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Bacillus Coagulans and its Spore as Potential Probiotics in the Production of Novel Shelf- Stable Foods

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Abstract

The synbiotic foods with therapeutic activities have been beneficial to gut health and immunity development, including Bacillus coagulans as the probiotic microorganism. It is preferred over other lactic acid bacteria (LAB) as it can produce spores. It is grown in the pH range of 5.5 to 6.2 and releases spores at 37°C. These microbial spores can withstand environments with high temperatures, acidic conditions, and salinity, making it a viable probiotic organism for production of novel shelf-stable foods. It has become an essential ingredient in the functional food industry due to its probiotic characteristics and great resistance to stressful conditions. For extensive commercial use and a wide range of food applications, apart from probiotic characteristics, a probiotic organism must be cost-effective, convenient and remain viable throughout the processing, storage and consumption. The non-spore- forming lactic acid bacteria can be utilized to make probiotic products and fermented dairy products under controlled processing and storage conditions. The spore- forming probiotic organism can be delivered into the human gut through novel food products derived from cereals, legumes, fruits and vegetables, confectionery products, and meat and non-dairy products. This has led to the development of convenient and shelf-stable non-dairy probiotics. These non-dairy-based probiotics are cheaper, resilient against various processing conditions, high in bioactive

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components, and can mitigate the risk of lifestyle diseases and reduce. Further, lactose intolerance is associated with the consumption of dairy probiotics. Therefore, this review aimed to assess the utilization of probiotic Bacillus coagulans spores in emerging shelf-stable novel non-dairy products with probiotic potential.

Introduction

Novel foods enriched with probiotic organisms or their spores are the emerging concept to develop functional foods. Since current food processing technology enables the manufacture of processed foods with health benefits besides that of native food with basic nutrients, which meets the demand of present day consumers.[1] It is important to consume functional foods for a healthy diet, as well as to avoid the adverse effects of harmful eating habits.[2,3] For the sake of health and wellness and as part of a functional diet, probiotic foods have seen exponential growth in manufacturing, marketing, and consumption globally.[4] Probiotics can be an important food ingredient in many food products as a way to promote health and well-being by stimulating the immune system, improving lactose tolerance, and fighting diabetes.[5] Probiotic bacteria reduce toxicity, lower cholesterol, improve digestion, improve mineral absorption, and protect against gastroenteritis.[6] According to the United Nations' Food and Agriculture Organization (FAO) and World Health Organization (WHO), probiotics are defined as "living microorganisms, the use of which in the necessary amount has a therapeutic effect on the human body. [7]" To influence both the gastrointestinal microflora and the defense mechanism, probiotics need to be alive and delivered in sufficient numbers of colony forming units; while the compounds produced in the gastrointestinal tract, dead cells have an anti-inflammatory impact.[8] According to recommendations, a minimum of 107 CFU/g of probiotic food should be consumed daily to reap the benefits of the probiotics.[9] The Food Safety and Standards Authority of India (FSSAI) has specified that the viable number of added probiotic organisms in food shall be ≥108 Colonies Forming Units in the daily serving size advised.[10]

Probiotification of foods with suitable probiotic microorganisms should be precisely monitored during processing and storage to obtain a desirable and safe functional food.[11] Growing at 37°C, probiotics survival at undesirable gastrointestinal system conditions (e.g., digesting enzymes, pancreatic juice, and low pH) and support to host environment health by increasing microbial diversity in addition to performing therapeutic activities; some probiotics attach to mucus that surrounds gastrointestinal epithelial cells.[12] Apart from antibiotics, lysozyme, and pancreatic enzyme resistance, stomach acidity and bile resistance are also important factors.[13] Furthermore, the aggregation properties of probiotics, which allow them to survive and fight pathogens, should be investigated.[14] Although dairybased probiotic foods occupied a major space in the global market, non-dairy probiotic foods have gained popularity in recent years, as more and more people have restricted diet plans towards consumption of milk based foods (includes vegetarians, vegans, and anyone who have milk protein allergies or lactose intolerance), and a diversified non-dairy foods have been developed and demonstrated as potential probiotic carriers.[15,16]

