

Food Storage, Processing and Genetic Stability Studies of *Bacillus* (*Heyndrickxia*) *coagulans* BCP92 (MTCC 25460)

Sohel S. Shaikh^{1*}, Chinmayi Joshi², Farhana Malek¹, Anis Malik¹, Manoj Gandhi¹

¹Pellucid Lifesciences Pvt Ltd, Plot No. 3538, Phase-4, GIDC Industrial Estate, Chhatral-382729 Dist.-Gandhinagar, Gujarat, India

²Smt. S. S. Patel Nootan Science and Commerce College, Sankalchand Patel University, Visnagar-384315, India

Abstract

Background and Objective: *Bacillus coagulans* are spore-forming probiotics that provide health benefits when consumed in adequate amounts. Therefore, they can be added to functional foods to enhance their nutritional values. The aim of the present study was to investigate stability of *Bacillus coagulans* BCP92 in various functional foods during food processing and storage conditions as well as genetic stability study of the strain using DNA fingerprinting method.

Material and Methods: *Bacillus coagulans* BCP92 was incorporated into a range of functional foods and beverages such as instant coffee, tea, sweet corn soups, oatmeal, upma, gummies, brownies, ice creams, non-alcoholic beverages, chocolates, peanut butter and shrikhand. Viability of the bacteria was assessed using pour plate method under various processing and storage conditions. Genetic stability of *B. coagulans* was assessed using DNA fingerprinting.

Results and Conclusion: The viability was shown in food processing conditions of teas (99.97%), coffees (99.45%), sweet corn soups (99.36%), oatmeal (98.81%), upma (99.57%), gummies (99.67%) and brownies (98.14%). In food-storage conditions, relative viability was as follows: fruit juices (98.91%), lassi (98.72%), energy drinks (98.70%), cold coffees (99.29%), milk chocolates (99.87%), white chocolates (100.13%), dark chocolates (99.20%), shrikhand (99.04%), ice creams (99.45%), and peanut butters (98.32%). Furthermore, DNA fingerprinting showed genetic stability of the probiotic *B. coagulans* BCP92. In conclusion, *B. coagulans* BCP92 has shown good viability in various food processing and storage conditions. Moreover, it is genetically stable, thus making it a good candidate for addition to functional foods.

Conflict of interest: The authors declare no conflict of interest.

How to cite this article

Shaikh SS, Joshi C, Malek F, Malik A, Gandhi M. Food Storage, Processing and Genetic Stability Studies of *Bacillus* (*Heyndrickxia*) *coagulans* BCP92 MTCC 25460. *Appl Food Biotechnol*. 2024; 11 (1): e22. <http://dx.doi.org/10.22037/afb.v11i1.44919>

Article Information

Article history:

- Received 20 Mar 2024
- Revised 6 May 2024
- Accepted 3 Jun 2024

Keywords:

- *Bacillus coagulans*
- Food matrices
- Functional foods
- Genetic stability
- Probiotics stability

Corresponding author:

Sohel S. Shaikh

Pellucid Lifesciences Pvt Ltd,
Plot No. 3538, Phase-4, GIDC
Industrial Estate, Chhatral-
382729 Dist.-Gandhinagar,
Gujarat, India

Tel: +91 98229 79295

E-mail:

sohel@pellucidlifesciences.com
sohels7392@gmail.com

1. Introduction

The potential health benefits of probiotics, which involve improving gut microflora, have been a topic of scientific interests for many years. However, it has only recently begun to receive scientific assessments [1]. Probiotics are living microorganisms that confer health benefits to the host when consumed in sufficient quantities [2]. Several studies have revealed that consumption of probiotics decreases risks of antibiotic-associated diarrhoea [3], symptoms of irritable bowel syndrome (IBS) [4], risks of lactose intolerance [5] and constipation [6] and risks of carcinogens and helps in decreases of obesity and

enhancement of immune responses and decreases of cholesterol levels [7]. To confer the specific health benefits of incorporated probiotics in food products, the recommended adequate levels of probiotics (10^6 – 10^7 CFU.ml⁻¹) should be provided in the final products [8]. Preserving viability of the probiotic cultures in foods until the end of shelf life is an important criterion for providing effective probiotic food products [9]. It has been observed that the viability of most probiotic bacteria is lost during processing and storage conditions. Furthermore, only a limited number of bacteria can survive harsh conditions of

the gastrointestinal tract (GIT) [10]. An effective way to deliver probiotic bacteria includes incorporation of them into food products, making it easier for the consumers to maintain their gut health, considering that many people choose probiotic food products instead of probiotic capsules and pills [11].

Awareness of the importance of maintaining gut health has led to great increases in demands for probiotic foods. Probiotics are either used as starter cultures in combination with traditional starters or alone and incorporated into dairy products, where many functional characterisations are improved by the addition of probiotics. However, there are several challenges linked to function and stability of the probiotics in dairy products [12]. It is generally accepted that probiotic products should include a minimum concentration of 10^6 – 10^7 CFU.g⁻¹ or CFU.ml⁻¹ and a total concentration should be 10^8 – 10^9 CFU.g⁻¹ consumed daily to exert the probiotic effects [13,14]. Numerous probiotic foods include dairy products such as ice creams, fermented milks, frozen desserts, yoghurts, cheeses, milk powders and cheesecakes, [12,15,16], as well as non-dairy products such as oat drinks, commercial fruit juices, soya milks [17–19]. However, spore-based *Bacillus*-based probiotics have shown higher survival rates than those others have. In a study by Hashemi et al. [20], it was observed that survival rates of the samples with *B. coagulans* were higher than those with *Lactobacillus acidophilus*. Similarly, Soares et al. detected that the *Bacillus* strains, which included probiotic characteristics, showed a greater viability than that the probiotic strains of *Bifidobacterium* and *Lactobacillus* did [21]. Stability of probiotics is always a concern during the storage and processing conditions. The present study focused on the stability assessment of *Bacillus coagulans* BCP92 under various food processing and storage conditions to assess its potential as a probiotic additive for enhancing the nutritional values of food products. Furthermore, assessment of genetic stability of the strain was carried out using DNA fingerprinting method.

