lactospore.com

LactoSpore®: Probiotic of Choice

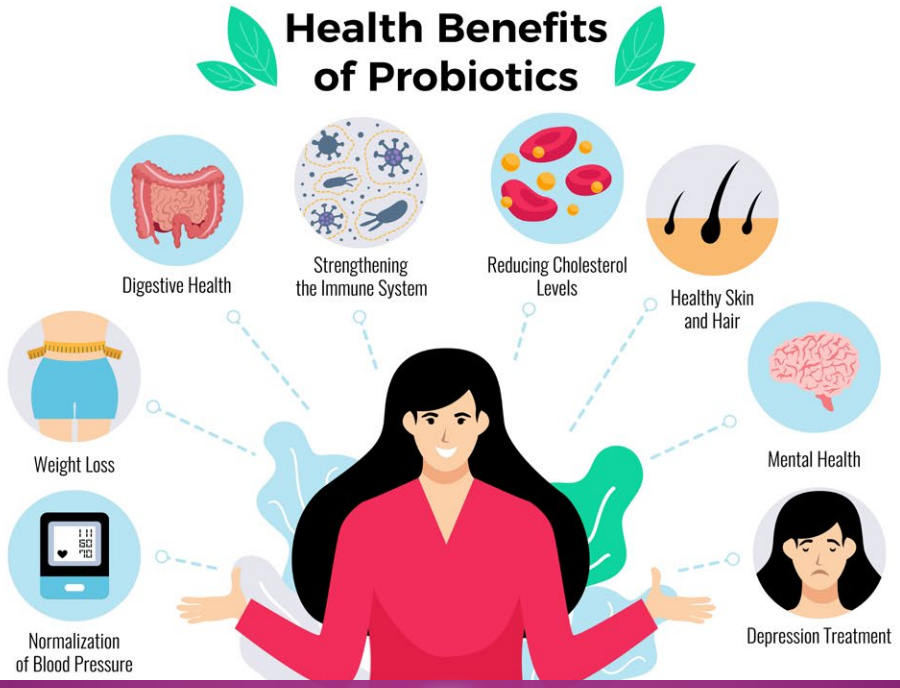
presented by

Stable LactoSpore® Probiotic

SABINSA®

INTRODUCTION

LactoSpore® is a patented commercial preparation of the probiotic bacterium *Bacillus coagulans* MTCC 5856 (formerly known as *Lactobacillus sporogenes* and now classified as *Heyndrickxia coagulans*) (Narsing Rao *et al.*, 2023). *Bacillus coagulans* is a Gram-positive, spore forming, rod shaped, microaerophilic bacterium (3.0–5.0 µm in size) that that produces L(+) lactic acid. The safety and efficacy of *B. coagulans* as a probiotic are well-established, supported by a long history of global use. LactoSpore® is a proprietary preparation of the strain, *Heyndrickxia coagulans* MTCC 5856, from Sami-Sabinsa Group Ltd. and is marketed worldwide as a dietary supplement or ingredient with clinically proven health benefits. LactoSpore® has also been granted Generally Recognized as Safe (GRAS) status by the U.S. Food and Drug Administration (FDA).



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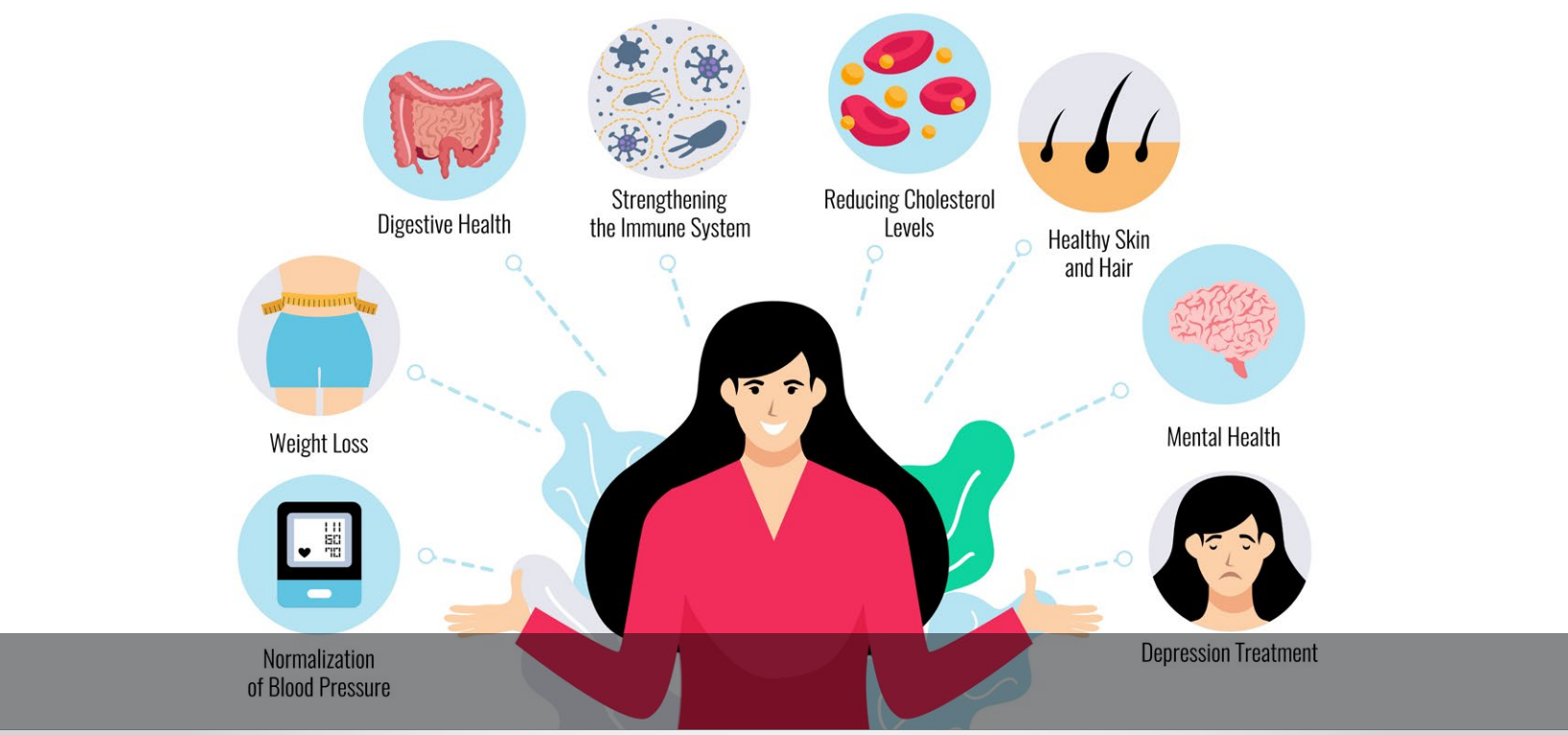
HOW DOES A PROBIOTIC WORK IN OUR BODY?

Gut microbiota has a profound impact on human physiology, nutrition, brain health, metabolism and immunity. The human gut hosts approximately 100 trillion bacteria from over 500 species, and each individual's gut microbiota is as unique as a fingerprint. Ninety percent of the vertebral microbiome consists of two phyla, Firmicutes and Bacteroidetes. Actinobacteria and Proteobacteria also contribute to the heterogeneity of the gut microbiome. Disruption of this microbial balance is often linked to a range of health issues, including gastrointestinal (GI) disorders, indigestion, malnutrition, obesity, metabolic conditions, and even intestinal cancers. Some physiological conditions like maternal environment, genotype, diet,



presence of drugs and antibiotics, stress, etc., alter the composition of normal gut microflora, which affects digestion, nutrient absorption, and overall health. A growing awareness of the gut microbiome's role in overall health has popularized the use of probiotics and their health-promoting benefits. Probiotics help in the restoration of a healthy gut microbiome via different mechanisms like altering the permeability of the intestinal epithelium, modulating the immune system and by producing enzymes and bioactive compounds. Recent research has highlighted the undeniable impact of gut microbiota on brain function.

The health benefits of probiotics are strain specific. Not all the strains of the same species give the same effectiveness against different health conditions. Therefore, the strain *Heyndrickxia coagulans* MTCC 5856, known for its multiple health benefits, stands out as one of the most effective probiotics available on the market. LactoSpore® offers a wide range of applications across nutraceuticals, cosmeceuticals, and the emerging category of nutricosmetics, which plays an important role in promoting skin health. Sabinsa's LactoSpore® has been selected as the beneficial probiotic ingredient by several food industries. In recent years, LactoSpore® is also gaining attention as a feed additive for improving the productivity and growth performance of animals (Zhou *et al*., 2020).

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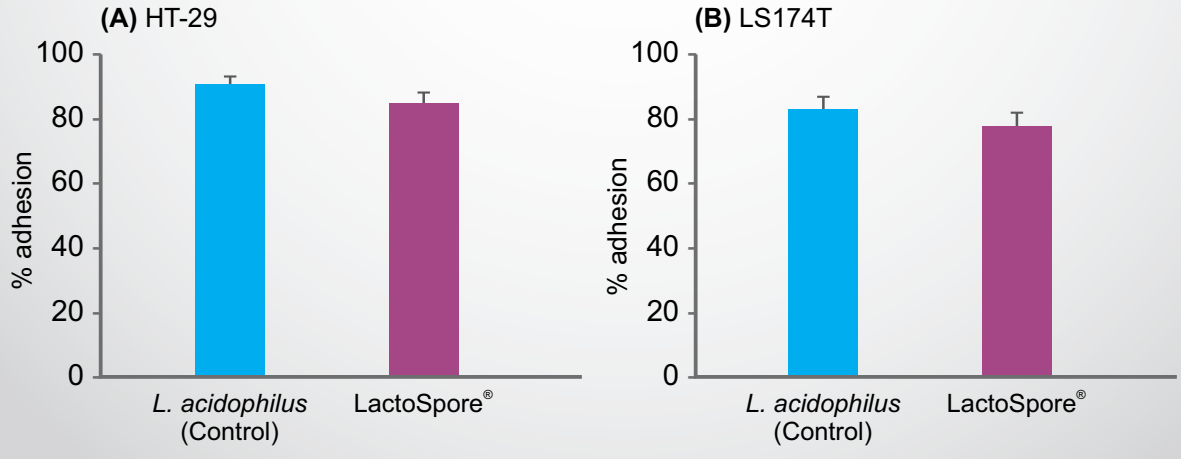
SCIENTIFICALLY EVALUATED FOR ITS UTILITY AS A PROBIOTIC

Microorganisms should possess certain characteristics to be considered a potent probiotic, including the ability to survive and thrive in the acidic, digestive environment of the gut. Additionally, they must be able to colonize the gut and adhere to the intestinal epithelium, ensuring the delivery of health benefits when consumed in adequate quantities (Patel *et al.*, 2009). The probiotic efficiency of LactoSpore® is well documented for its ability to withstand acid/an acidic pH and bile salts, susceptibility to antibiotics, nontoxicity to colon cells, and resistance to human pathogens. Moreover, LactoSpore® was found to be non-mutagenic, non-cytotoxic, negative for the presence of enterotoxin genes and showed genetic and phenotypic consistency over the decades of commercial production (Majeed *et al.*, 2016a).