Lactobacillus and *Bifidobacterium* are among the most widely employed genera of bacteria with potential probiotic properties, but most become inactive during manufacturing processes, storage, and other severe processing conditions associated with functional foods. Their weak resistance to stomach acid and high temperatures, however, can result in the substantial loss of viable probiotic bacteria.[6] In the current market of probiotic ingredients, this has become a detriment to the use of non-spore-forming probiotics in foods to maintain their survival and longevity. To enhance the survival of probiotics, several methods are available, including microencapsulation and cold chain management, but these outcomes are an additional expenditure to the consumer.

Why do Need Spore Forming Lab?

The use of probiotic spore-forming *Bacillus* species are becoming increasingly popular over non-spore-forming probiotics because they are more resistant to processing changes, storage, and digestive track conditions, apart from the antimicrobial activity and competition exclusion enhanced immunity, better nutritional values and growth, and protection against GIT diseases such as diarrhoea, irritable bowel syndrome (IBS), inflammatory bowel disease, ulcerative skin conditions, bacterial vaginoses and malignancies are also potential benefits of *Bacillus* species.[4,17] Spore-forming bacteria, particularly those of the genus *Bacillus*, can help various industrial producers address marketing and technological challenges.[18] The spore forming *Bacillus coagulans* belongs to the *Bacillus* genus, which releases the bacteriocin 'coagulin', which has a variety of antibacterial characteristics.[16,19] B. *coagulans* is an aerobic or facultatively anaerobic, positive to gram staining, lactic acid-producing bacterium (LAB), grown in the pH range of 5.5 to 6.2 and releases spores at 37°C. It has been designated as GRAS (Generally Regarded as Safe).[14,20] This microbe can withstand in environments with high temperatures, acidic conditions, and salinity, making it a viable candidate for usage in thermally processed products.[5,21] Moreover, B.*coagulans* can also be kept in storage without refrigeration.[22] In addition, this organism is also a certified probiotic for fermented food and beverages.[5] As spores of this bacteria germinate in the small intestine, they help digest carbohydrates in the human gastrointestinal tract (GIT).[23] As a probiotic, B.*coagulans* is more capable than most gut microbiota offermenting a wide range of plant substrates rich in cell wall components that are insoluble.[18] It has also been found to modulate innate immunity by binding and interacting with gut microbes.[24] This microbe can withstand high temperatures, and acidic, and salty environments, making it a viable candidate for usage in thermally processed products.[25] Aside from the health benefits, integrating B.*coagulans* spores has other advantages, including the generation of bacteriocins, peptides, fatty acids with a short chain, riboflavin, and hydrogen peroxide.[26] During manufacturing and storage procedures such as freezing, drying, thawing, and granulation, spore production promotes the survival of living cells. Furthermore, spores have a greater potential to survive and prolife rate in the digestive tract after passing through the stomach. As a result of their processing and storage stability, as well as their low production costs, *Bacillus* probiotics appeared to be more acceptable for use in the food sector.[27]

This review is an attempt to collate the scientific progress concerning probiotics using B. *coagulans* spores in non-dairy products.

Market Potential for *Bacillus Coagulans* Spores

Probiotics are becoming more widely recognised as a means of regulating gastrointestinal disorders and as nutritional supplements (e.g., capsules, tablets, sachets, syrups) or novel meals for health promotion. In 2020, the estimated market size for probiotics was $34.1 billion, and it is anticipated to grow at an annual rate of 8.6% to $73.9 billion by 2030.[28]