2. Materials and Methods

2.1 Microbial culture

Bacterial spores of *Bacillus coagulans* BCP92 (MTCC 25460) used in this study were produced at Pellucid Lifesciences, India. Concentration of the prepared *B. coagulans* spores was 150 billion CFU.g⁻¹ ($11.146 \log$ CFU.g⁻¹). Standard pour plate technique was used to assess the total viable bacterial count. The *B. coagulans* spores were thoroughly mixed in the food product and incubated at 75 °C for 30 min using water bath, followed by rapid cooling down to below 45 °C. Suspension was serially diluted in sterile peptone water. An appropriate quantity of diluents was poured into a sterile petri plate and mixed with

molten glucose yeast extract BC agar (Hi media M2102, India) in triplicate. Plates were incubated at 40 °C for 48–72 h. The mean of these viable counts was expressed as log₁₀ CFU.g⁻¹. All the Chemicals and reagents were purchased from Merck, Germany, and the microbiological media were purchased from Hi Media, India.

2.2 Assessment of the stability of *Bacillus coagulans* BCP92 under food processing conditions

The *B. coagulans* BCP92 was added to a variety of food products and beverages such as instant coffees, teas, sweet corn soup powders, oatmeal, upma, cornflakes, gummies and brownies to assess its stability under certain processing conditions. The *B. coagulans* BCP92 stability under food processing conditions was studied by comparing the initial count with that after the processing conditions.

2.2.1 Instant coffees and teas

Instant coffee (7.5 g) and tea (1.9 g) powders were mixed with 14 mg each of *B. coagulans* powder. The prepared mixtures were dissolved in 100 ml of hot water (80–85 °C). Samples from tea and coffee were collected at 0 and 30 min and viable counts of *B. coagulans* BCP92 were analysed using standard pour plate method.

2.2.2 Sweet-corn soup powders and oatmeal

Briefly, *B. coagulans* BCP92 (14 mg) was mixed uniformly with sweet-corn soup powder (10 g.serving⁻¹) and oatmeal (100 g.serving⁻¹) separately. Mixture was cooked in 150 ml hot water (80–85 °C). Cooked samples were collected at 0 and 30 min to analyse the viable cell count of *B. coagulans* BCP92 using pour plate method.

2.2.3 Upma and cornflakes

Ready-to-eat breakfast upma and cornflakes were purchased from a local market. Upma (50 g.serving⁻¹) was added into warm water (80–85 °C) and cooked for 2 min. Then, 14 mg of *B. coagulans* BCP92 were added to the mixture, stirred well and cooked for 2 min. Pour plate method was used to assess viable count of *B. coagulans* BCP92 in samples of 0 and 30 min. One serve of 100-g cornflakes was mixed with 150 ml of hot milk and cooked for 5 min. Then, 14 mg *B. coagulans* BCP92 were added to the mixture and set for 5 min. Samples were collected at 0 min and 30 min to assess the viable count of *B. coagulans* BCP92 using pour plate method.

2.2.4 Gummies

All the requirements for gummies such as sugar, flavour, sodium citrate, citric acid, corn syrup, water and pectin were mixed for a batch of gummies for 1 kg material to investigate the bacterial survival under processing conditions. All the ingredients of gummies were mixed and heated to dissolve all the contents. Once all the ingredients dissolved, they were mixed with *B. coagulans* BCP92 (14 mg.3 g⁻¹) at not greater than 85–90 °C in the final mixture before it solidifies. Viable count of *B. coagulans* BCP92



was measured in gummies and the molten sample once mixed using pour plate method.

2.2.5 Brownies

All the necessary ingredients for preparing brownies such as flour, salt, cocoa powder, eggs, brown sugar, vanilla essence, vegetable oil and butter were mixed thoroughly to prepare the batter, then *B. coagulans* BCP92 (14 mg·30 g·serving⁻¹) was added to the batter and baked at 175 °C for 22–25 min. Viable count of *B. coagulans* BCP92 was calculated in the brownies before and after baking using pour plate method.

2.3 Storage stability of *Bacillus coagulans* BCP92 in various food matrices

Stability of *B. coagulans* BCP92 was assessed under standard food storage conditions for ice creams (-20 °C), peanut butters (22–25 °C), non-alcoholic beverages, chocolates and shrikhand (4 °C) based on the ICH Guideline Q1A(R2) [22].

2.3.1 Ice creams

A batch of ice cream was homogenously mixed with *B. coagulans* BCP92 (14 mg.100 ml serving⁻¹) and 100 ml of ice cream were dispensed in a sterile ice cream cup. The ice cream was stored at -20 °C for 6 m. The primary bacterial count of the *B. coagulans* BCP92 was carried out immediately after mixing of *B. coagulans* BCP92. Samples were collected monthly for enumeration up to 6 m of storage. Pour-plate technique in triplicates was used to carry out the bacterial count.

2.3.2 Non-alcoholic beverages

Four various types of commercial beverages of fruit juices, energy drinks, lassi and cold coffees were purchased from a local market. The *B. coagulans* BCP92 (14 mg.serving⁻¹) was inoculated into four sterile flasks containing fruit juices (100 ml), energy drinks (250 ml), cold coffees (200 ml) and lassi (180 ml). All flasks were sealed and stored at 4 °C. Viability of *B. coagulans* BCP92 in all beverages was assessed using pour plate method. Stored samples were collected for analysis on Days 0, 30, 60, 90, 120 and 180. All analyses were carried out with three replicates.

