L. rhamnosus* has an impressive resume of health benefits. This is one species that certainly has some strain-specific health benefits and attributes.

### **Location**

- *L. rhamnosus* is primarily found in the small intestine, female vaginal tract and urinary tract.

### **Mechanism**

- It protects against the invasion of harmful microorganisms by stimulating gut lining cells to produce mucin, a mucous coating that also prevents toxins from reaching the blood.

- *L. plantarum* creates a healthy barrier in your colon to keep dangerous bacteria from penetrating the lining of your intestines and entering your blood stream. As well as it may be helpful in reducing bacterial translocation, a benefit dependent on bacterial adhesion to the intestinal wall. It enhances gut immune function, is anti-inflammatory and is able to reduce the ability of pathogens to prosper. It may also reduce gastrointestinal side effects commonly associated with antibiotics.
- It produces lactic acid and short-chain fatty acids. It has also shown the rare ability to produce *L. lysine*, a beneficial amino acid.

#### **Clinical Practice**

- It has been shown to be highly effective in preventing soy-related allergies.
- This species is of benefit to pregnant women, newborns, children with ADD and ADHD and ASD.
- It has been shown to be an effective treatment for IBS, Crohn's disease and colitis. It has the ability to destroy pathogens and to preserve critical nutrients, vitamins and antioxidants.

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- *L. rhamnosus* stimulates the production of antibodies and also assists in the process of phagocytosis. It decreases T cell proliferation, induces helper (cd4+) T cell hypo responsiveness, increases phagocytic activity in peripheral white blood cells, killing activity in natural killer cells, and inhibits pro-inflammatory cytokines, increases the natural killing activity of spleen cells, which may help to prevent tumor formation.
- It reduces intestinal inflammation in infants possibly due to improved intestinal barrier function leading to decreases in antigen translocation, benefits atopic dermatitis and eczema in infants, increases colonic *B. breve* populations and promotes healthy bifidobacteria diversity in infants.
- *L. rhamnosus* is capable of interrupting the gastrointestinal transportation of the variety of enterococcus that is resistant to the antibiotic vancomycin.

#### **Clinical Practice**

- It offers considerable assistance with the immune system, particularly in combating intestinal and urinary tract pathogens.
- It enhances the effectiveness of oral vaccinations against rotavirus, as an oral therapy for viral gastroenteritis, treats and prevents rotavirus diarrhea, antibiotic-associated diarrhea, diarrhea caused by *C. difficile*.
- It is helpful for constipation, IBD, lactose intolerance, food allergies, inhibits the bacteria involved in vaginal and UTIs.

## **LACTOBACILLUS SALIVARIUS**

### **Characteristics**

- *L. salivarius* is a highly effective probiotic bacteria, which plays an important role in maintaining a healthy digestive system. It works to restore the proper balance in the intestines, attacking many of the unfriendly bacteria and other detrimental invaders while prompting the growth of beneficial organisms.

### **Location**

- It is found in the mouth and in the small intestine.

### **Mechanism**

- It breaks down proteins, helps digest foods, makes nutrients more assimilable; breaks down undigested protein and disengages the toxins produced by protein putrefaction, produces alpha-galactosidase.
- It enhances calcium absorption and produces B vitamins.
- It adheres to the intestinal wall, thereby forming a living matrix that helps protect the mucosal lining.
- It protects against *Salmonella* and *H. pylori* bacteria, highly resistant to antibiotics.
- Research has shown that the addition of this species along with other probiotic species suppressed proinflammatory cytokines and further suppressed bacterial overgrowth in the small intestine leading to a reduction in bacterial translocation.

### **Clinical Practice**

- It is used to relieve the symptom flatulence in individuals suffering from irritable bowel syndrome.
- *L. salivarius* has been shown to improve bleeding gums, tooth decay, bad breath, oral thrush and canker sores.

## **LACTOBACILLUS LACTIS**

### **Characteristics**

- This is a Gram-positive bacteria used extensively in the production of buttermilk and cheese.

### **Mechanism**

- It produces nisin, a bacteriocin which is active against a wide range of food pathogens.
- It inhibits the growth of *Salmonella* and *E. coli* bacteria, as it has strong acid producing capabilities.

### **Clinical Practice**

- It is effective in treating maldigestion and has been shown to be effective in treating chemotherapy induced dysbiosis.

## BIFIDOBACTERIA SPECIES

### BIFIDOBACTERIA BIFIDUM

#### Characteristics

- Bifidobacterium bifidum is a bacterial species of the Bifidobacterium genus. It is a Gram-positive bacterium that is not motile, anaerobic and not spore-forming. The bacterium is rod-shaped and can be found living in clusters, pairs or even independently. It is also found in breast milk.