Non-Dairy Thermostable Probiotics

Because of the ease of product handling and storage, the paradigm change towards vegetarianism, and health difficulties associated with dairy products, including lactose intolerance, allergenic due to proteins in milk, and LDL levels and cholesterol management, nondairy thermally stable probiotic meals are in high demand. The fundamental issue in producing thermally stable probiotic meals, however, has been the probiotic bacteria's heat sensitivity throughout varied thermal processing and storage circumstances. To protect probiotic bacteria in foods during thermal processing with a probiotic edible coating or additions of microencapsulated probiotics [29,30] have been tested successfully. The multiple preparation stages that may obstruct their industrial applicability are the principal restrictions of the aforementioned approaches. Another option is to include thermally stable probiotic strains such as B. *coagulans* and its spores directly in the novel shelf-stable functional food products (Table 1). Owing to its characteristic spore-forming ability, B. *coagulans* and its spores can persist even after heat processing.[22] Once securely inside the small intestine, the viable spore can germinate and generate new vegetative cells, allowing it to give its numerous health advantages.

Table 1: Novel Thermostable probiotic foods

Product	**B.*coagulans* strain**	**Substrate**	**Published references**
Soy drink	B.*coagulans* VHProbi C08	Soy protein concentrate	33
Tea	B.*coagulans* GanedenBC30	Herbal Tea	45
Dried apple snack	B.*coagulans* BC410 MLD spores	Apple cubes	5
Chapati	B.*coagulans* MTCC 5856 spores	Indian flat bread (Chapati)	4
Noodles	B.*coagulans* MTCC 5856 spores	Noodles seasoning	4
Dried Strawberry halves	B.*coagulans* BC450 MLD spores	Strawberry halves	16
Orange Juice	B.*coagulans* GBI-30 6086	Orange Juice	35
Probiotic chocolate fruit snack	B.*coagulans* GBI-30,	Dark chocolate synbiotic snack with raspberry fruit content	34
Raisin juice powder	B.*coagulans*	Raisin juice powder	36
Synobiotic food	B.*coagulans* MTCC5856	Green banana resistant starch	38
Probiotic biofilm	B.*coagulans*	Cashew gum	39
Jelly candies	B.*coagulans* GBI-30 6086	Palm Juçara Passion fruit	41
Pasta	B.coaguIns GBI-30	Wheat	31
Rock candy	B.*coagulans* Unique IS-2	Nabat	17
Tea	B.*coagulans* MTCC 5856	Brewed Tea	46
Coffee	B.*coagulans* MTCC 5856	Brewed Coffee	46
Part-baked synobiotic breads	B.*coagulans* GanedenBC30	Wheat	4
Dried date paste	B.*coagulans* BC4	Dates	40

Cereal and Legumes Based Probiotic Food Products

Wheat

The ready-to-eat bakery industry is quickly expanding around the world, but to stay competitive, companies must innovate to meet consumers' need for healthier foods. Functional bread containing thermally stable probiotic strains and other bioactive compounds is an emerging marketing trend aimed at adding variety to the existing types of bread. Functional bread meets the demands of customers and particularly those who avoid dairy products that are normally enriched with probiotics.[29] Wheat flour and B.*coagulans* Ganeden BC 30 (9 Log_{10}CFU/g) were used to make a part-baked probiotic bread. The dough was partially baked at 230°C for 7 minutes, after fermentation and sufficient proofing and then the bread was cooled to ambient temperature before being packaged in plastic bags. The part-baked loaves of bread were quickly frozen at -33°C for 2 h on a plate, then immediately packed in polythene covers and kept at 20°C to assess the storage life at different (2, 14, 28, and 56) days.[30] The frozen part-baked bread was taken away from the freezer and thawed at ambient temperature for 1 hour, then baked in a preheated electrical oven at a temperature of 230°C for 8 minutes until a golden crust developed. The viability of probiotic cells in the bread prepared through a process of frozen storage part-baking and re-baking was 7.45 Log10CFU/g ($p < .05$) even after 3 days of storage at ambient temperature. The probiotic Indian flatbread (chapatti) from whole wheat flour was prepared using B. *coagulans* MTCC 5856 spores and ensured a total cell count of 1.5×10^{10} CFU/g in the chapatti dough. The spores survived during the preparation of chapati, which contained live cells of 6.92 Log_{10}CFU/g from the starting cell count of 7.78 Log_{10} CFU/g and reported 88.94 % viability in chapatti even after baking at temperature ranges from 205 °C to 230 °C. Authors also reported 94.56% viable cells of B. *coagulans* MTCC 5856 spores in commercial Noodle (noodle block) cooked with the seasoning (masala) containing Probiotic spores and cooked at a temperature of 90-95 °C for 1 min.[4] Among wheat-based processed products, pasta is extensively consumed because it is simple to produce, transport, cook, and store. Along with adults, kids in particular enjoy eating spaghetti or pasta as a breakfast meal. The complex carbohydrates present in the pasta modulate the gut microbes; hence, pasta is an excellent vehicle for the delivery of probiotic microorganisms in the gut. The probiotic pasta is prepared by using a pasta maker with freeze-dried *Bacillus coagulans* GBI-30 (108 CFU/g) and semolina of durum wheat. The extruded pasta was dried in an oven through a two-stage drying process at 50 °C for 30 minutes with intermittent tempering. The number of spore-forming cells counted did not considerably vary during the pasta-making process. B. *coagulans* GBI-30 retained its viability while making pasta (8.58 log_{10} CFU/g), upon 6 months of storage period (7.51 log_{10} CFU/g.) and after 11 minutes of cooking (6 logs CFU/g.). The findings showed that for B. *coagulans* GBI-30 count was higher than the maximum established limit for probiotic food items (6 log_{10}CFU/g) even after cooking.[31]