2.3.3 Chocolates

Three types of chocolates were purchased from a local market, including white, dark and milk chocolates. Chocolate bars were melted by heating at 45–50 °C. Then, *B. coagulans* BCP92 (14 mg.100 ml serving⁻¹) powder was thoroughly mixed with the molten chocolates. The mixture of chocolate and probiotics was stored at 4 °C. Viability of *B. coagulans* BCP92 in chocolates was assessed using pour plate method. Stored samples were collected for analysis on Days 0, 30, 60, 90, 120 and 180. All analyses were carried out with three replicates.

2.3.4 Peanut butter

Peanut butter was purchased from the local market. Samples (32 g·serving⁻¹) were inoculated with *B. coagulans* BCP92 (14 mg·serving⁻¹). Peanut butter and probiotic powder were mixed uniformly and stored at room temperature (RT). Then, *B. coagulans* BCP92 viability was assessed after 0, 30, 60, 90, 120 and 180 days of storage.

2.3.5 Shrikhand

Shrikhand was purchased from a local market, added into probiotic *B. coagulans* BCP92 (14 mg.100 g.serving⁻¹) and stored at 4 °C. Stored samples were collected for analysis on Days 0, 30, 60, 90, 120 and 180. All analyses were carried out with three replicates.

2.4 Genetic stability of *Bacillus coagulans* BCP92

For the comparisons from two various stages of the production process, primary cultures in the form of VIAL and final product batch samples were used. Generally, DNA was isolated from each sample. Quality of the DNA was assessed on 1.0% agarose gel and a single band of high-molecular weight DNA was observed. Moreover, DNA fingerprinting of the cultures was carried out using rep-PCR method and MSP-PCR fingerprinting. Two types of rep-PCR fingerprinting were applied [23] using BOX (50-CTACGGCAAGGCGACGCTGACG-30) and (GTG)5 primers (5-GTGGTGGTGGTGGTG-3) [24]. Briefly, 20 ul of PCR amplicons were separated on 2% agarose gel and banding patterns were analyzed using Gel-analyzer software. The dendrogram was plotted using unweighted pair-group method and arithmetic averages with correlation levels expressed as proportions of the Pearson correlation coefficient.

2.5 Statistical analysis

Viable count of *B. coagulans* BCP92 was expressed as log₁₀ CFU.serving⁻¹ in food processing conditions and log₁₀ CFU g⁻¹.ml⁻¹ in food storage conditions. All analyses were carried out with three replicates. Results included averages of the three independent determinations. Differences between the two values were calculated using student's t-test. Level of the significance for all statistical tests was $p < 0.05$.

3. Results and Discussion

3.1 Stability of *Bacillus coagulans* BCP92 in food processing conditions

Stability of *B. coagulans* BCP92 was studied by measuring viability of the bacteria in various food matrices under certain processing conditions (Figure 1). The primary viable count of *B. coagulans* BCP92 in tea was 9.31 ±0.02 log₁₀ CFU serving⁻¹ and in coffee was 9.38 ±0.02 log₁₀ CFU serving⁻¹. After 30 min, these values were 9.30 ±0.05 and 9.33 ±0.02 log₁₀ CFU serving⁻¹, respectively. The *B. coagulans* preserved 99.97% of its viability in tea. In instant



coffee, viability of *B. coagulans* after processing was preserved up to 99.45%. Viability of *B. coagulans* BCP92 was assessed in sweet corn soup and oatmeal during processing. The *B. coagulans* was incorporated into corn soup powder and oatmeal by adding hot water. In soup preparation, the primary *B. coagulans* of $9.38 \pm 0.03 \log_{10}$ CFU serving⁻¹ preserved a 99.36% viable count after 30 min. The oatmeal primary concentration was $9.25 \pm 0.03 \log_{10}$ CFU serving⁻¹ and after 30 min, it preserved 98.81% viability (Figure 1).

Upma primary concentration was $9.20 \pm 0.05 \log_{10}$ CFU serving⁻¹ and after 30 min, it preserved 99.57% viability. The viability studies on cornflakes showed a primary count of $9.22 \pm 0.02 \log_{10}$ CFU.serving⁻¹, and after 30 min, it preserved 99.78% of viability (Figure 1). Viability was studied in gummies as well. For gummies before and after processing, they showed 99.67% of relative viability per gummy. The viable count of *B. coagulans* BCP92 spores in gummy processing conditions showed slight non-significant decreases in count (Figure 1). Viability studies in brownies showed that the primary concentration of probiotics in each brownie was $9.22 \log_{10}$ CFU serving⁻¹. After heating, this showed a 98.14% relative survival rate (Figure 1). Studies have shown use of probiotics in functional foods. Polo et al. [25] reported use of *B. coagulans* in herbal teas. Majeed et al. [26] reported viability of *B. coagulans* up to 2 y of shelf life when stored with tea and coffee powders. Kahraman et al. [27] and Miranda et al. [28] studied *B. coagulans* stability in gummies and showed *B. coagulans* survivals during production and processing. Majeed et al. [29] reported stability of *B. coagulans* in various food matrices such as hot fudge toppings, chocolate fudges (97.23%) and peanut butters (99.6%) with viability over 95% as well as its viability in apple juices (99.3%) of baked products. Almada

et al. [30] showed that eight strains of *Bacillus* in various baking, cooking and drying processes affected γ of the *Bacillus* strains; of which, *B. coagulans* reported higher resistance. Foods containing spore probiotics are becoming popular due to their resistance to heat processes, low water activity, acidic pH and heat stability [31]. In this study, *B. coagulans* BCP92 showed high viability in various foods during food processing conditions, with viabilities ranging 98.14–99.97% in food products such as teas, coffees, sweet corn soups, oatmeal, upma, gummies and brownies.