LACTOSPORE® ADHERES TO INTESTINAL CELLS

The ability of LactoSpore® to attach to suitable surfaces and survive in the gastrointestinal (GI) tract is well characterized. The cell adhesion ability of LactoSpore® was confirmed *in vitro* using human mucus-secreting colonic adenocarcinoma cell lines, HT-29 and LS174T cells. LactoSpore® showed excellent adhesion to both cell lines at 85.8 ± 2.1% and 80.6 ± 1.45% respectively, after a contact period of 4 hours without any negative influences on the viability of either of the colonic cell lines (Figure 1) (Shinde *et al*., 2019a).

The viable count of probiotics is effectively enhanced by natural plant-based fibers which were confirmed to have prebiotic potential as they are resistant to both acid and enzymatic hydrolysis. Galactomannan from fenugreek seeds (*Trigonella foenum-graecum* L.) exhibited prebiotic activity with LactoSpore® in a synbiotic combination and enhanced its probiotic potential (Majeed *et al.*, 2018a).



Values are means ± SEM of three replicate experiments.

FIGURE 1. ADHESION OF LACTOSPORE® TO HT-29 (A) AND LS174T (B) CELLS AFTER 4 HOURS. (SHINDE *et al*., 2019a)

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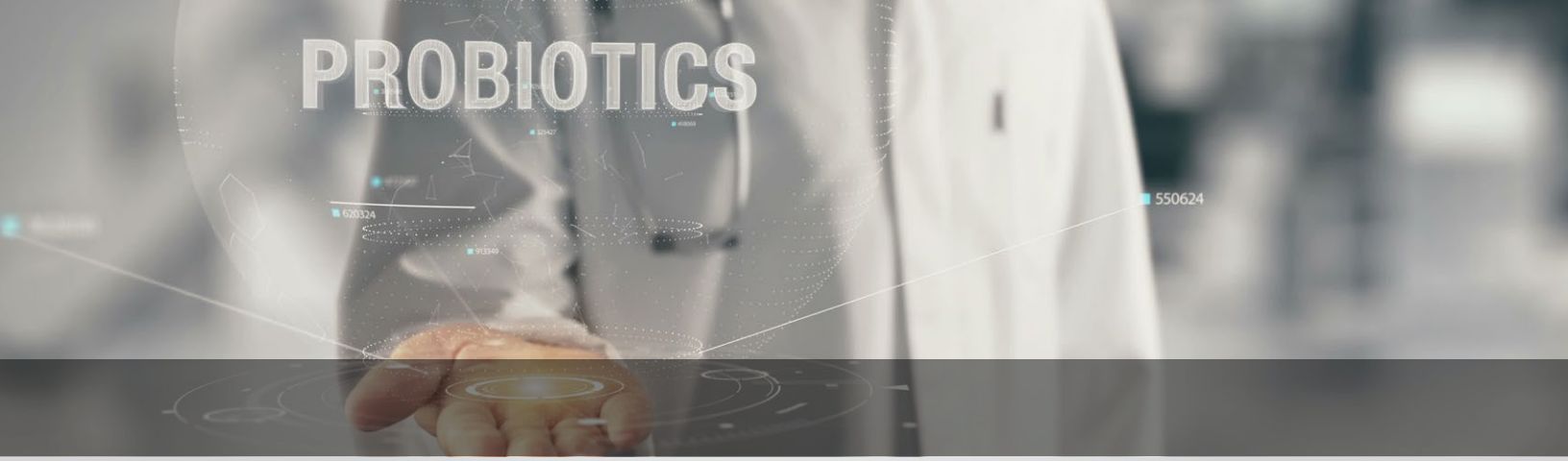
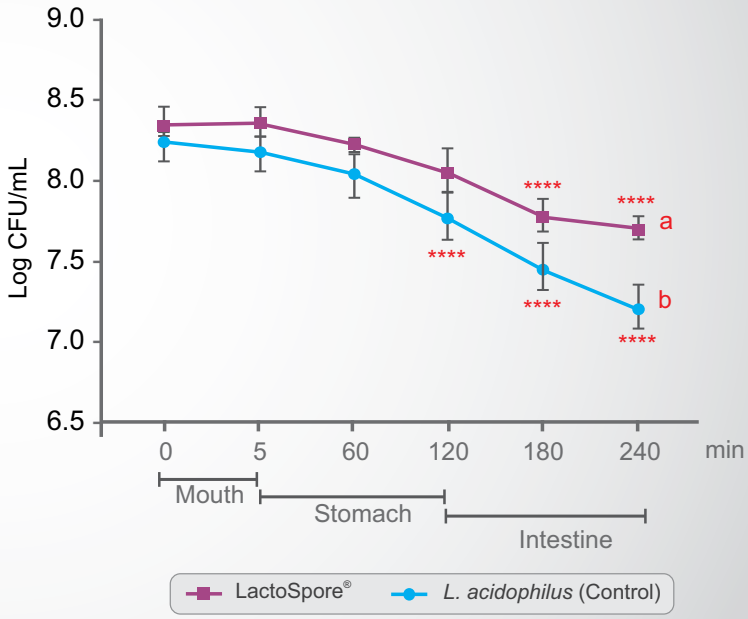
STABILITY OF LACTOSPORE® ASSURES QUALITY & QUANTITY

For the last two decades, LactoSpore® has been in the market as a dietary ingredient and is a room-temperature stable, lactose-free, gluten-free and non-GMO probiotic preparation with GRAS status. LactoSpore® can withstand high heat and other harsh environments and hence, it has been included in cooked foods as a functional component (Majeed and Prakash, 2008). The spores of *Bacillus species* can remain dormant for many years, allowing them to withstand the harsh conditions of food processing, manufacturing, and storage. When growth conditions are favorable, the spores resuscitate through germination. The remarkable stability of *B. coagulans* spores may be attributed to one or more layers of proteinaceous spore coats, which protect the spores from harsh conditions. Moreover, the ability to remain as viable but non-culturable (VBNC) bacteria also helps the bacteria to thrive in unfavorable conditions for several



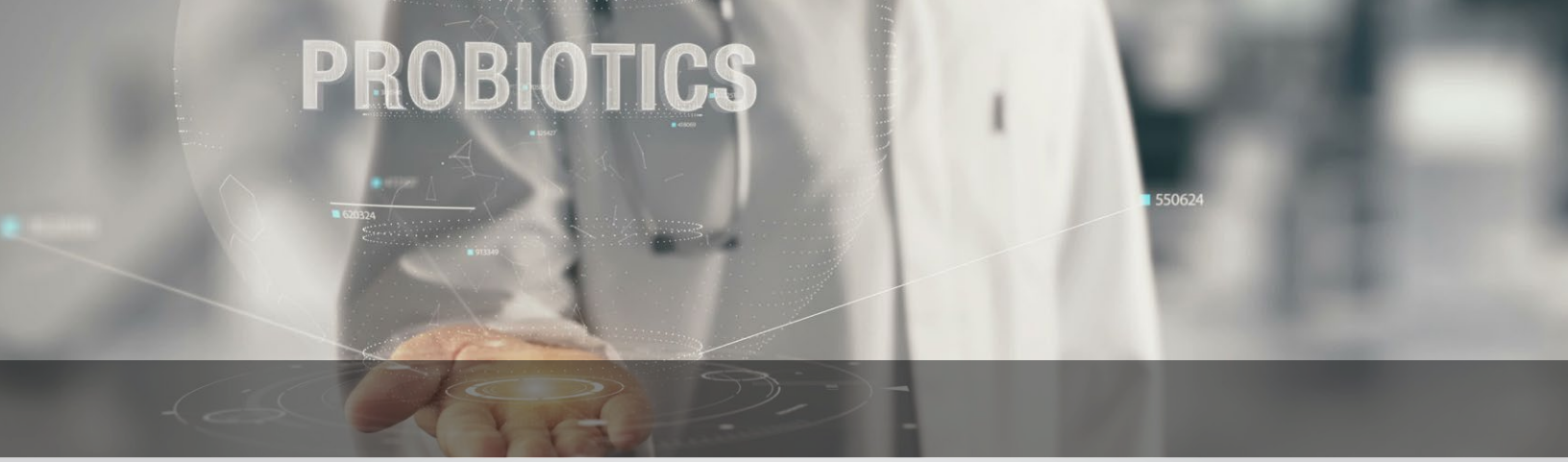
decades (Majeed *et al.*, 2018b). VBNC bacteria retain the characteristics of living cells and can regain cultivability by a process known as resuscitation. The presence of VBNC bacteria in LactoSpore®, when used in commercial formulations, was confirmed using a reliable and accurate method of flow cytometry (FCM). It effectively explains the protective mechanism adapted by LactoSpore® in response to stressful environmental circumstances, such as acidic and alkaline conditions, high temperatures and in commercial formulations like compressed tablets and capsules (Majeed *et al.*, 2018b).

LactoSpore® was subjected to a simulated digestion process that imitates saliva, gastric and small intestinal conditions. The substantial resistance of LactoSpore® to the simulated digestive conditions encountered during the process was approximately five times greater compared with those of the *L. acidophilus* control. It showed no significant drop in the viable spore count even after 240 minutes of mouth, gastric and intestinal phases of digestion (Figure 2). The survival rate of LactoSpore® after the simulated digestion process was 92.4% compared to 87.6% survival for *L. acidophilus* (Shinde *et al.*, 2019a).