Soy Drink

Soybeans are a traditional Asian food with a high protein content (30–40%). Since soy protein concentrate has a distinctive taste and excellent nutritional qualities, it has been used as a component in meat, beverages, and dairy products. The microbial fermentation of soy proteins breakdown into amino acids and bioactive peptides increases the nutritional value of beverages.[32]

The researchers investigated the suitability of soy protein and fructo oligo saccharide for B. *coagulans* VH Probi C08 manufacture of a probiotic plant-based drink.[33] The sporulation rate of B. *coagulans* VHProbi C08 was 90.03 % when it was isolated from sour cabbage. The retention rate was 6.86 log CFU/mlof drink after being treated at 95 °C for 15 minutes. VH Probi C08 fermented plant-based drink offers antibacterial and antioxidant properties. The viable count of FPBD did not change significantly during 28 days of storage and the count was greater than10^8 CFU/ml. This suggested that VHProbi C08's probiotic efficiency had not changed throughout storage. VHProbi C08 exhibited various health functions, according to gene prediction. After 16 hours of fermentation, VHProbi C08 significantly improved the antibacterial and antioxidant activities of a plant-based beverage prepared from soy protein and fructooligo saccharide. The drink also contained significantly more active substances, such as Leucodopa chrome, Cytidine, Xanthine, Cotinine glucuronide, Gallic acid, 2-Hydroxycinnamic acid, and others.

Fruits and Vegetable-Based Probiotic Food Products

Probiotics have been integrated into fruit-based snacks, particularly dried fruits, via impregnation with a probiotic suspension, even simple impregnation, vacuum impregnation, osmotic dehydration-assisted impregnation, and other probiotic carrier coatings. The probiotic dried apple fruit snack contains greater cell count (8.0 log_{10} CFU/portion) even after three months of storage at room temperature when combined with *Bacillus coagulans* were reported in the literature.[5] Dehydrated probiotic strawberry halves are prepared by four different treatment processes such as impregnating probiotic B. *coagulans* BC4 50 MLD spores, then oven drying and freeze-drying; coating with probiotics, then oven drying and freeze-drying.[16] The simple impregnation procedure managed to retain a greater cell count than a coating and then impregnated with B.*coagulans* BC4 50 MLD spores, followed by freeze-drying treatment, preserved the greatest probiotic cell counts (8.0 log_{10}CFU/g) after processing and, consequently, during 6 months of storage. Dried strawberry halves from all four treatments were able to deliver probiotics to the small intestine atcounts higher than 6 log CFU/g, revealing that all four treatments might be used to make probiotic fruit snacks.