3.2 Storage stability of *Bacillus coagulans* BCP92 in various food storage conditions

Incorporation of *B. coagulans* BCP92 into a food product was studied to assess viability and stability of *B. coagulans* BCP92 during storage and its possible use as a food ingredient (Table 1). The relative viability of *B. coagulans* in ice creams was 99.41%, The primary viable count of *B. coagulans* in ice creams was $7.32 \pm 0.04 \log_{10}$ CFU ml⁻¹ and the final count was $7.28 \pm 0.06 \log_{10}$ CFU ml⁻¹ over 6 m of storage (Table 1). Studies show use of ice creams as vehicles for probiotics. Due to exposure of the cells to various stress factors associated with formulation, overrun, melting and storage, losses in viability occur [9]. Fruit juices, energy drinks and cold coffees showed primary counts of 7.34 ± 0.01 , 6.92 ± 0.01 and $7.00 \pm 0.03 \log_{10}$ CFU ml⁻¹, respectively. After storage up to 6 m, the preserved viability rates were up to 98.87, 98.74 and 99.23%, respectively (Table 1). Viability of *B. coagulans* was assessed in various types of chocolates. Results revealed that *B. coagulans* BCP92 preserved its high viability throughout the entire 180-d storage time. Viability of *B. coagulans* in milk, white and dark chocolates were 99.99, 100 and 99.16%, respectively (Table 1).

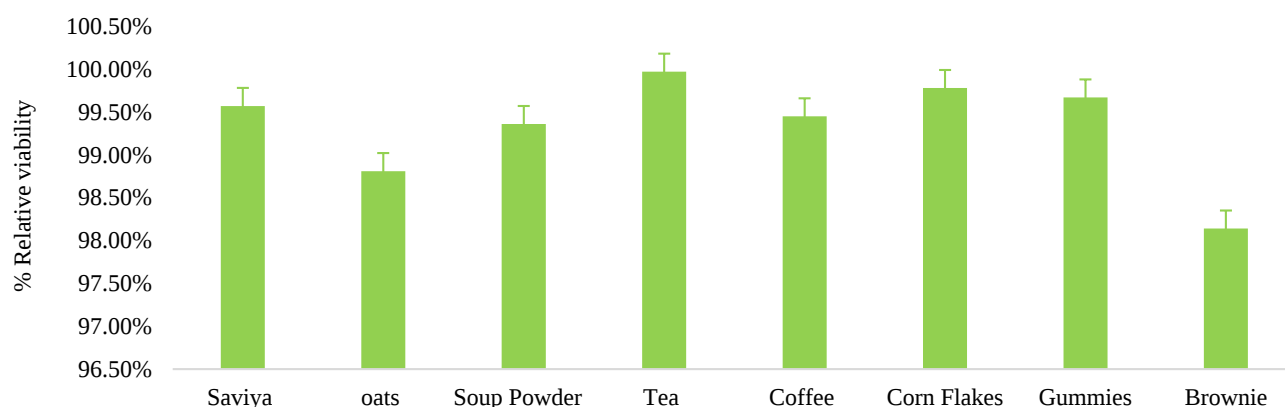


Figure 1. *Bacillus coagulans* BCP92 stability under food processing conditions



Table 1. Stability of *Bacillus coagulans* BCP92 under food storage conditions up to 180 days

	O day	30 Days	60 Days	90 days	120 days	150 Days	180 Days	Survival rate (%)
Fruit Juice	7.34±0.01 ^a	7.28±0.07 ^a	7.26±0.01 ^a	7.12±0.02 ^a	7.06±0.01 ^a	7.12±0.04 ^a	7.26±0.01 ^a	98.87%
Lassi	7.03±0.02 ^b	7.00±0.02 ^b	6.93±0.02 ^b	6.95±0.03 ^b	6.94±0.03 ^b	6.95±0.02 ^b	6.94±0.03 ^b	98.80%
Energy Drink	6.92±0.01 ^c	6.91±0.02 ^c	6.91±0.04 ^c	6.90±0.01 ^c	6.88±0.01 ^c	6.85±0.03 ^c	6.83±0.05 ^c	98.74%
Cold Coffee	7.00±0.03 ^d	7.01±0.03 ^d	6.97±0.03 ^d	6.97±0.01 ^d	6.96±0.01 ^d	6.95±0.06 ^d	6.95±0.01 ^d	99.23%
Chocolate (Milk)	7.59±0.07 ^e	7.57±0.07 ^e	7.58±0.05 ^e	7.56±0.03 ^e	7.58±0.03 ^e	7.56±0.04 ^e	7.58±0.03 ^e	99.99%
Chocolate (White)	7.51±0.02 ^f	7.56±0.03 ^f	7.54±0.05 ^f	7.55±0.04 ^f	7.55±0.04 ^f	7.52±0.05 ^f	7.52±0.04 ^f	100%
Chocolate (Dark)	7.54±0.02 ^g	7.56±0.04 ^g	7.49±0.03 ^g	7.54±0.06 ^g	7.55±0.06 ^g	7.46±0.03 ^g	7.48±0.07 ^g	99.16%
Shrikhand	7.32±0.08 ^h	7.31±0.08 ^h	7.32±0.02 ^h	7.32±0.04 ^h	7.27±0.04 ^h	7.26±0.02 ^h	7.25±0.04 ^h	99.08%
Ice Cream	7.32±0.04 ⁱ	7.32±0.04 ⁱ	7.34±0.01 ⁱ	7.31±0.06 ⁱ	7.33±0.06 ⁱ	7.29±0.08 ⁱ	7.28±0.06 ⁱ	99.41%
Peanut Butter	7.76±0.02 ^j	7.68±0.03 ^j	7.64±0.02 ^j	7.63±0.01 ^j	7.63±0.03 ^j	7.63±0.03 ^j	7.63±0.05 ^j	98.40%

Values are presented as mean ± standard deviation. Means in the row with the same superscripts are not significantly different ($p < 0.05$).