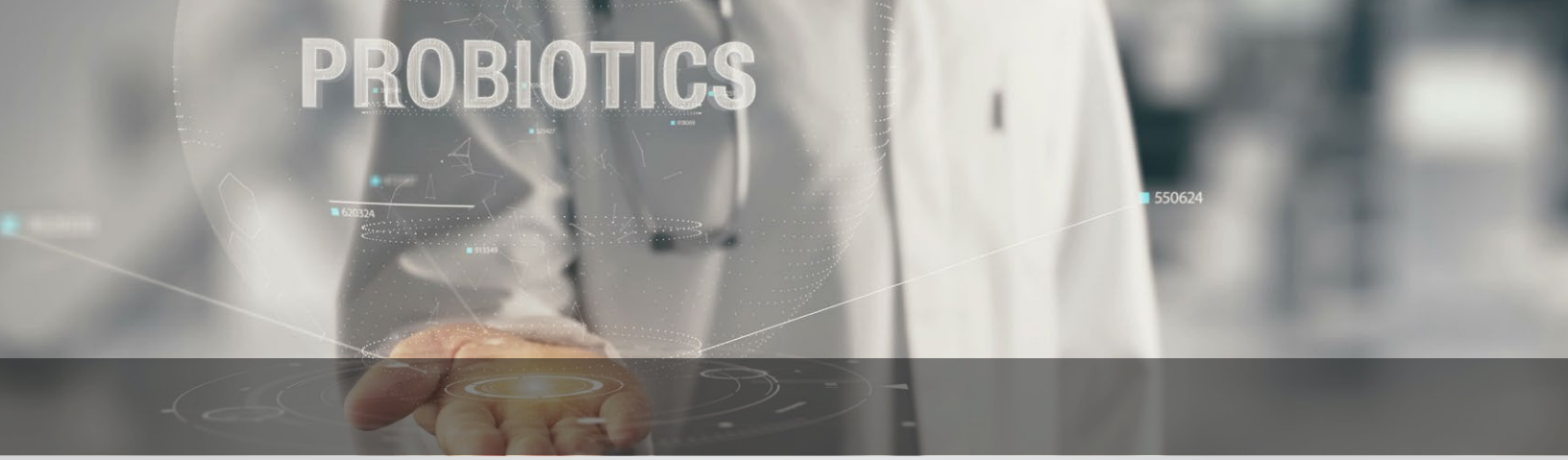
*P < 0.05 compared to 0 min.

FIGURE 2. SURVIVAL OF LACTOSPORE® IN THE THREE COMPARTMENTS OF DIGESTION SIMULATED *IN VITRO*: MOUTH, STOMACH AND INTESTINE. *L. acidophilus* SERVED AS A CONTROL FOR OVERALL SURVIVAL OF INDIVIDUAL BACTERIA. (SHINDE *et al.*, 2019a)

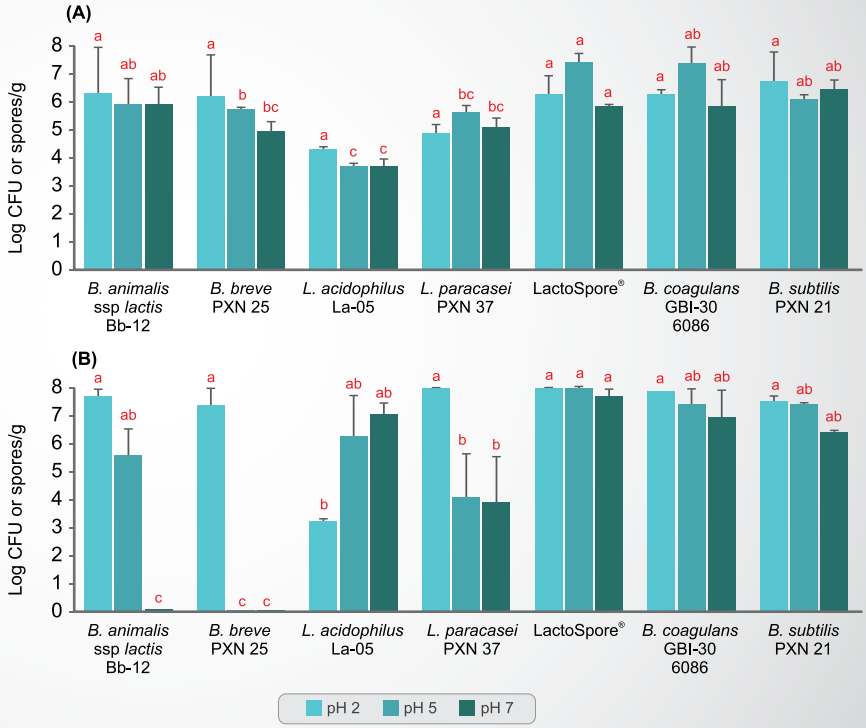


Similarly, the stability of fermented dairy products containing LactoSpore® and *Streptococcus thermophilus*, as auxiliary starter culture (4:1 ratio) was evaluated *in vitro*. The products demonstrated strong resistance to acidic pH and bile salts, maintaining over 8 Log_{10} CFU (colony-forming units)/g of viable microorganisms throughout the 60-day storage period (Lavrentev *et al.*, 2021). Likewise, LactoSpore® was used to make fermented soymilk and it was found viable even after the fermentation temperature of 50°C (for 9 hours) (Joyce *et al.*, 2021).

Majeed *et al.* (2016b) investigated the stability of LactoSpore® during the processing and storage of functional foods such as baked foods, beverages, and fruit preserves to which LactoSpore® was added to achieve the desired concentration of 15×10^9 CFU/g of the finished product. LactoSpore® was found stable during processing and the respective storage conditions of waffles, muffins, chocolate fudge frosting and hot fudge topping, vegetable oil, apple juice, concentrated glucose syrup and even in brewed coffee. LactoSpore® showed 92% and 86% stability during baking and storage at frozen conditions of banana muffins and waffles, respectively, for up to 12 months. Brewing coffee at 90°C for 2 min did not reduce the viability of LactoSpore® significantly and was 66% viable even after maintaining the temperature at 77°C for 4 h. It showed over 95% viability in chocolate fudge frosting, hot fudge topping, peanut butter, strawberry preserve and vegetable oil at room temperature for 12 months. LactoSpore® was found stable in refrigerated apple juice for up to 6 months and maintained its viability in concentrated glucose syrup at both refrigerated and room temperatures for 24 months (Majeed *et al.*, 2016b). In another study, LactoSpore® was found stable during the manufacturing and storage of a variety foods such as "Requeijão cremoso" cheese, orange juice and homemade breads.



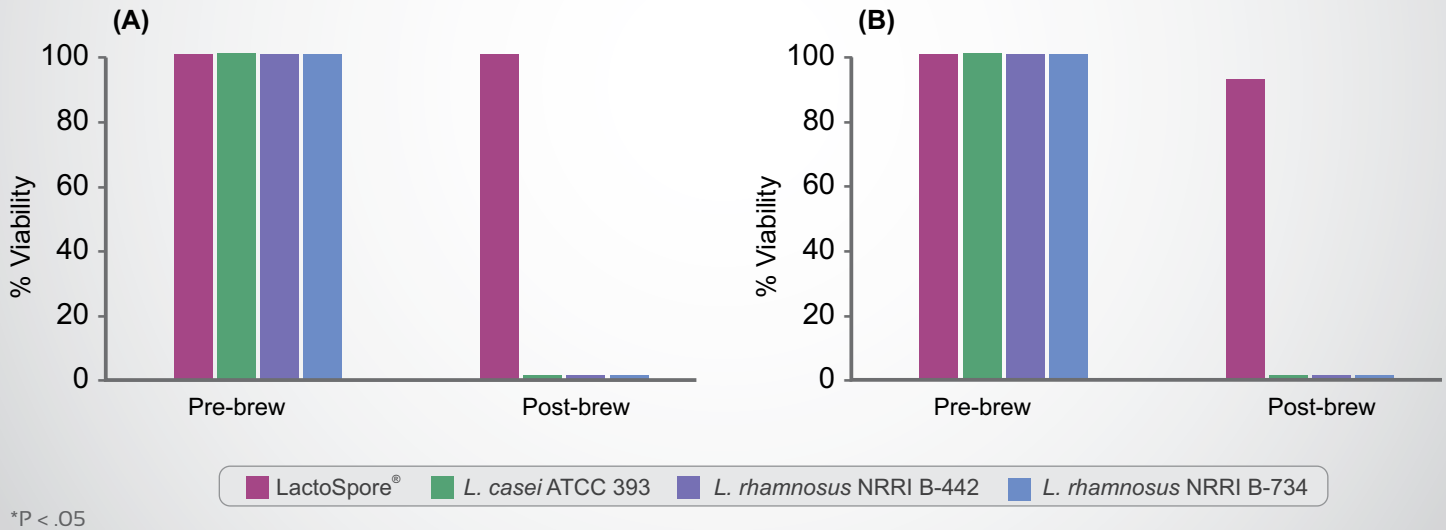
The viability of LactoSpore® was not significantly impacted by shelf life, storage conditions (whether refrigerated or not), or the composition and manufacturing processes used. LactoSpore® was added to "Requeijão cremoso" cheese when the mean pH was 6.1 (at 50°C) and it remained viable during storage at 6°C for 45 days (Figure 3). Viability of LactoSpore® was not significantly affected by the low pH and high acidity of orange juice (pH 4.15) and apple juice (pH 3.15) and was relatively constant during its shelf-life period under refrigerated temperature (7 days and 6 months, respectively). Similarly, the probiotic potential of LactoSpore® remained unaltered in breads when stored at room temperature (approximately 25°C) for 7 days. Also, LactoSpore® in these food matrices showed higher stability under GI tract simulatory conditions at the beginning and the end of the shelf life (Soares *et al.*, 2019). The authors also observed subtle differences in the stability of *Bacillus* species, with *B. coagulans* MTCC 5856 showing higher stability compared to GBI-30 6086, which, in turn, was better than *B. subtilis* PXN 21 (Soares *et al.*, 2019).



[a, b, c] small letters denote significant difference ($P < .05$) between different probiotic strains at different pH values.