The synobiotic dark chocolate made with interior prebiotic inulin and lyophilized raspberry fruit fraction and the exterior layer of dark chocolate also contains a probiotic cell count of 108CFU/gof B. *coagulans* GBI-30 6086.[34] The synobiotic dark chocolate maintained at room temperature (20°C) was capable of releasing probiotics in concentrations of more than 5.4 X 8 log_{10} per gram.

Pasteurized orange juice (11°Brix) was used as a substrate in probiotic drinks and yogurt containing B. *coagulans* GBI-30, 6086 spores and evaluated their efficacy in healthy rats.[35] The composition of food is very important for the distribution of spores produced by probiotic bacteria. When compared to orange juice, yoghurt was an efficient carrier of B. *coagulans* GBI-30 6086, which is probably owing to the presence of other lactic bacteria and the chemical characteristics of yoghurt. Due to its health benefits and economic advantages over conventional liquid counter parts, such as reduced volume, better handling, shelf life, and lower transportation costs, the development of functional fruit juice powder has been gaining traction in the market. The survivability of *Bacillus coagulans* in raisin juice powder was tested.[36] The probiotic cell carrier's pectin (PC;0, 5, and 10% w/w) and maltodextrin (MD; 0, 10, and 20 %w/w) at different compositions were blended with raisin juice and fed to the spray dryer at inlet and outlet temperatures of 150 °C and 90 °C, respectively. After spray drying, all combinations of powders had viable cell counts of more than 4.0 log_{10} CFU/g. The spray-dried probiotic raisin powder containing 10% maltodextrin's and 5% pectin was shown to be effective as coating carriers for protecting probiotic cell count and also preserving the product qualities.

Green banana flour is high in resistant starch (RS), which works as a prebiotic component in the gut microbiome. Several clinical investigations have shown the advantageous effects of resistant starch from various food sources on colon health. [37]

A synobiotic meal containing a prebiotic green banana resistant starch and spores of B. *coagulans* MTCC5856 as a probiotic was supplemented by complimentary treatment to the dextran-sulfate sodium (DSS)-prompted colitis in mice. Supplementing with a synbiotic food containing B. *coagulans* MTCC5856 and green banana resistant starch reduced the chronic inflammatory condition of the induced inflammatory bowel disease in mice.[38] Synobiotic supplementation reduced aberrant immune responses and colonic damage while also increasing metabolite and short-chain fatty acid synthesis. This opens the door to further research into its application in the treatment of inflammation in humans with inflammatory bowel disease and paves the way for the development of shelf-stable functional foods to improve human gut health.

Several researchers are investigating various edible films and coating materials as cell wall materials for carrying probiotic microorganisms. Probiotic survival during production and storage is dependent on the basis of the protective film composition around the bacteria, as well as the bacteria's capability to withstand processing and storage conditions. B. *coagulans* encapsulated with a biopolymer mixture consisting of cashew gum and bacterial cellulose. The cashew gum derived from exudates from cashew trees was employed as a probiotic carrier matrix film.[39] The film dispersion mixture was dried for 150 minutes at 80 degrees Celsius in an oven with a vacuum pump attached and a pressure of 200 mm Hg. For at least 45 days of storage, the biopolymer film protected viable cell levels of above 7 log CFU/g. The films had no harmful effect on Caco-2 cells, according to the study. [40]