Stability studies in shrikhand and lassi showed consistency of *B. coagulans* BCP92 as the primary concentration of *B. coagulans* was 7.32 ± 0.08 and $7.03 \pm 0.02 \log_{10}$ CFU ml⁻¹ and after the study, it preserved its 99.08 and 98.80% relative viabilities, respectively (Table 1). Stability studies in peanut butters demonstrated a good viability of 98.40% from primary $7.76 \pm 0.02 \log_{10}$ CFU ml⁻¹ per serving after 6 m of storage (Table 1).

Various studies report stability of non-spore-forming probiotics, showing survival of probiotics during storage [32,33]. However, probiotic *Bacillus* strain showed a higher survival rate [20, 21] than *Lactobacillus* and *Bifidobacterium* during storage under GIT conditions when studied in cheeses, pasteurized orange juices and breads [21]. A study with *L. acidophilus* and *B. coagulans* in ice creams stored at -18 °C for 90 d showed a higher survival rate in *B. coagulans* than *L. acidophilus* [20]. Marcial-Coba et al. [33] microencapsulated *Akkermansia muciniphila* and *L. casei* in dark chocolates. In another study, Cielecka-Piontek et al. [34] demonstrated stability of *B. animalis* subsp. *Lactis*, *Saccharomyces boulardii* and *B. coagulans* GBI-30, 6086 in chocolates. Similar results were reported by Silva et al. [35] in *L. acidophilus* LA3 and *B. animalis* subsp. *lactis* BLC1, showing the highest viabilities of approximately 7.7 and 7.3 log CFU.g⁻¹ in semisweet chocolates, respectively. Lavrentev et al. [36] showed use of *B. coagulans* as a starter culture and its viability for 60 d and reported satisfactory results for stability and quality characteristics of the product. Maity et al. reported the *B. coagulans* stability in various food matrices under processing conditions, including lemon iced teas (99.46%), green teas (98.48%), masala teas (98.96%), lemon teas (99.59%), instant coffees (99.79%), upma (99.89%), corn soups (99.79%) and noodles (99.68%) [37]. The *B. coagulans* BCP92 preserved its stability over 6 m in foods such as fruit juices, lassi, energy drinks, cold coffees,

chocolates, shrikhand, ice creams and peanut butters with relative viabilities ranging 98.32–100.13%. In the present study, non-significant decreases were observed in all the food matrices under food processing and storage conditions, showing the versatile nature of *B. coagulans* BCP92.

3.3 Genetic stability

Samples of “VIAL” (primary sample) and “final product” were provided for the study. Generally, DNA was extracted and DNA fingerprinting was carried out using BOX primers sets, followed by PCR analysis and dendrogram plotting. Genotype of each strain could be differentiated by the distribution of PCR bands and the two samples were closely linked to each other based on DNA fingerprinting patterns in the experiments (Figures 2 and 3). Genomic safety and probiotics attributes showed that *B. coagulans* BCP92 was safe [38]. Genomic fingerprinting also showed genetic stability of *B. coagulans* BCP92 in the production cycle; in which, it showed the genetic stability in primary and final samples of production. Majeed et al. reported genetic stability of three various sample batches [24]. Genetic stability studies using DNA fingerprinting verified stability of *B. coagulans* BCP92.

4. Conclusion

The present study reported stability of *B. coagulans* BCP92 in various food matrices under processing and storage conditions. The *B. coagulans* BCP92 tolerated the low pH of juices, low-temperature storage and heating during food processing conditions. These findings focused on the potential of these food products as carrier vehicles for the delivery and stability of spore-forming probiotics. The *B. coagulans* BCP92 was also genetically stable in the production process, which was a positive indication of genetic stability of culture. Hence, this finding suggests use of spore-forming probiotic *B. coagulans* BCP92 in



functional foods for gut health improvement and gastrointestinal disorders. Further studies can be carried out on incorporating *B. coagulans* BCP92 in food products and assessing them on human subjects to gauge their effects on human health. Additional studies on food supplemented with *B. coagulans* BCP92 can help deeper understanding of its potential benefits for human health and sensory profiling, ultimately leading to advancements in the field of nutrition and wellness.

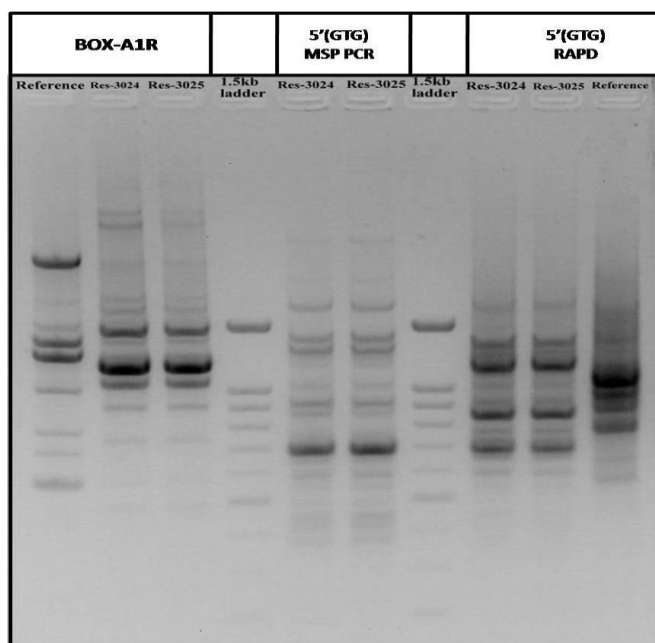


Figure 2. The DNA fingerprinting patterns of primary and final products. Res-3024, VIAL; and Res-3025, final product

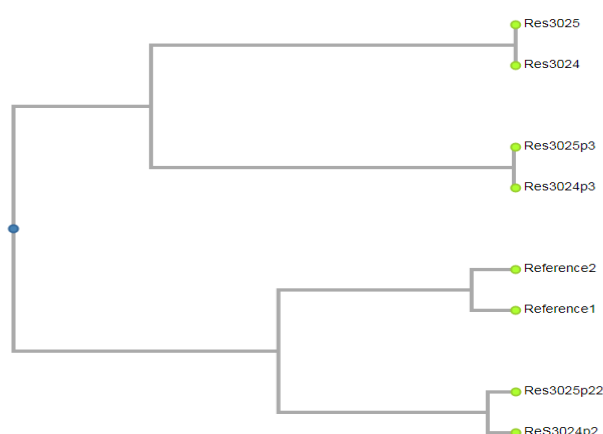


Figure 3. Cluster analysis of digitized banding patterns of the samples generated by PCR using BOXA1R and (GTG)5 primers. Dendrogram was constructed using unweighted pair-group method and arithmetic averages with correlation levels expressed as proportion values of the Pearson correlation coefficient. Arrows show bands shared by a majority of the isolates

5. Conflict of Interest

The authors report no conflict of interest.