FIGURE 3. VIABILITY OF DIFFERENT MICROORGANISMS WITH CLAIMED PROBIOTIC PROPERTIES (LOG CFU/G) INOCULATED IN "REQUEIJAO CREMOSO" CHEESE SAMPLES (A) AND ORANGE JUICE (B) AT THE END OF SHELF LIFE, TO THE SIMULATED DIGESTION (DIFFERENT pH VALUES) OF THE GASTROINTESTINAL TRACT.
(SOARES *et al.*, 2019)

LactoSpore® demonstrated the ability to withstand brewing conditions and grow in the harsh environment of the gastrointestinal tract, utilizing tea and coffee as its sole nutritional source. The growth increased even further when water soluble fibers were added at the time of brewing. LactoSpore® showed high resistance to the brewing conditions compared to other probiotic bacteria (Figure 4). It showed over 99% viability ($P > 0.05$) in tea and coffee powder at room temperature up to 24 months of storage (Figure 5) suggesting the possible use of LactoSpore® in other processed foods (Majeed *et al.*, 2019a).



*P < .05

FIGURE 4. : EFFECT OF BREWING CONDITIONS ON THE VIABILITY OF LACTOSPORE® AND OTHER PROBIOTIC BACTERIA. LACTOSPORE® SHOWED HIGH RESISTANCE TO THE TEA (A) AND COFFEE (B) BREWING CONDITIONS. (MAJEED *et al.*, 2019a)

LACTOSPORE® HAS EXCELLENT GENOMIC STABILITY

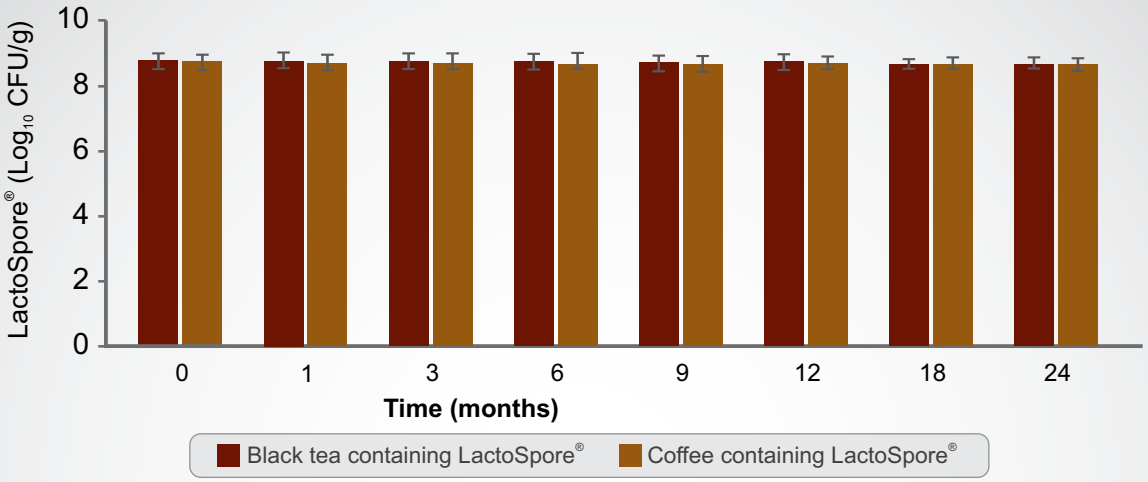


FIGURE 5: EFFECT OF STORAGE CONDITIONS (25 ± 2°C WITH 60 ± 5% RH, RELATIVE HUMIDITY) ON THE VIABILITY OF LACTOSPORE® INCORPORATED IN BLACK TEA AND COFFEE POWDER. (MAJEED *et al.*, 2019a)

Bacterial species are vulnerable to intra-species variability over the years of commercial production. However, the phenotypic and genotypic stability of LactoSpore® collected from five different production lots of different years was investigated using various techniques such as biochemical profiling, 16S rRNA sequencing, $(GTG)^5$ primer, BOX PCR fingerprinting, and Multi-Locus-Sequence typing. All these analyses confirmed the strain purity and consistency over the period of commercialization. *In vitro* evaluation of the probiotic potential of LactoSpore® confirmed its resistance to the harsh gut environment and antimicrobial activity against human pathogens. It also showed the production of L-lactic acid, a property exclusive for *B. coagulans* MTCC 5856. All these results ensure the strain purity and stability of LactoSpore® for commercial production and truly comply with its label claim in the market (Majeed *et al.*, 2016a).

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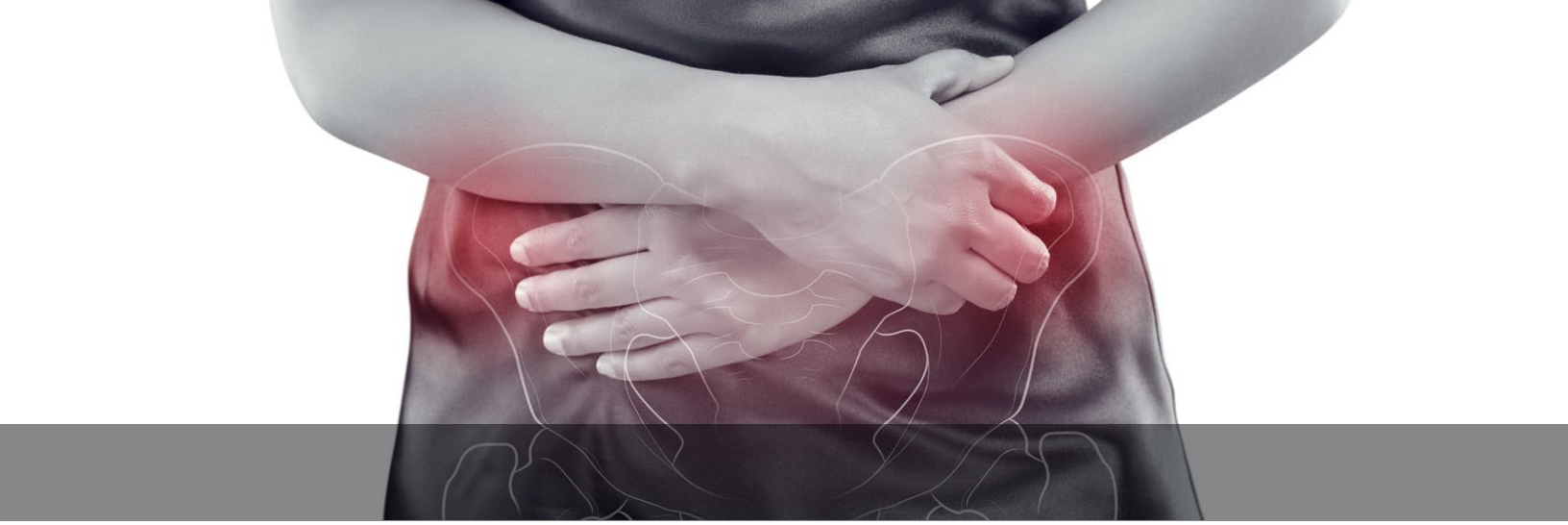
LACTOSPORE® AS A THERMOSTABLE PROBIOTIC WITH HEALTH CLAIMS

Probiotics were initially marketed as microbial preparations that provide beneficial effects not only on the GI tract but also on systemic areas such as the liver, vagina and bloodstream. In recent decades, numerous promising studies have highlighted the diverse health applications of LactoSpore®. The beneficial effects of LactoSpore® on human health is supported by adequate animal and clinical data which highlight the possible use of LactoSpore® against diarrhea, irritable bowel diseases (IBD), mental health, obesity, healthy sugar control, and metabolic syndrome.

IMPROVED GUT HEALTH

Probiotics support the healthy maintenance of GI function and integrity by modulating intestinal microbial communities, suppressing pathogens, and nourishing and strengthening colonic enterocytes. Probiotics are known to produce short chain fatty acids (SCFA) that support the growth of healthy microbiota and reduce inflammation. Majeed *et al.* found that LactoSpore® produces SCFA (propionate, butyrate and acetate) by fermenting plant-based natural fibers, which may enhance the beneficial effects on the gut microbiome (Majeed *et al.*, 2019b).



Helicobacter pylori is a Gram-negative bacterium and is regarded as the etiologic factor for the development of peptic ulcer disease, gastric adenocarcinoma, and the lymphoma of mucosal lymphoid tissue. Double antibiotic therapy along with proton pump inhibitors are used to eradicate the infection. Despite therapeutic improvements, *H. pylori* is associated with a high infection rate worldwide. Moreover, the infection remains asymptomatic in 80% of individuals. Treatment failures are high and are associated with antibiotic resistance and adverse effects of the drugs. Several studies have reported that certain probiotic strains can exhibit inhibitory activity against *H. pylori* infections with concomitant decrease in antibiotic associated adverse effects (Homan and Orel, 2015). LactoSpore® was found to be effective in inhibiting *H. pylori* growth *in vitro*. When combined with β-glucogallin and mucic acid gallates, the effectiveness was significantly greater than when used individually, highlighting the synergistic effect of the composition (Figure 6) (Majeed *et al.*, 2017).