Sugar and Confectionery

Sugar and confectionery products like candy, gummies, and chewing gum are popular ready-to-eat foods, having greater acceptance among consumers. Then the confectionery products are preferable vehicles for the inclusion of probiotic microbes to formulate functional foods for people of all ages. Prepared a probiotic jelly sweet by heating a pulp of passion fruit and juçara combined with gelatin and other confection components. The jelly mixture was then cooled to 70°C and immediately added B. *coagulans* GBI-30 6086 (10^9 CFU/g) before being put into silicone moulds and oven-dried for 48 hours at 25°C. After this time, the sweets were wrapped in sugar and small quantities of citric acid and preserved in light-protected glass containers. The jelly sweets' physicochemical properties, total anthocyanin's content as well as their antioxidant capacity remained unchanged. During 90 days of storage, the B. *coagulans*cell viability was above 6.4 $\log_{10}$ CFU/g.[41] Similarly, another study reported that spores of B. *coagulans* MTCC 5856 in a concentrated glucose syrup had greater viability when held at 4 °C for 24 months but decreased by 0.85 $\log_{10}$ CFU/g when stored for 24 months at 30 °C.[42] According to these studies, adding fruit pulps and B. *coagulans* to jelly candies or other confections is a practical, creative, and promising alternative for the confectionery sector because it provides a range of functional confectionery products to all age groups for health and well -being.

For the preparation of Nabat (rock candy), the spores of B. *coagulans* Unique IS-2 were chosen as a probiotic organism that is tolerant to thermal processing and osmotic conditions.[17] According to the findings, B. *coagulans* strain Unique IS-2 had a count of around 10^9CFU/g after 6 months of production of probiotic rock candy, with only a 1-log reduction and 90.39 percent viability compared to the first inoculation. A product like rock candy containing probiotic strains would be well accepted in the consumer market for a wider variety of functional benefits to promote good health. In a further investigation, B. *coagulans* BC4 was added to an intermediate moisture food (dry date paste), and the results showed excellent stability during storage at room temperature and no discernible variations in spore viability after 45 days under aerobic and anaerobic conditions.[40]

Meat-Based Probiotic Food Products

It was investigated that the viability of spores of B. *coagulans* ATCC 31284 in thermal processed sausages with different percentages of meat using domestic cooking methods such as boiling at 95°C for 5 minutes, microwaving (2450MHz and output power of 900W w for 1 minute), and deep-fat frying at 180°C for 3 minutes. The boiling approach resulted in the maximum survivability and retention of the cells in the samples of cooked sausage.[43]

Tea and Coffee Beverages

Non alcoholic beverages containing unconventional elements such as fruit-based ingredients, amino acids, dietary fibers, vitamins, minerals and addition of probiotic organisms are known as functional beverages. By fermenting coffee brews with vegetative cells or by using *Bacillus* spores to create non-fermented formulations, probiotic coffees can be created. Because *Bacillus* spores can withstand heat during brewing, it is simpler to produce non-fermented compositions. Incorporating probiotics into coffee provides numerous potential benefits in light of current health trends. Coffee can be fermented with vegetative probiotics or left unfermented. Probiotic viability in coffee brews is harmed by heat, nutritional insufficiency, and acidity. *Bacillus* probiotics may boost the functions of coffee components and amino acids by bio-transforming them into functional bioactive components.[44] Tea is a great carrier for B. *coagulans* Ganeden BC30, a probiotic bacterium. Spores of B. *coagulans* Ganeden BC30 withstand infusion treatments very well.[45] When tea infusion temperature increased from 70 to 100°C, the probiotic cell density in tea infusion of 200 ml was 9.5 Log_{10} CFU/tea bag (2g) and 8.5 Log10 CFU/teabag (2g) respectively. At 75°C, the decimal reduction time (D value) of B.*coagulans* Ganeden BC30 was 26 minutes and 36 seconds, whereas, at 100°C, it was 2 minutes and 54 seconds. The probiotic colonised mucosal compartments immediately and remained after 4 days of washout, according to Q-PCR with specific primers. B.*coagulans* MTCC 5856 has been clinically studied for its usefulness in treating IBS and diarrhoea, as well as its role in short-chain fatty acid production, bile salt de-conjugation, cholesterol binding to cells, and assimilation of cholesterol.[30,42] The viability of B. *coagulans* MTCC 5856 spores after brewing of coffee and tea at 80°C and their subsequent growth in a hostile environment in the gastrointestinal tract (GIT) was investigated. It was discovered that the B. *coagulans* MTCC 5856 spores were remarkably stable, remaining 99% survived after brewing.[46] They also found that following tea and coffee brewing, *Lactobacillus* species were reduced by 2.0 Log_{10} CFU/serving.