References

- Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M. Probiotics: Properties, examples and specific applications. Cold Spring Harb perspect Med. 2013; 3: a010074. <https://doi.org/10.1101/cshperspect.a010074>
- Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. October 2001. <https://www.igb.es/digestivo/pdfs/probioticos.pdf> [Accessed 06 May 2024].
- Mekonnen SA, Merenstein D, Fraser CM, Marco ML. Molecular mechanisms of probiotic prevention of antibiotic-associated diarrhea. Curr Opin Biotechnol. 2020; 61: 226-234. <https://doi.org/10.1016/j.copbio.2020.01.005>
- Le Morvan de Sequeira C, Kaeber M, Cekin SE, Cekin SE, Enck P, Mack I. The effect of probiotics on quality of life, depression and anxiety in patients with irritable bowel syndrome: A systematic review and meta-analysis. J Clin Med. 2021; 10: 3497. <https://doi.org/10.3390/jcm10163497>
- Ahn SI, Kim MS, Park DG, Han BK, Kim YJ. Effects of probiotics administration on lactose intolerance in adulthood: A meta-analysis. J Dairy Sci. 2023; 106(7): 4489-4501. <https://doi.org/10.3168/jds.2022-22762>
- Venkataraman R, Shenoy R, Ahire JJ, Neelamraju J, Madempudi RS. Effect of *Bacillus coagulans* unique IS2 with lactulose on functional constipation in adults: a double-blind placebo-controlled study. Probiotics Antimicrob Proteins. 2023; 15(2): 379-386. <https://doi.org/10.1007/s12602-021-09855-8>
- Cao J, Yu Z, Liu W, Zhao J, Zhang H, Zhai Q, Chen W. Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases. J Funct Foods. 2020; 64: 103643. <https://doi.org/10.1016/j.jff.2019.103643>
- Palanivelu J, Thanigaivel S, Vickram S, Dey N, Mihaylova D, Desseva I. Probiotics in functional foods: Survival assessment and approaches for improved viability. Appl Sci. 2022; 12(1): 455. <https://doi.org/10.3390/app12010455>
- Mohammadi R, Mortazavian AM, Khosrokhavar R, da Cruz AG. Probiotic ice cream: viability of probiotic bacteria and sensory properties. Ann Microbiol. 2011; 61: 411-424. <https://doi.org/10.1007/s13213-010-0188-z>
- Han S, Lu Y, Xie J, Fei Y, Zheng G, Wang Z, Liu J, Lv L, Ling Z, Berglund B, Yao M. Probiotic gastrointestinal transit and colonization after oral administration: A long journey. Front Cell Infect Microbiol. 2021; 11: 609722. <https://doi.org/10.3389/fcimb.2021.609722>
- Rodgers S. Incorporation of probiotic cultures in foodservice products: An exploratory study. J Foodserv. 2007; 18: 108-118. <https://doi.org/10.1111/j.1745-4506.2007.00055.x>