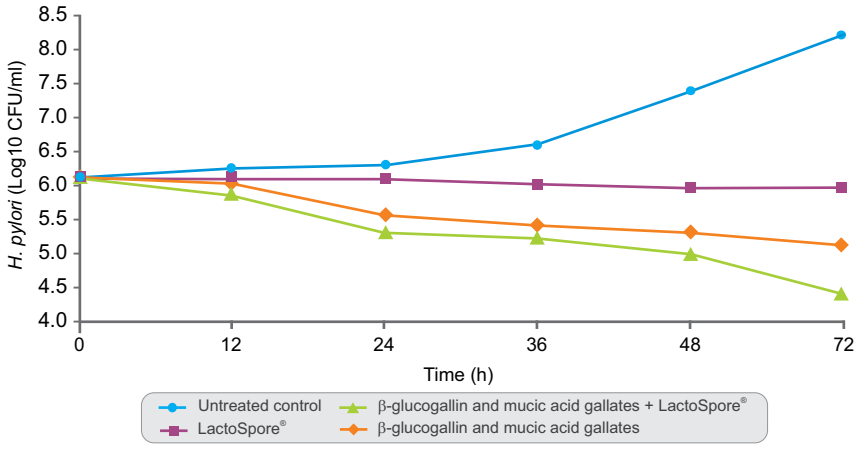
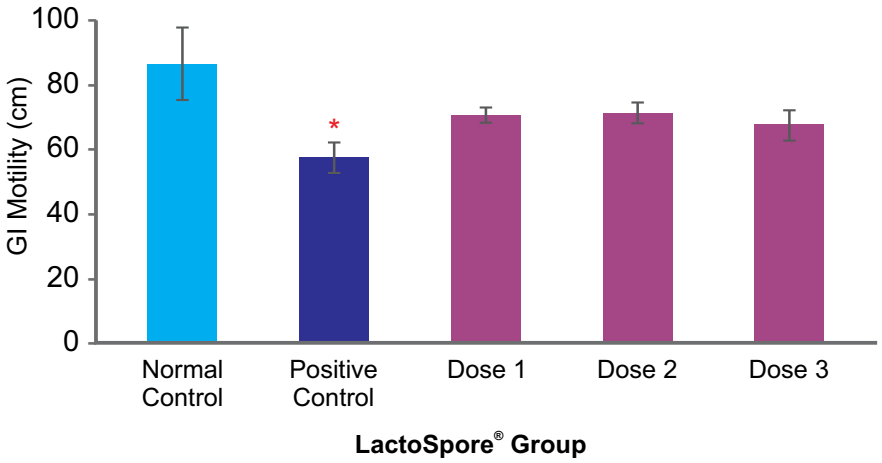


FIGURE 6. GRAPHICAL REPRESENTATION SHOWING THE INHIBITION IN GROWTH OF *H. pylori* BY A COMBINATION CONTAINING LACTOSPORE® AND β-GLUCOGALLIN. (MAJEED *et al.*, 2017)

Several studies have evaluated the uses of LactoSpore® in the prevention and treatment of GI related discomforts. LactoSpore® showed significant antidiarrheal activities *in vivo*. Diarrhea is characterized by an increase in volume, frequency and water content of feces, resulting in dehydration and loss of body fluids and electrolytes. LactoSpore® supplementation showed 33% inhibition of fecal output and 22% reduction in GI motility in Wistar rats at a dose of 160 × 10^6 CFU/kg body weight (Figure 7) (Majeed *et al.*, 2016c).



Data in mean ± standard error, *p<0.05 Vs normal control.

FIGURE 7. DOSE 1, 2 AND 3 WERE 40, 80 AND 160 ×10^6 CFU/KG BODY WEIGHT, RESPECTIVELY. (MAJEED *et al.*, 2016c)

The long-term consumption of LactoSpore® along with cinnamon extract (Cinnulin®*) reduced inflammation of the colon in mice with experimentally-induced colitis. The LactoSpore® combination significantly reduced the shortening of the colon and increased the survival rates of rats (Figure 8). The results indicate the possible use of LactoSpore® to improve the conditions of gastrointestinal disorders, including colitis (Vaclav and Jana, 2013).

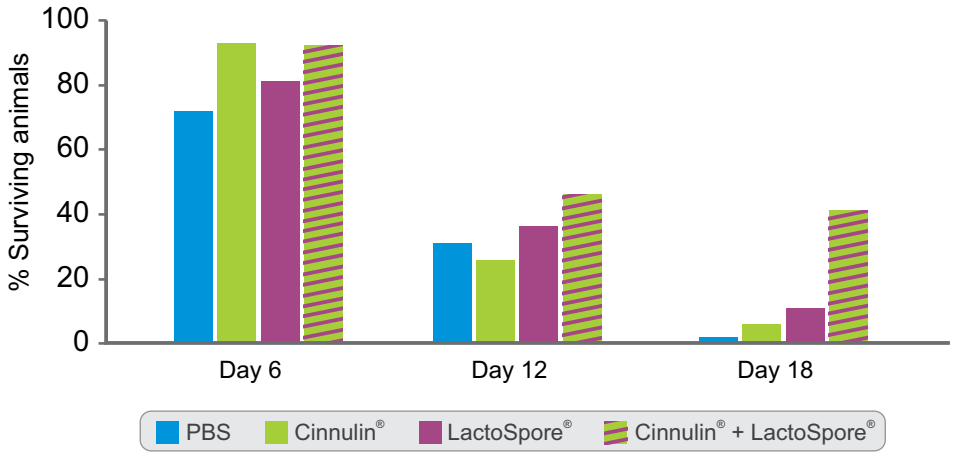


FIGURE 8. EFFECT OF LONG-TERM FEEDING WITH CINNULIN®, LACTOSPORE®, OR CINNULIN®/ LACTOSPORE® COMBINATION ON SURVIVAL RATE IN MICE WITH EXPERIMENTALLY-INDUCED COLITIS. (VACLAV AND JANA, 2013)

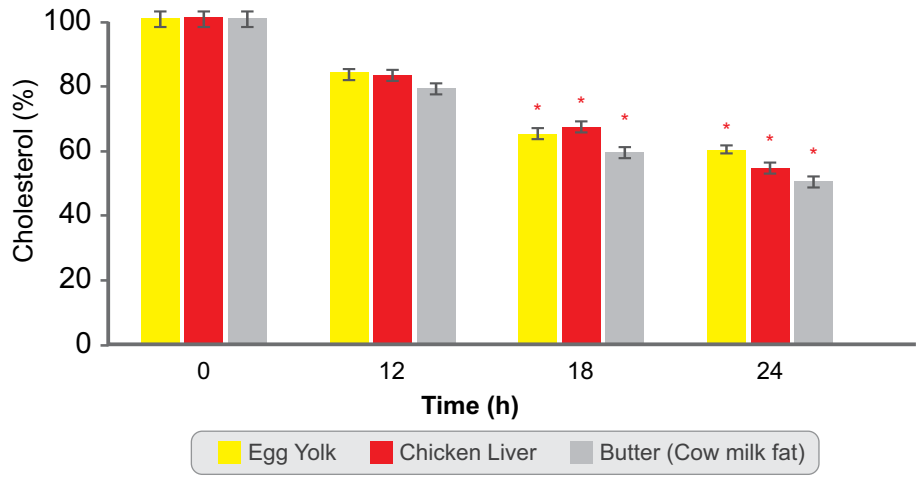
A synbiotic combination containing LactoSpore® and prebiotic whole plant sugar cane fibers (PSCF) significantly altered the expression of tight junction proteins and modulated serum IL-1, IL-10 and C-reactive protein (CRP) levels in mice with DSS-induced colitis. The synbiotic supplementation reduced disease severity, colonic damage, and inflammatory mediators while modulating the metabolite and short chain fatty acid (SCFA) profiles of IBD mice (Shinde *et al.*, 2019b).

*Cinnulin® is a registered trademark of Integrity Nutraceuticals, Inc., a Florida corporation

The efficacy of LactoSpore® as an antidiarrheal agent was further confirmed in humans. A total number of 36 patients with IBD were enrolled in a double-blind, placebo-controlled study where 18 patients received LactoSpore® at a dosage of 2×10^9 CFU/day for 90 days while the other group received placebo. The diarrhea prominent IBD patients who consumed LactoSpore® experienced remarkable relief from the clinical symptoms like bloating, vomiting, diarrhea, stool frequency and abdominal pain (Majeed *et al.*, 2016e). Another clinical study in 30 healthy volunteers also supported the beneficial shift of gut microflora towards healthy bacteria after the consumption of LactoSpore® capsule for a period of 28 days (Majeed *et al.*, 2023a). Further, LactoSpore® consumption significantly reduced the functional gas and bloating symptoms in subjects when they received LactoSpore® tablets for 30 days (Majeed *et al.*, 2023b). Thus, all these studies provide ample data for the effectiveness of LactoSpore® for improving gut health. Considering the unwanted side effects of synthetic and conventional antidiarrheal and antigastritis medicines, LactoSpore® may be consumed as an alternative or supportive measure for the problems associated with the gut.

HEALTHY CHOLESTEROL LEVELS

The global number of deaths from cardiovascular disease (CVD) has risen exponentially over the past two decades. The World Health Organization (WHO) estimates that nearly 23 million people will be affected by CVD and it will become a major disease burden globally by 2030. Increased serum cholesterol level is associated with CVD and other disabilities (Mendis *et al*., 2011) associated with heart health. The side effects associated with pharmacological agents for hypercholesterolemia, such as statins, have increased the demand for safe and effective alternatives, making LactoSpore® a promising option for supportive therapy. Bile salt hydrolase (BSH) activity is considered a biomarker for the selection of probiotic strain adjuncts to manage hypercholesterolemia. LactoSpore® was found to be a potent producer of BSH *in vitro*. LactoSpore® significantly ($P < 0.05$) reduced cholesterol levels in a culture medium by 48.42%. Interestingly, even the resting probiotic (live but suspended in buffer) and heat-killed bacteria were able to reduce cholesterol by 36.47% and 8.5%, respectively. Furthermore, LactoSpore® significantly reduced the cholesterol levels in cholesterol-rich foods, such as egg yolk (39.79%), chicken liver (45.44%) and butter (49.51%), when incubated for 24 hours in conditions mimicking the *in vivo* environment (Figure 9) (Majeed *et al*., 2019c).



Each value represents the mean ± SD (n = 3). *P < 0.05.

FIGURE 9. EFFECTS OF LACTOSPORE® ON THE REMOVAL OF CHOLESTEROL FROM DIFFERENT FOOD SOURCES (EGG YOLK, CHICKEN LIVER AND BUTTER). (MAJEED *et al.*, 2019c)

Another study revealed the synergistic effects of LactoSpore® in combination with an aqueous extract of cinnamon significantly lowered the levels of total cholesterol, triglycerides and LDL in hypercholesterolemic mice (Vaclav and Jana, 2013). Further, supplementation of LactoSpore® along with yacon as a potential additive to a low-energy dietary intervention program of 21 days, showed a clinically meaningful improvement in lipid profiles associated with reduced blood pressure (Balliett and Burke, 2013). All of these studies suggest that LactoSpore® could be used as a dietary ingredient for managing hypercholesterolemia, as well as associated coronary heart disease and other related conditions.