Challenges in the Probiotic Industry

Selection of Probiotic *Bacillus Coagulans* Strain

The ability of probiotic bacteria to bind to the mucosa of the intestine and epithelial cells is among the most important characteristics for their selection. This adhesion stops gastrointestinal motility from carrying probiotic cells away, allowing temporary colonisation, immunity modulation, and the exclusion competitive pathogens.[47]

Toxicity

The evaluation of a strain's safety and probiotic qualities are required for human and animal uses. Heat resistance, lysozyme resistance, biofilm formation, cell aggregation properties, pH levels, bile salt concentrations, antibacterial activity, antibiotic resistance, metabolic activity, and toxicological characteristics are the main criteria for probiotic organism selection.[14,48] The majority of B. *coagulans* strains have been designated as GRAS and are now approved for use in a range of functional foods and nutritional supplements.[49,50] Based on an analysis of the whole genome, phenotypic data, and probiotic properties of B. *coagulans* 13002, no toxin-producing, virulence-factor, or active antibiotic resistance genes were discovered.[49] Its safety was further, validated by an acute oral toxicity, antibiotic resistance assays and hemolytic assay. The presence of adhesion-related genes, stress-responsive genes and antimicrobial gene clusters in the genome revealed probiotic features including severe temperature, bile salts, acid, alkaline, and oxidative stressors.

Potato, pickle, maize, and tomato were the sources of four isolated and recognised B.*coagulans* strains using taxonomy assays that included spore-forming rods, positive to gram staining, acid production, catalase and consumption of starch.[14] Furthermore, *in vitro* probiotic activities, heat resistance and characteristics of toxic effects of the strains were investigated and compared to the commercial probiotic strain B. *coagulans* GBI-30, 6086. These organisms have demonstrated probiotic features such as thermal stability, withstand acidic pH, lysozyme, antibiotic and bile resistance, production of biofilm and exhibited antibacterial properties. All four strains displayed adhesion, auto-, and coaggregation characteristics, however the degree of each varied with incubation time. These strains are safe because no enterotoxin genes (bceT, ces, entFM, and cysts) or enterotoxins (Hbl, and the) were discovered.

Human irritable bowel syndrome (IBS) can be effectively treated by supplementing B. *coagulans* LBSC, which is a phenotypically and genetically safe probiotic organism.[51] B. *coagulans* R11 protects intestinal villi from lead damage. 52 Its safety in vivo was tested in a study, and a dose of 9.52 X1011 cell count was found to be safe and well-tolerated for an adult weighing 70 kg.[53] Furthermore, B. *coagulans* genome investigation also revealed that no additional genes with potential safety risks were found, and the antibiotic resistance genes in this species were not easily transferable to other bacteria.[19] Several studies have reported the *in vitro* fermentation properties of B. *coagulans* strains, including B. *coagulans* MTCC 5855, B. *coagulans* PTA- 6086, and B. *coagulans* 15B are safe to use in the food industry.[18] Nonpathogenic B. *coagulans* has been reported, and it is found on the European Union Food Safety Authority's (EFSA) Qualified Presumption of Safety (QPS)list and four B. *coagulans* strains have been designated as Generally Recognized as Safe by the US Food and Drug Administration (http://www.fda.gov/).[54] B. *coagulans* is included in the Korean Ministry of Food and Drug Safety's list of food materials. B. *coagulans* can be used in the food sector, notably in functional meals, because of safety features. Recent research has found that B. *coagulans* modulates the microbial flora, host immunity, and physiological metabolism to have an impact on intestinal diseases including chronic diarrhoea, antibiotic-related diarrhoea, irritable bowel syndrome, constipation, and gastroenteritis. Further, more toxicological studies and a significant number of investigations have shown that *Bacillus coagulans* is non-mutagenic, non-teratogenic, and non-genotoxic.[55]