#### Location

- B. bifidum is found in the colon, lower small intestine, breast milk and often in the vagina.

#### Mechanism

- It produces B vitamins that help the body in the absorption of calcium, iron, magnesium, etc.
- Research carried out by the Yakult Company showed that their patented strain of B. bifidum had significant antioxidant action and was able to protect the intestinal lining from lipid peroxidation in iron overloaded mice.
- Bifidobacterium bifidum activates B cells making them more responsive to transforming growth factor-1 and interleukin-5 for IgA secretion, supplementation enhances white cell phagocytic activity. It enhances IgA response to C. difficile toxin A, suppresses total and allergen-specific IgE secretion, as well as stimulates macrophages and increases the production and function of T-cells and NK cells.

#### Clinical Practice

- It reduces inflammation in the colon, is beneficial for IBD and reduces incidence of acute diarrhea in infants.
- It is important in managing allergies and eczema.

### BIFIDOBACTERIA BREVE

#### Characteristics

- This anaerobic and non-motile species is found in the large intestine but it is also present in the vagina, where it helps to inhibit overproduction of candida albicans, also known as the primary cause of yeast infections in women.

#### Location

- Large intestine and vagina.

#### Mechanism

- B. breve possesses a superior ability to break down many types of food, even plant fibres that are normally considered non-digestible.
- B. breve ferment sugars in the GIT and produce lactic acid and acetic acid.
- B. breve appears to inhibit E. coli. It antagonizes rotavirus and reduces intestinal populations of anaerobic bacteroides and clostridium. It has been shown to repress the growth of ulcer-inducing bacteria.
- It can colonize the immature bowel of infants very effectively; it causes better weight gain in very low birth weight infants.
- It increases the immune response of Peyer's patch cells, stimulating B cell proliferation and antibody production.

#### Clinical Practice

- It represses the growth of ulcer-inducing bacteria.
- It boosts immune function.

### BIFIDOBACTERIA INFANTIS

#### Characteristics

- B. infantis generally survives stomach and bile acids, and is generally able to adhere to intestinal tissues.

#### Location

- It is found in the large intestine of infants, found in small amounts in the vagina.

**Mechanism**

- B. infantis produces predominantly acetic acid, with other acid production more dependent on the type of prebiotic food it consumes.
- Bactericidal activity has also been observed against Clostridia, Salmonella, E. coli and Shigella.

**Clinical Practice**

- This species is highly successful in forming colonies; it reduces the growth of bacteroides common in inflammatory bowel disease. It is effective adjunct therapy in acute diarrhea and IBS symptoms such as bloating, gas, diarrhea, constipation, urgency and abdominal discomfort.
- It reduces the risk of necrotizing enterocolitis in premature or low birth weight babies, assists in nitrogen retention, weight gain in infants and contributes to the production of B vitamins.

**BIFIDOBACTERIA LACTIS****Characteristics**

- Bifidobacterium lactis is used as a probiotic in a variety of foods, particularly dairy products like yogurt and is also included in baby food. It well suited for intestinal survival, with high tolerance to gastrointestinal conditions (acid and bile).

**Location**

- Found mostly in the large intestine.

**Mechanism**

- B. lactis resists the digestion of acid and the action of bile salts, while allowing it to travel to the colon in larger doses to offer its protection from harmful toxins or inflammation. It has the ability to protect epithelial cells from damage by gliadin exposure.
- The tumor killing ability of the lymphocytes and phagocytes are also increased, especially in poor immune function. It raises T-helper cell levels and activates lymphocytes that kill fungi and yeast in the blood and intestines.

**Clinical Practice**

- It is very helpful for the colon in that it prevents diarrhea, relieves constipation and decreases chronic pain and inflammation of the colon.
- It has been shown to be beneficial in preventing and treating atopic eczema in children with food allergies.
- It stimulates the immune response in the intestine and the blood, and has proven particularly effective in enhancing cellular immunity in the elderly.

**BIFIDOBACTERIA LONGUM****Characteristics**

- It is one of the most prevalent healthy bacteria in the intestinal tract of breast-fed infants. It is able to eliminate the nitrates commonly found in foods ingested by humans.

**Location**

- GI tract and vagina.