IMPROVED MENTAL HEALTH

Probiotics do more than improve gut health; they also enhance brain function (Sarkar *et al*., 2016). The complex interactions between the gut microbiota and the central nervous system are referred to as the gut-brain axis. Although the mechanism of the gut-brain axis is not yet fully understood, it is believed that the production of certain neurotransmitters, hormones, neuropeptides, and short-chain fatty acids (SCFAs) by probiotics may help alleviate symptoms of clinical depression. However, evidence-based studies strongly suggest the connections between GI and emotional health. For instance, more than 50% of people with irritable bowel syndrome also develop depression and anxiety. Therefore, the effects of probiotics on mental health and neurological conditions are poised to reveal an entirely new approach for developing personalized medicines tailored to an individual's gut microbiota. The

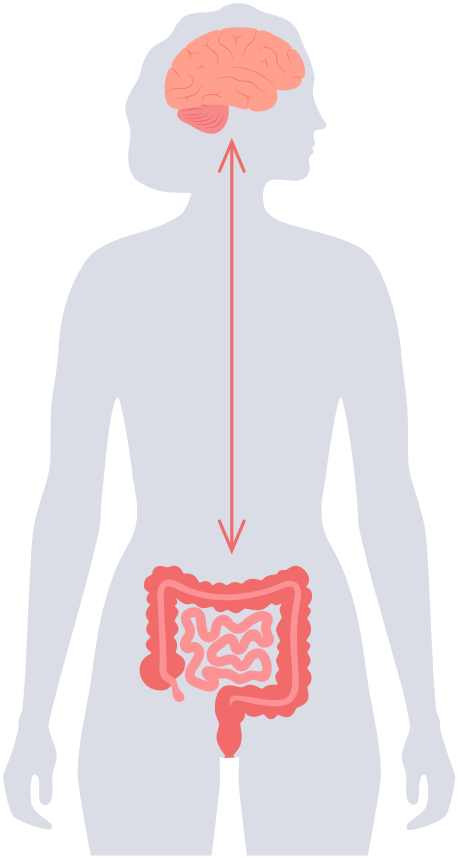


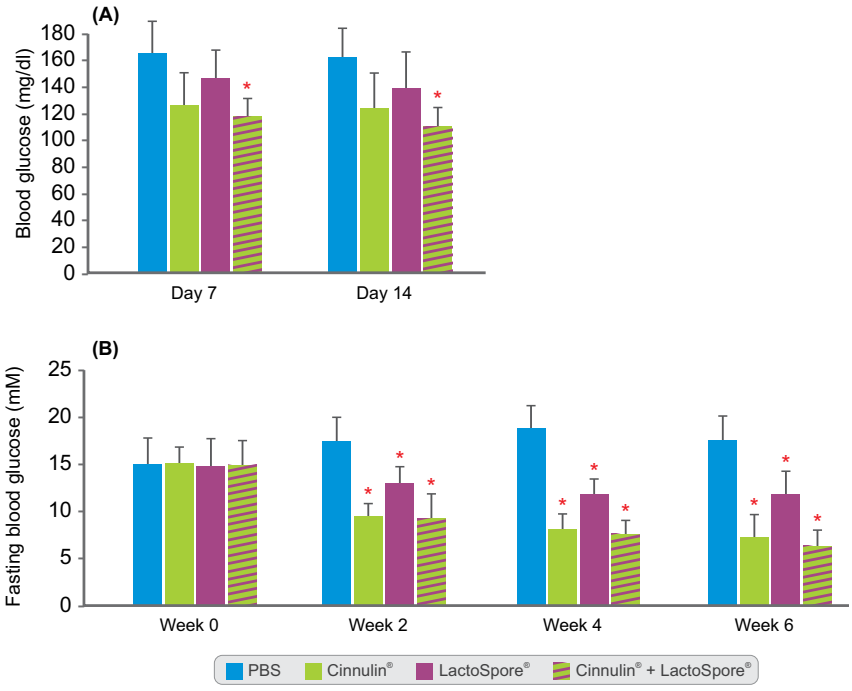
TABLE 1: EFFECT OF LACTOSPORE® ON SERUM MYELOPEROXIDASE.

Serum myeloperoxidase (ng/mL)			
	LactoSpore® (n = 20)	Placebo (n = 20)	P value
Baseline (Day 0)	12.6 ± 4.31	9.81 ± 4.10	P = 0.0460
End of the study (Day 90)	7.7 ± 2.57	9.8 ± 4.65	P ≤ 0.01
Change from baseline to day 90	P ≤ 0.01	P = 0.9886	P ≤ 0.01

psychobiotic properties of LactoSpore® were analyzed in IBD patients with major depressive disorder (MDD), characterized by increased medical morbidity, mortality, feelings of guilt, low mood, reduced quality of life, and disturbed sleep or appetite. MDD is often not effectively treated with standard antidepressants, while alternative therapies may take weeks or even months to show results. Patients with IBD consumed LactoSpore® tablets (600 mg) in the morning as a dietary supplement for a period of 90 days. Both primary and secondary efficacy measures, such as the Hamilton Rating Scale for Depression (HAM-D), Montgomery-Asberg Depression Rating Scale (MADRS), and the Center for Epidemiological Studies Depression Scale (CES-D), demonstrated significant improvements in the LactoSpore® group compared to the placebo group. Moreover, the LactoSpore® group showed a significant reduction in the level of serum myeloperoxidase (Table 1), an inflammatory marker associated with oxidative stress and depression, after 90 days of treatment. Thus, the study suggests clinically meaningful effects of LactoSpore® in improving the symptoms of depression (Majeed *et al.*, 2018c).

IMPROVED GLUCOSE METABOLISM

Type 2 diabetes is a major global health burden, with the International Diabetes Federation projecting that it will affect 10.4% of the total population (Ogurtsova *et al.*, 2017). The persistence of altered glucose metabolism may be a root cause for several other diseases including hypertension, heart diseases (stroke), kidney failure and retinopathy. Recent research has uncovered the crucial role of gut microflora as a key environmental factor influencing the development of lifestyle diseases like diabetes, by regulating molecular interactions and host energy homeostasis (Clarke *et al.*, 2014). The LactoSpore® and Cinnulin® combination was found to be effective in reducing blood sugar and fasting glucose levels in hyperglycemic mice induced with streptozotocin (STZ) (Figure 10) (Vaclav and Jana, 2013).



* Represents statistical difference from control (PBS).

FIGURE 10. EFFECT OF LONG-TERM FEEDING WITH CINNULIN®, LACTOSPORE®, OR CINNULIN®/ LACTOSPORE® COMBINATION ON BLOOD GLUCOSE LEVELS (A) AND ON FASTING GLUCOSE LEVELS (B) IN MICE WITH EXPERIMENTALLY-INDUCED HYPERGLYCEMIA. EACH VALUE REPRESENTS THE MEAN OF THE THREE INDEPENDENT EXPERIMENTS ± SD. (VACLAV AND JANA, 2013)

IMPROVED BODY COMPOSITION

Obesity is a major societal challenge in modern populations and can lead to potentially life-threatening conditions such as type 2 diabetes, hypercholesterolemia, high blood pressure, metabolic syndrome, and even heart disease. The prevalence of overweight and obesity is rising rapidly, not only among the elderly but also among adults and children. Hence, several new strategies are being explored for body weight management in different populations. A low-energy-density dietary intervention plus regimented supplementation program included LactoSpore® as a dietary supplement to target the potential role of gut microbiota in obesity. The 21-day intervention program in 50 participants showed a significant reduction in fat mass while maintaining lean body mass. Anthropometric measurements were improved significantly after the consumption of LactoSpore® (Table 2). Further, a 28% increase in serum testosterone levels (from 400.8 to 511.8 µg/dL) was observed for men, which is a strong indicator of a reduced risk of developing obesity-related diseases (Balliett and Burke, 2013).

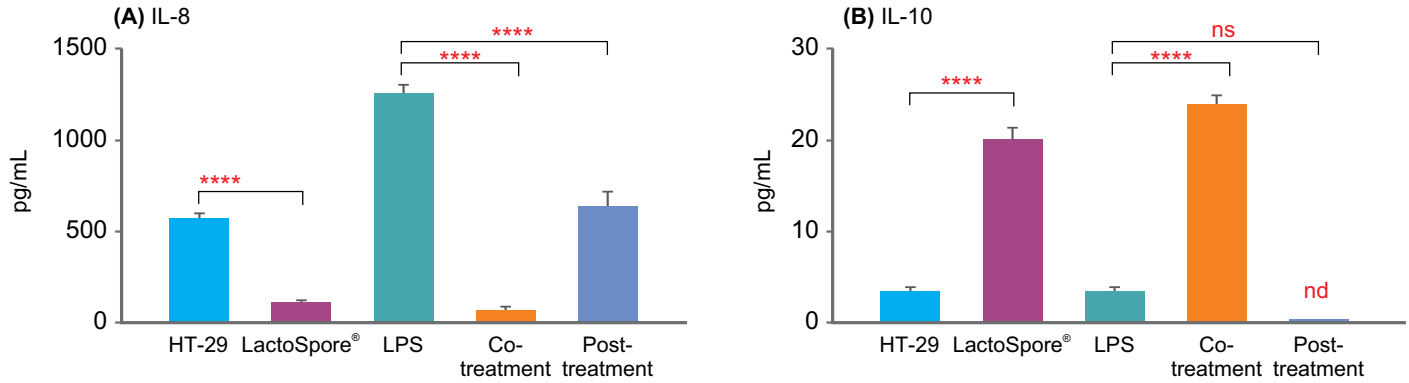
TABLE 2: ANTHROPOMETRIC MEASUREMENTS (MEAN VALUES ± SD)

	Pre-intervention	Post-intervention	Difference
Weight (lb)	175.4 ± 38.31	166.7 ± 36.69	-8.7 ± 5.54* (7.16-10.34)
Waist (in)	36.4 ± 5.74	34.9± 5.81	-1.5 ± 1.14* (1.13-1.78)
Hip (in)	42.0 ± 3.93	40.8 ± 3.71	-1.2 ± 1.24 * (.80-1.51)
Waist-hip ratio	0.86 ± 0.081	0.85 ± 0.087	-0.01 ± 0.033* (.002-.021)
Height (in)	66.23 ± 2.946	66.25 ± 2.936	N/A
Waist-height ratio	0.55 ± 0.087	0.53 ± 0.088	-0.02 ± 0.018* (0.017-0.027)
BMI (kg/m^2)	28.0 ± 5.29	26.6 ± 5.09	-1.4 ± 0.81* (1.2-1.6)

* Significant at P < .05; 95th confidence intervals of the difference in parentheses.