Probiotic Stability

Sporulation is a mechanism that allows a strain to live in an environment with low nutrition levels. The spore coat is consists of the hydrophobic spore core, mineral ions, and spore proteins.[56] Thus, the relationship between resistance and spore DNA saturation with a/b-type small acid-soluble spore proteins that guard DNA against moist heat damage.[4] For spore-probiotics to have therapeutic effects on the host, they must survive and germinate in the upper gastrointestinal tract (GIT). The survival and germination of spores under fed and fasted GIT conditions were investigated using flow cytometry and the agar plate method in an *in vitro* simulator of the Human Intestinal Microbial Ecosystem (SHIME®) model. Lactate and short-chain fatty acids (SCFA) were used as metabolic activity indicators. The highest spore germination was observed under fasted situations as compared to fed state in an in -vitro simulated model of the gastrointestinal tract .Underfed and fasted upper GIT circumstances, *Bacillus coagulans* Unique IS2 showed 102 % and 99 % urvival, respectively. In the small intestine a high degree of spore germination takes place owing to the spores having not yet fully developed into vegetative cells but helping with lactose and fructose digestion.[57]

As previously stated, the upper portion of the small intestine is where B. *coagulans* spores can germinate. The usual duration between oral delivery to germination is approximately 4-6 hours, and around 85% of the spores make it to the gut. *Bacillus coagulans* has been found to boost nutrient absorption in the gut and aids in the digestion process with gut microflora.[58] B. *coagulans* can facilitates the effective utilisation of eaten meals, owing to their ability to create enzymes such as galactosidase, -β-galactosidase, -amylase, lipase, and alkaline proteases.[25] B. *coagulans*, on the other hand, cannot colonise the intestine and can only live there for a short time. B. *coagulans* is eliminated in stools for around 7 days after a single oral treatment. As a result, to accomplish the long-term treatment, B. *coagulans* spores must be taken orally every day.[55] *Bacillus* strain spores can remain latent for years before germinating if given the right stimuli.[59] B. *coagulans*, like other *Bacillus* strains, can be prompted to germinate by a variety of stimuli, including nutrition, non-nutrient substances, and physical treatments.[25]

Sensory Criteria

The presence of probiotic bacteria in the generated items did not affect the texture, appearance, or organoleptic qualities, according to the finding s. Regardless of the health benefits of probiotics, a few LAB strains may alter the sensory characteristics of foods, particularly texture, which appears to be mostly connected to lactic acid bacteria's production of exopoly saccharides. As natural thickeners, emulsifiers, stabilisers, binders, gelling agents, coagulants, and suspending agents, polysaccharides obtained from food-grade LAB are well-known non-toxic, biodegradable, and environmentally acceptable ingredients in the food and cosmetics industries. EPS from LAB bacteria can be isolated and utilised for starchy foods to prevent syneresis and enhance the look and feel of starch (e.g., bread, pasta, noodles, soup, salad dressings, jellies, puddings, white sauce, and ketchup).[1]

Conclusion and Future Prospects

Consumer food habits have been changed due to their growing interest in healthier and functional foods. The formulation of probiotic food items is critical for acceptance of consumers, food industry, and research institutions concerned in the product development and promoting the therapeutically functional advantages of probiotic microorganisms on host health. Every probiotic strain that must be sufficiently characterized; i) safe for the intended use ii) supported by at least one successful human clinical trial carried out in accordance with generally accepted scientific standards; and, where applicable, in accordance with recommendations and regulations made by local/national authorities; and (iii) alive in the product at an effective dose for the duration of the shelf life under prescribed storage conditions. Probiotic B. *coagulans* releases spores and is thermo stable which may provide an advantage for preparation of novel heat processed probiotic meals. B. *coagulans* is a popular probiotic organism, because of their tolerance to acidic gastric juices and stability against high temperatures. It is more antibiotic-resistant than the other LAB genera. Furthermore, the novel shelf-stable probiotic foods containing B. *coagulans* in the food and beverage sector are becoming more popular due to their low cost and potential as substitutes for other chemical sources.

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Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

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