12. Gao J, Li X, Zhang G, Sadiq FA, Simal-Gandara J, Xiao J, Sang Y. Probiotics in the dairy industry-advances and opportunities. *Compr Rev Food Sci Food Saf.* 2021; 20: 3937-3982
<https://doi.org/10.1111/1541-4337.12755>
13. Ranadheera CS, Evans CA, Baines SK, Balthazar CF, Cruz AG, Esmerino EA, Freitas MQ, Pimentel TC, Wittwer AE, Naumovski N, Graca JS. Probiotics in goat milk products: delivery capacity and ability to improve sensory attributes. *Compr Rev Food Sci Food Saf.* 2019; 18(4): 867-882.
<https://doi.org/10.1111/1541-4337.12447>
14. Byakika S, Mukisa IM, Byaruhanga YB, Muyanja C. A Review of criteria and methods for evaluating the probiotic potential of microorganisms. *Food Rev Int.* 2019; 35: 427-466.
<https://doi.org/10.1080/87559129.2019.1584815>
15. Haldar L, Gandhi DN. Development of vacuum-dried probiotic milk powder with *Bacillus coagulans*. *Int J Dairy Technol.* 2020; 73: 283-291
<https://doi.org/10.1111/1471-0307.12671>
16. Hasgucmn CK, Sengun IY. Viability of probiotic strain *Lactobacillus rhamnosus* and its impact on sensory properties of cheesecake during storage at -20° C and 4° C. *LWT.* 2020; 134: 109967.
<https://doi.org/10.1016/j.lwt.2020.109967>
17. Bernat N, Cháfer M, González-Martínez C, Rodríguez-García J, Chiralt A. Optimisation of oat milk formulation to obtain fermented derivatives by using probiotic *Lactobacillus reuteri* microorganisms. *Food Sci Technol Int.* 2015; 21(2):145-157
<https://doi.org/10.1177/1082013213518936>
18. Pham TT, Shah NP. Biotransformation of isoflavone glycosides by *Bifidobacterium animalis* in soymilk supplemented with skim milk powder. *J Food Sci.* 2007; 72(8): M316-M324.
<https://doi.org/10.1111/j.1750-3841.2007.00476.x>
19. Malganji S, Sohravandi S, Jahadi M, Nematollahi A, Sarmadi B. Effect of refrigerated storage on sensory properties and viability of probiotic in grape drink. *Appl Food Biotechnol.* 2016; 3(1): 59-62.
<https://journals.sbmu.ac.ir/afb/article/view/10544>
20. Hashemi M, Gheisari HR, Shekarforoush SS. Survival of *Lactobacillus acidophilus* and *Bacillus coagulans* in probiotic and low-fat synbiotic ice creams. *Food Hygiene.* 2013; 3: 57-65.
21. Soares MB, Martinez RC, Pereira EP, Balthazar CF, Cruz AG, Ranadheera CS, Sant'Ana AS. The resistance of *Bacillus*, *Bifidobacterium* and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Res Int.* 2019; 125: 108542.
<https://doi.org/10.1016/j.foodres.2019.108542>
22. The international conference on harmonization (ICH) harmonized tripartite guideline. stability testing of new drug substances and products Q1A (R2). Current Step 4 version, 2003. Available from:
<https://database.ich.org/sites/default/files/Q1A%28R2%29%20G%20u%20id%20line.pdf> (Accessed on 11th April 2018).
23. Louws FJ, Fulbright DW, Stephens CT, De Bruijn FJ. Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas pathovars* and strains generated with repetitive sequences and PCR. *Appl Environ Microbiol.* 1994; 60: 2286-2295.
<https://doi.org/10.1128/aem.60.7.2286-2295.1994>
24. Majeed M, Nagabhushanam K, Natarajan S, Sivakumar A, Eshuis-de Ruyter T, Booij-Veurink J, de Vries YP, Ali F. Evaluation of genetic and phenotypic consistency of *Bacillus coagulans* MTCC 5856: A commercial probiotic strain. *World J Microbiol Biotechnol.* 2016; 32(4): 60.
<https://doi.org/10.1007/s11274-016-2027-2>
25. Polo A, Cappello C, Carafa I, Da Ros A, Baccilieri F, Di Cagno R, Gobbetti M. A novel functional herbal tea containing probiotic *Bacillus coagulans* GanedenBC30: An *in vitro* study using the simulator of the human intestinal microbial ecosystem (SHIME). *J Funct Foods.* 2022; 88: 104873.
<https://doi.org/10.1016/j.jff.2021.104873>
26. Majeed M, Majeed S, Nagabhushanam K, Arumugam S, Beede K, Ali F. Evaluation of probiotic *Bacillus coagulans* MTCC 5856 viability after tea and coffee brewing and its growth in GIT hostile environment. *Food Res Int.* 2019; 121: 497-505.
<https://doi.org/10.1016/j.foodres.2018.12.003>
27. Kahraman B, Korkmaz K, Daştan D, Toker OS, Dertli E, Arici M. Production and characterization of probiotic jelly candy containing *Bacillus* species. *Food Measure.* 2023; 14: 5864-73.
<https://doi.org/10.1007/s11694-023-02076-3>
28. Miranda JS, Costa BV, de Oliveira IV, de Lima DCN, Martins EMF, Júnior BRDCL, do Nascimento Benevenuto WCA, de Queiroz IC, da Silva RR, Martins ML. Probiotic jelly candies enriched with native Atlantic Forest fruits and *Bacillus coagulans* GBI-30 6086. *LWT.* 2020; 126: 109275.
<https://doi.org/10.1016/j.lwt.2020.109275>
29. Majeed M, Majeed S, Nagabhushanam K, Natarajan S, Sivakumar A, Ali F. Evaluation of the stability of *Bacillus coagulans* MTCC 5856 during processing and storage of functional foods. *Int. J. Food Sci.* 2016; 51: 894-901.
<https://doi.org/10.1111/ijfs.13044>
30. Almada Érix CN, Almada CN, Pedrosa GTS, Dos Santos P, Schmieles M, Clerici MTP, Martinez J, Lollo PC, Magnani M, Sant'Ana AS. Quantifying the impact of eight-unit operations on the survival of eight *Bacillus* strains with claimed probiotic properties. *Food Res Int.* 2021; 142: 110191.
<https://doi.org/10.1016/j.foodres.2021.110191>
31. Blaiotta G, De Sena M, De Girolamo F, Aponte M, Romano R. Probiotic *bacilli* incorporation in foods: is really so easy? *Food Microbiol.* 2023; 114: 104342.
<https://doi.org/10.1016/j.fm.2023.104342>
32. Madureira AR, Pereira CI, Truszkowska K, Gomes AM, Pintado ME, Malcata FX. Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. *Int Dairy J.* 2005; 14: 921-927.
<https://doi.org/10.1016/j.idairyj.2004.08.025>
33. Marcial Coba MS, Saaby L, Knøchel S, Nielsen DS. Dark chocolate as a stable carrier of microencapsulated *Akkermansia muciniphila* and *Lactobacillus casei*. *FEMS Microbiol Lett.* 2019; 366(2): 1-6.
<https://doi.org/10.1093/femsle/fny290>