IMMUNOMODULATORY EFFECTS

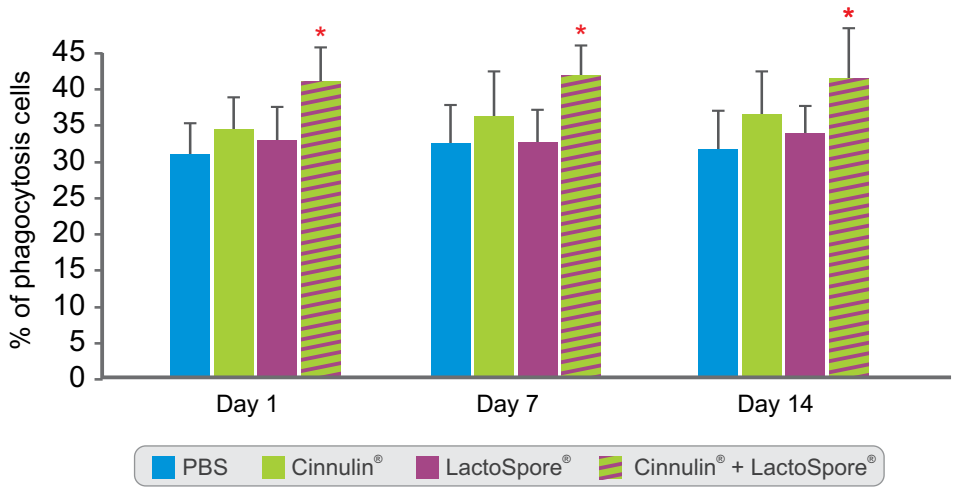
The gut microbiota plays a critical role in maintaining the host-diet-microbiota balance, and disruptions in this relationship can transform a healthy state into one prone to diseases, including cancer (Boleij and Tjalsma, 2012). Intestinal microflora has a pivotal effect on the host's immune homeostasis. It profoundly influences not only the local intestinal immune system, but also has a significant impact on systemic immune responses. Alterations in gut microbial communities are linked to immune dysregulation and increased susceptibility to pathogen invasion. A potent probiotic modulates immune and inflammatory mechanisms, reducing the development of inflammatory biomarkers and potentially preventing unnecessary activation of the immune system. The specific interactions between probiotics and the immune system suggest potential probiotic-based therapeutic strategies for treating allergies and autoimmune diseases (Cristofori *et al.*, 2021). The LactoSpore® treatment led to a significant reduction in the release of the pro-inflammatory cytokine IL-8 under normal and LPS-stimulated conditions in HT-29 cells. LactoSpore® also induced anti-inflammatory IL-10 secretion by HT-29 cells in both inflamed and non-inflamed conditions (Figure 11) (Shinde *et al.*, 2019a).



Data are represented as mean ± SEM of three repeated measurements. (nd=non-detected, ns=non-significant, **** P < 0.0001).

FIGURE 11. IMMUNO-MODULATORY EFFECTS OF LACTOSPORE®. A) IL-8, B) IL-10 RELEASED BY HT-29 CELLS (NEGATIVE CONTROL), LACTOSPORE® TREATED HT-29 CELLS, LPS-STIMULATED HT-29 CELLS (POSITIVE CONTROL) AND LPS-STIMULATED AND TREATED WITH LACTOSPORE® (CO- AND POST-TREATMENT). (SHINDE *et al.*, 2019a)

The synergistic effects of LactoSpore® and cinnamon extract (Cinnulin®) in immune modulation were demonstrated *in vivo* by stimulating the phagocytic activity of peripheral blood neutrophils. Phagocytosis is one of the most important immunological reactions traditionally associated with the effects of natural immune modulators. The combination of LactoSpore® + Cinnulin® significantly induced the phagocytosis of 2-hydroxyethyl methacrylate (HEMA) particles by peripheral blood cells compared to the individual ingredients (Figure 12) (Vaclav and Jana, 2013).

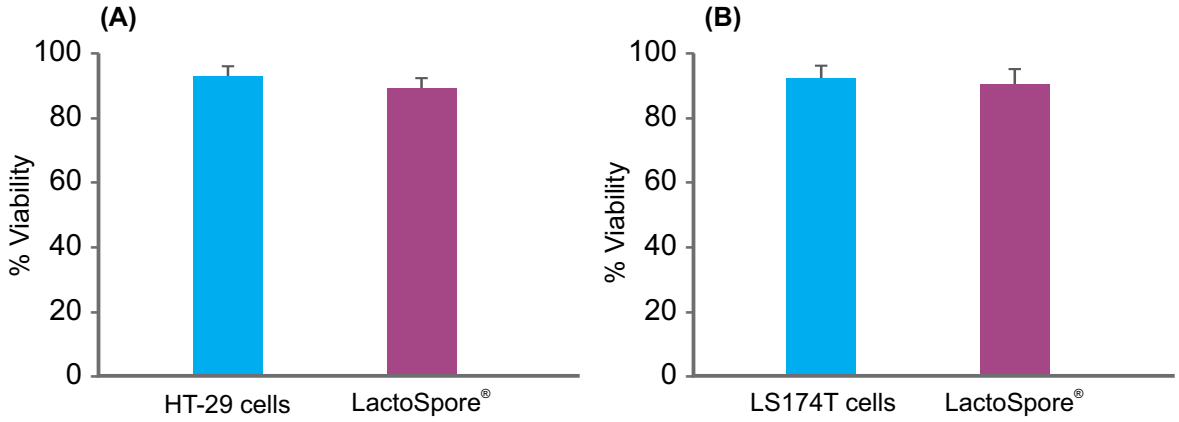


Each value represents the mean of the three independent experiments ± SD.
*Represents statistical difference from control (PBS).

FIGURE 12. EFFECT OF LONG-TERM FEEDING WITH CINNULIN®, LACTOSPORE®, OR CINNULIN®/ LACTOSPORE® COMBINATION ON PHAGOCYTOSIS OF HEMA PARTICLES BY PERIPHERAL BLOOD NEUTROPHILS. (VACLAV AND JANA, 2013)

LACTOSPORE® - TRUST BEGINS WITH SAFETY

Over the years, a couple of *in vitro* and *in vivo* studies, including human trials, affirmed the safety of LactoSpore® as a probiotic. The extensive investigations on the antibiotic susceptibility, absence of enterotoxin genes, and effects on intestinal cells confirmed the non-toxicity of LactoSpore® (Majeed *et al.*, 2016a). *In vivo* studies have revealed that LactoSpore® causes no harm to the intestinal cells at a dosage of 2×10^9 CFU/mL even after the treatment period of 8 hours (Figure 13) (Shinde *et al.*, 2019a). No increase in extracellular LDH was observed in the cell culture after the treatment, indicating no damage to the cell membrane or cell death. This was further confirmed by the Trypan blue exclusion test. These results showed that LactoSpore® is non-toxic to human colonic cell lines (Shinde *et al.*, 2019a).



Data are represented as mean ± SEM of three replicate experiments

FIGURE 13. EFFECT OF LACTOSPORE® ON CELL VIABILITY. VIABILITY OF (A) HT-29 AND (B) LS174T CELLS AS DETERMINED BY TRYPAN BLUE EXCLUSION TEST. (SHINDE *et al.*, 2019a)

Further, LactoSpore® did not show any mutagenic effect in the Ames test and was found stable up to 36 months at ambient temperature confirming its shelf life for 3 years. Thus, the extensive *in vitro* safety studies revealed that LactoSpore® is safe to use and eminently qualified as a probiotic strain (Majeed *et al.*, 2016a).

The safety and tolerability of LactoSpore® at a dose of 2×10^9 CFU/day was evaluated in 40 healthy adults. The healthy volunteers received either a LactoSpore® tablet or placebo for a supplementation period of 30 days. Several safety parameters were evaluated including blood hematology, clinical chemistry parameters, anthropometric measures (weight, BMI, blood pressure and heart rate), adverse events, Bristol stool score, tolerability questionnaire and bowel habit diary. The treatment compliance was 99% for the LactoSpore® group and 97% for the placebo group. There were no significant differences in any of the safety parameters between the LactoSpore® group and placebo group.

Moreover, the LactoSpore® tablets were found easy to swallow and did not show any toxic effects in the stomach during the 30 days of supplementation (Majeed *et al.*, 2016d).

A double-blind, placebo controlled study also verified the safety of LactoSpore® at a dose of 2×10^9 CFU/day over a relatively long 90-day supplementation period in patients with IBS and depression. The study did not report any adverse events or changes in safety blood parameters and vital signs (blood pressure and heart rate) during the period of LactoSpore® consumption (Majeed *et al.*, 2018c). Similarly, several other human studies also ensure the safety of LactoSpore® consumption at a dose of 2 billion CFU/day (Majeed *et al.*, 2016e).

In 2008, European Food Safety Authority granted the Qualified Presumption of Safety (QPS) status to *B. coagulans*, and the Japanese Ministry of Health and Welfare also approved *B. coagulans* for the treatment of abnormalities associated with intestinal microflora and dysbiosis. The U.S FDA issued GRAS status for the use of LactoSpore® in foods at a maximum level of approximately 2×10^9 CFU/serving after a comprehensive review of safety and toxicology data (Majeed *et al.*, 2018c). Additionally, LactoSpore® has received approval from various global regulatory authorities, including the Natural Health Product Directorate (NNHPD) in Canada, the Therapeutic Goods Administration (TGA) in Australia, and Food Standards Australia New Zealand (FSANZ).