**Mechanism**

- B. longum ferments sugars into lactic acid thereby lowering pH levels in the intestine. It assists the body in maintaining healthy balance of intestinal flora by producing lactic acid, hydrogen peroxide and acetic acid that increase the acidity of the intestine and inhibit the reproduction of many harmful bacteria. It breaks down bile salts and helps to synthesize B-complex vitamins.
- It stimulates the immune system through its effects on immunoglobulin A (IgA), regulates the immune response to respiratory antigens and inhibits neutrophil elastase.



- It has been shown to inhibit the action of Vero Cytotoxin produced by some strains of E. coli which can cause hemorrhagic colitis and hemolytic uremic syndrome in humans. It has been reported to stimulate IL-1, decrease the number of Clostridia, Bacteroides and Coliform and decrease some enzymatic activity in feces suspected to be involved in carcinogenesis.

#### Clinical Practice

- B. longum is commonly used for diarrhea prevention, cholesterol reduction and proper immune function.
- It has been found to improve constipation, increase the frequency of stools and decrease transit time.

## SOIL-BASED ORGANISMS

### BACILLUS COAGULANS (PREVIOUSLY KNOWN AS LACTOBACILLUS SPOROGENES)

#### Characteristics

- Bacillus coagulans (B. coagulans) is a bacterium used in many industrial applications. Some strains are used as probiotics. This is a gram-positive, spore-forming bacillus, is resistant to high temperatures and to acid (grows optimally at 37°C and pH 5.5-6.2), and no refrigeration is required. Like other Bacillus species that are sometimes called "soil organisms", it is more-or-less rod-shaped and has the ability to form endospores, very tough outer shells, when conditions are unfavourable. When conditions are favourable for growth (requires a nutritionally complex environment), the endospores germinate into vegetative cells which can rapidly multiply. It grows very well in aerobic conditions, but can also function in a low-oxygen environment. B. coagulans exists either as single organisms or in short chains and forms colonies. It is one of the Bacillus species that is motile; that is, it can move independently because it has a whip-like propelling feature. It produces lactic acid, but is not considered to be a lactic-acid bacterium (LAB) such as Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus, Pediococcus, etc. because it forms endospores and the lactic acid bacteria do not.

#### Location

- Found to concentrate throughout the large and small intestine.

#### Mechanism

- The main mechanism of survival and proliferation of B. coagulans is "competitive exclusion".
- It produces bacteriocins like coagulin, which has antibacterial activity, and short chain fatty acids.
- It reduces colonization of some strains of vancomycin-resistant enterococcus.
- In vitro, it also reduces fungal growth of species of fusarium (a large genus of filamentous fungi).
- Plays a key role in digestion of food and absorption of nutrients.

#### Clinical Practice

- It is useful for constipation, abdominal distention, neonatal diarrhea, antibiotic induced diarrhea, gut dysbiosis, vaginitis, aphthous stomatitis, and urinary tract infections (via coagulin).
- Controls common inflammatory bowel conditions (IBS< Crohn's and UC), in addition to its potent immune boosting activity.
- It is able to lower cholesterol levels, producing a significant reduction in LDL and small increase in HDL.
- Improves the body's ability to utilize calcium, phosphorus and iron, and stimulates both gastric juices and gastric motility.

### BACILLUS CLAUSII SNZ 1971

#### Characteristics

- This gram positive, rod-shaped, non- pathogenic bacteria has been used in Europe since the 1960s.
- Its source of origin is soil.
- B. clausii is the largest selling strain of probiotics in the world and is done so as a prescription drug in most countries.

- The spores of *B. clausii* and other related Bacilli are used as probiotics to improve intestinal microbial balance during periods of antibiotic use, to modify the immune system within the GI tract, and to act as a direct anti-microbial agent.

**Location**

- Can be found in the large intestine and GI tract.

**Mechanism**

- *B. clausii* has been found to produce antimicrobial substances that are active against gram positive bacteria including *Staphylococcus aureus*, *Enterococcus faecium*, and *Clostridium difficile*.
- Anti-microbial activity occurs through the secretion of multiple antimicrobial and bacteriocins like inhibitory substances (such as Subtilin and Coagulin).

**Clinical Practice**

- *B. clausii* is recommended to be used during antibiotic treatment due to its ability to resist damage from a variety of common antibiotics including those used for SIBO eradication.
- *B. clausii* has been shown to help mitigate damage caused by antibiotic treatments for *H. pylori*.
- It is also used as a primary treatment for diarrhea from a multitude of other causes.
- It is currently being studied for its use in treating respiratory infections.
- This species possesses both antimicrobial and immunomodulatory activities and can inhibit the growth of pathogens in the GI tract.