34. Cielecka Piontek J, Dziedziński M, Szczepaniak O, Kobus-Cisowska J, Telichowska A, Szymanowska D. Survival of commercial probiotic strains and their effect on dark chocolate synbiotic snack with raspberry content during the storage and after simulated digestion. *Electron J.* 2020; 48: 62-71.
<https://doi.org/10.1016/j.ejbt.2020.09.005>
35. Silva MP, Tulini FL, Marinho JF, Mazzocato MC, De Martinis EC, Luccas V, Favaro-Trindade CS. Semisweet chocolate as a vehicle for the probiotics *Lactobacillus acidophilus* LA3 and *Bifidobacterium animalis subsp. lactis* BLC1: Evaluation of chocolate stability and probiotic survival under *in vitro* simulated gastrointestinal conditions. *LWT.* 2017; 75: 640-647.
<https://doi.org/10.1016/j.lwt.2016.10.025>
36. Lavrentev FV, Ashikhmina MS, Ulasevich SA, Morozova OV, Orlova OY, Skorb EV, Iakovchenko NV. Perspectives of *Bacillus coagulans* MTCC 5856 in the production of fermented dairy products. *LWT.* 2021; 148: 111623.
<https://doi.org/10.1016/j.lwt.2021.111623>
37. Maity C, Bagkar P, Dixit Y, Tiwari A, Gupta AK. Process and storage stability of *Bacillus coagulans* LBSC in food matrices and appraisal of calorific restriction. *Appl Food Biotechnol.* 2020; 8: 57-69.
<https://doi.org/10.22037/afb.v8i1.31212>
38. Shaikh SS, Jhala D, Patel A, Chettiar SS, Ghelani A, Malik A, Sengupta P. *In silico* analysis of probiotic attributes and safety assessment of probiotic strain *Bacillus coagulans* BCP92 for human application. *Lett Appl Microbiol.* 2023; 77(11): ovad145.
<https://doi.org/10.1093/lambio/ovad145>



بررسی ذخیره سازی، فرایند و پایداری ژنتیکی باسیلوس (Heyndrickxia) کوگولانس BCP92 (MTCC 25460)

سهل اس. شیخ^{۱*}، چینمایی جوشی^۲، فرحنا ملک^۱، انیس مالک^۱، منوج گاندی^۱

۱. شرکت Pellucid Lifesciences Pvt با مسئولیت محدود، قطعه ۳۵۳۸، فاز ۴، شهرک صنعتی GIDC، منطقه ۳۸۲۷۲۹، گندهیناگار، گجرات هند

۲. کالج علم و تجارت Smt. S. S. Patel Nootan، دانشگاه سانکال چاند پاتل، ویسناگار ۳۸۴۳۱۵، هند

تاریخچه مقاله

دریافت ۲۰ مارس ۲۰۲۴

داوری ۶ می ۲۰۲۴

پذیرش ۳ ژوئن ۲۰۲۴

واژگان کلیدی

- باسیلوس کوگولانس
- ماتریس غذایی
- غذاهای فراسودمند
- پایداری ژنتیکی
- پایداری زیست‌یاریها

نویسنده مسئول

سهل اس. شیخ

شرکت Pellucid

Lifesciences Pvt با مسئولیت

محدود، قطعه ۳۵۳۸، فاز ۴،

شهرک صنعتی GIDC.

منطقه ۳۸۲۷۲۹، گندهیناگار،

گجرات هند

پست الکترونیک:

sohel@pellucidlifescience

s.com

sohels7392@gmail.com

چکیده

سابقه و هدف: باسیلوس کوگولانس زیست‌یاریهایی با توانایی تشکیل اسپور یا هاگ می‌باشند که در صورت مصرف، به‌میزان کافی، فواید سلامتی دارند. بنابراین، می‌توان آن‌ها را به غذاهای فراسودمند^۱ اضافه کرد تا ارزش غذایی آنها افزایش یابد. هدف از این مطالعه بررسی پایداری باسیلوس کوگولانس BCP92 در غذاهای فراسودمند گوناگون در هنگام فرایند و نگهداری مواد غذایی و همچنین بررسی پایداری ژنتیکی سویه با استفاده از روش انگشت نگاری DNA بود.

مواد و روش‌ها: باسیلوس کوگولانس BCP92 در طیف وسیعی از غذاها و نوشیدنی‌های فراسودمند مانند قهوه فوری، چای، سوپ ذرت شیرین، بلغور جو دوسر، آپما^۲، آدامس، براونی، بستنی، نوشیدنی‌های غیرالکلی، شکلات، کره بادام زمینی و شریکند^۳ افزوده شد. زنده‌مانی باکتری‌ها در شرایط گوناگون فرایند و نگهداری به‌روش پورپلیت ارزیابی شد. پایداری ژنتیکی *B. coagulans* با استفاده از انگشت نگاری DNA بررسی شد.

یافته‌ها و نتیجه‌گیری: قابلیت زنده‌مانی در هنگام فرآوری مواد غذایی چای (۹۹/۹۷ درصد)، قهوه (۹۹/۴۵ درصد)، سوپ ذرت شیرین (۹۹/۳۶ درصد)، بلغور جو دوسر (۹۸/۸۱ درصد)، آپما (۹۹/۵۷ درصد)، آدامس (۹۹/۶۷ درصد) و براونی (۹۸/۱۴٪) نشان داده شد. در شرایط نگهداری مواد غذایی، زنده‌مانی نسبی به شرح زیر بود: آب میوه (۹۸/۹۱ درصد)، لاسی (۹۸/۷۲ درصد)، نوشیدنی‌های انرژی‌زا (۹۸/۷۰ درصد)، قهوه‌های سرد (۹۹/۲۹ درصد)، شکلات‌های شیری (۹۹/۸۷ درصد)، شکلات‌های سفید (۱۰۰/۱۳ درصد)، شکلات تلخ (۹۹/۲۰ درصد)، شریکند (۹۹/۰۴ درصد)، بستنی‌ها (۹۹/۴۵ درصد)، کره بادام زمینی (۹۸/۳۲ درصد). علاوه بر این، انگشت‌نگاری DNA پایداری ژنتیکی پروبیوتیک BCP92 *B. coagulans* را نشان داد. در نتیجه، BCP92 *B. coagulans* زنده‌مانی خوبی در شرایط گوناگون فرایند و نگهداری مواد غذایی نشان داده است. علاوه بر این، از نظر ژنتیکی پایدار است، بنابراین انتخاب خوبی برای افزودن به غذاهای فراسودمند است.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافع مرتبط با انتشار این مقاله ندارند.

¹ Functional foods

² Upma

³ Shrikhand