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RECENT ADVANCEMENTS IN THE UTILITY OF LACTOSPORE®

The past few years have witnessed a remarkable increase in the demand for functional foods fortified with probiotics. Incorporating probiotics into foods has become an effective strategy to provide functional health benefits beyond basic nutrition. Integrating probiotics into shelf-stable foods and products that require high-temperature processing presents a challenge, as it is crucial to maintain the viability of the spores during their passage through the GI tract. For over two decades, LactoSpore® has been safely and effectively used as an ingredient in various range of products like capsules, tablets, Tutti Frutti frozen yogurt, and bread. LactoSpore® has

expanded its applications to a wide range of functional foods and beverages as well. LactoSpore® was successfully incorporated into baked goods, beverages, vegetable oil, and concentrated glucose syrup without losing its viability. It was found to remain stable during the processing of functional foods and under storage conditions of low/room temperature for up to 12-24 months, without changing key sensory parameters such as flavor and aroma (Majeed *et al*., 2016b). The use of LactoSpore® in fermented products helps overcome stability issues of commonly used probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium*, which lose their viability due to the harsh gut environment. LactoSpore® showed good viability and stability during the processing and storage of fermented dairy and non-dairy products without compromising their organoleptic qualities and overall acceptability. Thus, fermented products can be an excellent carrier of LactoSpore® that may offer health benefits when consumed as part of a diet (Lavrentev *et al*., 2021; Joyce *et al*., 2021).

LactoSpore® was successfully included in brewed tea and coffee without changing the organoleptic properties of the product. LactoSpore® remained 94.94% and 94.04% viable after brewing

(immediately) in unroasted green and roasted coffee, respectively. The physical appearance and nutritional value (carbohydrate, protein, ash, moisture and fat) of the tea and coffee remained unaltered, even after 24 months of storage, indicating the shelf stability of LactoSpore® in tea and coffee. No significant difference was observed in tea or coffee with or without LactoSpore® for the appearance, color, aroma, taste and overall acceptability (Majeed *et al*., 2019a).

Recently, a shelf-life study of LactoSpore® was conducted after being added to purees made from fruits or vegetables. In the study, purees of ahuyama, fig or carrot were fortified with LactoSpore® as a functional ingredient, intended for use in infants aged 6 to 12 months. The safety and viability of LactoSpore® were studied via microbiological analysis after ultra-high temperature (UHT) pasteurization. It showed functional effects during storage with a shelf life up to 90 days in ahuyama and up to 30 days in carrots (López Marín *et al*., 2020).

Given the changing lifestyles and eating habits of modern societies, the proven safety and efficacy of LactoSpore®, along with its stability during the processing and storage of functional foods, present a promising new strategy for introducing probiotics into the body in an effortless and kid-friendly way.

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USE AS VETERINARY PROBIOTIC

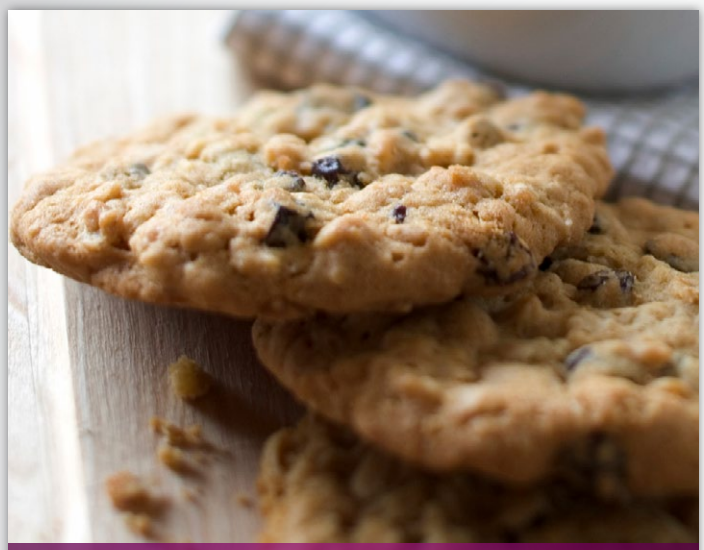
In pets and farm animals, administration of LactoSpore® would facilitate the establishment of a healthy gastrointestinal microfloral profile, reduce digestive upsets, improve feed utilization and support a healthy immune system. In controlled studies with *L. sporogenes* fed to farm animals and birds, feed efficiency and healthy growth were enhanced. Fecal counts of *Lactobacilli* increased while those of putrefactive cultures fell in animals and birds that received the probiotic (Zhou *et al.*, 2020).

CONCLUSIONS

The role of probiotics has expanded from use in gut health maintenance and supplementation in the event of dysbiosis (microfloral imbalance) during antibiotic therapy, to a wide range of health applications. With the increasing body of knowledge and research on known and emerging probiotic strains, future trends envisage their increased inclusion in dietary supplements and functional foods targeting diverse preventive health maintenance needs. Current innovations in functional foods with probiotics, including yogurts, beverages, bakery products, preserves, pickles, breakfast cereals, nutrition bars and other convenience products, present attractive delivery options for these healthful ingredients. A few examples of LactoSpore® in food-based products follow.

FOOD APPLICATIONS

CONTAINING LACTOSPORE®



ProbiOaties by BiteMarket, USA

Tutti Frutti, Worldwide

Perman Bread (white and wheat), Latin America

Beverages, Worldwide

MECHANISM OF ACTION

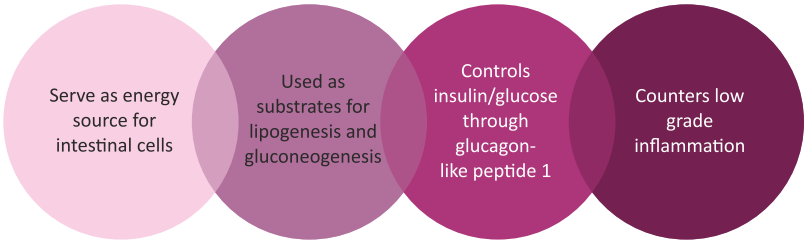
The beneficial activities of LactoSpore® in various conditions like gastroenteritis or irritable bowel syndrome may be attributed to the following factors:

Improves Gut Microflora

Probiotics are known to stimulate the growth of other beneficial bacteria like *Faecalibacterium prausnitzii* in the human gut. Thus, establishment of healthy microflora eventually helps in reducing toxins as well as the growth of other bacterial/viral pathogens. This proves beneficial in the treatment/ management of diseases like irritable bowel syndrome, which is attributed to a disrupted gut flora. In addition, probiotics also facilitate elimination of acids, antibiotics and other substances that might compete for colonisation sites and nutrients (Keller *et al.*, 2010).

Production of Short-chain Fatty Acids

Probiotic strains can produce short-chain fatty acids (SCFAs), the benefits of which have been summarized in the figure below (Kasińska and Drzewoski, 2015).



Studies indicate that LactoSpore® can produce various SCFAs (acetate, butyrate and propionate) by fermenting plant-based fibers (*Trigonella foenum-graecum* and *Vaccinium macrocarpon*) (Majeed *et al.*, 2018a and 2018d).

PATENTS

- HYPOLIPIDEMIC EFFECTS OF *Bacillus coagulans*
  US 11,213,553; CA3065162; NZ760204; JP7220677

- PROCESS FOR ENHANCING THE VIABLE COUNTS OF LACTIC ACID BACTERIA AND USEFUL COMPOSITION THEREOF
  US 10,323,227; US 10,293,008; US 9,717,766; AU2015308650; EA034017; EP3185877, IDP000066793; JP6630723; KR10-2089566; MX376004, MY-174855-A; NZ748360; PH1-2017-500059; SG11201700006U; UAEP6000202/2017; VN33287; ZL201580042813.X

- *Bacillus coagulans* MTCC 5856 FOR THE MANAGEMENT OF MAJOR DEPRESSIVE DISORDER
  US 10,166,261; AU2016413572; CA3023818; EA038255; EP3478304; JP6908633; KR10-2533865; NZ748553; PH12018502419; SA2019/00501; SG11201809841Q

- PROCESS FOR THE THERAPEUTIC MANAGEMENT OF DIARRHEA PREDOMINANT IRRITABLE BOWEL SYNDROME USING *Bacillus coagulans*
  US 9,579,352; AU2016358297; CA2967952, EA037312; EP3240553; HK1240122; JP6629875; KR10-2157043; NZ732234; MX384468; MY-182658-A; PH1-2017-501328 B1; SG10-2157043; SG11201706099Q; ZL201680008035.7

- STABLE PROBIOTIC COMPOSITION FOR THE MANAGEMENT OF LACTOSE INTOLERANCE
  US 11,202,810

- ANTI-POLLUTION COMPOSITIONS CONTAINING *Bacillus coagulans*
  CA3074267; JP7262445; KR10-2376076

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- MODULATION OF IMMUNE FUNCTION BY *Bacillus coagulans*
  US 11,298,381; AU2019262918; JP7183301; KR10-2558669; SA2020/07348

- BEVERAGE COMPOSITIONS CONTAINING *Bacillus coagulans* MTCC 5856
  US 11,122,814; US10609935; JP7076148; KR10-2648066; NZ758787

- COMPOSITION FOR MANAGEMENT OF *Helicobacter pylori* INFECTION
  US 10,792,295; AU2018281141; CA3061613; EP3634444; JP6839329; KR10-2403453

- COMPOSITIONS AND METHODS FOR REDUCING FLATULENCE
  US 10,806,760; AU2018282018; JP6987888, KR10-2452792, NZ759306

- STABLE PROBIOTIC COMPOSITION CONTAINING *Bacillus coagulans* MTCC 5856 AND METHOD OF DETECTION THEREOF
  US 10,668,115

- ALCOHOLIC BEVERAGE COMPOSITION CONTAINING *Bacillus coagulans*
  US 11,332,702; AU2018337853; CA3075642; EA045649; JP7135079; KR10-2515209

- COMPOSITIONS AND METHODS FOR MANAGEMENT OF ATROPHIC GASTRITIS AND COLITIS
  US 11,413,318

- METHOD AND COMPOSITION FOR THERAPEUTIC MANAGEMENT OF GLUTEN INTOLERANCE
  US 11,419,906; JP7390398; KR10-2684843

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Sabinsa, founded in 1988, provides alternative and complementary natural products for human nutrition and well-being. Sabinsa has pioneered the introduction of more than 120 ingredients, ranging from standardized botanicals, natural cosmeceuticals, to multi-enzyme blends and production of a shelf-stable probiotic. To support these products, there are numerous privately funded clinical studies in conjunction with prestigious institutions studying these products in a very consistent manner. Sabinsa is globally positioned with 1,800 people working in manufacturing and distribution facilities, and 120 full-time scientists conducting on-going research in India and the United States. Ingredients by Sabinsa are both Kosher and Halal certified.

For more information: info@sabinsa.com

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