## BACILLUS SUBTILIS SNZ 1972

**Characteristics**

- *Bacillus subtilis* were one of the first type of beneficial bacteria to ever be studied.
- *B. subtilis* are rod-shaped, gram positive bacteria with a rigid cell wall.
- Is considered a soil based organism which endospore formation provides a means to ensure long term survival.
- *B. subtilis* has been extensively used in the livestock and poultry industries as antibiotic alternatives.
- Readily found in soil as well as fermented foods such as natto.

**Location**

- Found throughout the GI tract.

**Mechanism**

- This bacterium is considered fungicidal, and possesses immunostimulant activity widely used for gastrointestinal and urinary tract diseases prior to the introduction of antibiotics.
- Supports gut repair by increasing IgA and butyrate.
- Produces over 12 antibiotics, fermented into Vitamin K in the gut, and has a positive impact on inflammatory and autoimmune markers.

**Clinical Practice**

- Is widely used, safe and effective.
- Has DNA protective and antioxidant (superoxide scavenging) activity.
- Helps promote the balance of intestinal flora, enhance innate immunity and general disease resistance.

## OTHER PROBIOTICS

### SACCHAROMYCES BOULARDII

**Characteristics**

- *Saccharomyces boulardii*, or *S. boulardii*, is an important probiotic that can recolonize and sustain flora in the large and small intestine. The only probiotic non-pathogenic yeast; it belongs to the Brewer's Yeast family, and is not of the group that *Candida* belongs. *S. boulardii* is classified as being non-systemic and non-pathogenic.

- It confines itself to the intestinal tract, instead of spreading throughout the body. It survives gastric acidity, is not adversely affected or inhibited by antibiotics, and no refrigeration is required.

**Location**

- Large and small intestine.

**Mechanism**

- One of its biggest advantages, especially when taking antibiotics, is that it is not affected by antibiotics since it is a yeast. Thus it can keep pathogenic microorganisms and yeasts such as Candida from overwhelming the body when the antibiotics kill most of the good bacteria and most, but not all, of the bad bacteria in the GI tract. It will however be affected by antifungal medications.
- It produces some B vitamins and has an overall probiotic effect.
- It diminishes the expression of inflammation cytokines such as IL-8, IL-6 and IL-1 $\beta$ , tumour necrosis factor-alpha (TNF-a), and interferon-gamma (IFN-g).
- It improves resistance to tight junctions of the intestinal barrier, restores membrane permeability altered by infectious agents.
- It secretes proteases and other substances that break down bacterial enterotoxins and inhibit their binding to intestinal receptors.
- It stimulates D-glucose and sodium absorption and produces short-chain fatty acids such as butyrate and acetate that nourish colon mucosa.
- It enhances the numbers of healthful Bifidobacteria in the colon while simultaneously suppressing populations of pathogenic Clostridia, E. coli, Salmonella and Candida albicans.

**Clinical Practice**

- It modulates the immune system (increases IgA levels).
- It treats and prevents diarrhea, including rotaviral diarrhea in children, diarrhea caused by bacterial overgrowth in adults, traveller's diarrhea, antibiotic-associated diarrhea and diarrhea associated with enteral tube feedings.
- It is used for general digestion problems, IBS, IBD, Crohn's disease, UC, Lyme disease, relapsing C. difficile colitis and bacterial overgrowth in short bowel syndrome.
- It is used for lactose intolerance, urinary tract infections (UTIs), vaginal and Candida-related yeast infections, high cholesterol, hives, fever blisters, canker sores and adolescent acne.

## STREPTOCOCCUS THERMOPHILUS

**Characteristics**

- Streptococcus thermophilus, along with Lactobacillus bulgaricus, were originally the starter strains used to make yogurt. The cell structure of Streptococcus thermophilus allows the bacteria to endure elevated temperatures.

**Location**

- Large and small intestine.

**Mechanism**

- Streptococcus thermophilus' unique abilities is that it can break down casein, the protein in dairy products like cheese.
- It aids recovery from malnutrition due to short-term fasting and reduce the associated intestinal atrophy.
- Used in combination with L. bulgaricus, it helps in the prevention and treatment of diarrhea (Pseudomonas, E. coli, S. aureus, Salmonella and Shigella).
- In addition to the natural ability of Streptococcus thermophilus to prevent transformation of nitrates into cancer-causing nitrites, the bacterium has other exciting cancer-related applications.

**Clinical Practice**

- It has been shown to have antioxidant and antitumor activities.
- Used to treat chemotherapy induced mucositis.
- It is used to prevent and treat of diarrhea.