August 9. 2023

The U.S. Food and Drug Administration
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition (HFS-200)
5100 Campus Drive, College Park, MD 20740

To Whom it May Concern:

In accordance to 21 CFR Part 170, Subpart E, Pellucid Lifesciences Pvt., Ltd., with me as their consultant/agent, hereby provide notice of a claim that the addition of *Bacillus coagulans* strain BCP92 to conventional foods is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act. Pellucid Lifesciences Pvt., Ltd., has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As per the requirement, one copy of the GRAS monograph and one signed copy of the conclusion from the members of the Expert Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the signed statement of the Expert Panel.

If you have any questions regarding this notification, please feel free to contact me at 541-829-9121 or

Sincerely,

Brinda Mahadevan. Ph.D., ERT, Fellow ATS
Brincor Associates, LLC
6056 Haybury Drive, New Albany OH 43054

Encl.

GENERALLY RECOGNIZED AS SAFE (GRAS) EVALUATION OF *BACILLUS COAGULANS* BCP92 AS A FOOD INGREDIENT

Submitted by:
Pellucid Lifesciences Pvt., Limited
Plot No. 3538, Phase - 4, GIDC Industrial Estate
Chhatral – 382729, Gandhinagar
Gujarat, India

Submitted to:
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Campus Drive
College Park, MD 20740
USA

Prepared by:
Brincor Associates, LLC
6056 Haybury Drive
New Albany, OH 43054
USA

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GENERALLY RECOGNIZED AS SAFE (GRAS) EVALUATION OF *Bacillus coagulans* BCP92

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1. Part I – SIGNED STATEMENTS AND CERTIFICATION

1.1. Submission of GRAS Notice

In accordance with 21 CFR § 170 Subpart E, consisting of §170.203 through 170.285, Pellucid Lifesciences Pvt. Ltd., hereby informs the FDA that *Bacillus coagulans* BCP92 spores preparations manufactured by Pellucid Lifesciences, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Pellucid Lifesciences' view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.3 below.

1.2. Name and Address of Notifier

Mr. Anis Malik
Executive Director
Pellucid Lifesciences Pvt. Limited
Plot No. 3538, Phase – 4, GIDC Industrial Estate
Chhatral – 382729, Gandhinagar
Gujarat, INDIA
Phone: +91 9924124304
E-mail: malikanis@pellucislifesciences.com

US Consultant/Agent:
Brinda Mahadevan, MS, PhD, ERT, FATS
Brincor Associates, LLC
6056 Haybury Drive
New Albany, OH 43054
Phone: +1 541-829-9121
E-mail: bmahadevan@peak.org

1.3. Name of Notified Substance

The common name of the substance of this GRAS assessment is *Bacillus coagulans* strain BCP92 spore preparation that will be marketed as standardized powder. The strain was deposited in the Indian Microbial Type Culture Collection and Gene Bank (MTCC) (recognized as an International Depositary Authority (IDA) under the Budapest Treaty), with reference number MTCC 25460.

In this GRAS notice, *Bacillus coagulans* strain BCP92 is referred to by shorter names such as *Bacillus coagulans* BCP92, *B. coagulans* BCP92, *B. coagulans* MTCC 25460 or *Weizmannia coagulans*. The tradename is Pelluspore.

1.4. Intended Conditions of Use

Bacillus coagulans BCP92 spore preparation (*B. coagulans*) is intended to be used in the following food categories:

Baked goods and baking mixes, breakfast cereals, beverages and beverage bases, coffee and tea; milk and milk products, dairy product analogs, fruit juices, condiments and relishes,

confections and frostings, frozen dairy desserts and mixes, fruit and water ices, drinking water, sports drinks, gelatins, jams and jellies, puddings and fillings, alcoholic beverages, grain products and pastas, hard candy, soft candy, chewing gum, extracts, and flavorings, herbs, seeds, spices, seasonings, blends, nuts and nut products, plant protein products, processed fruits, processed vegetables and vegetable juices, snack foods, soups and soup mixes, sugar and sweet sauces, toppings, and syrups at a maximum level of approximately 1×10^8 to 2×10^9 colony forming units (cfu)/serving. Based upon the estimated number of servings of food consumed per day in the US and the highest intended addition level of *Bacillus coagulans* per serving, the estimated daily intake (EDI) of the strain is 36.4×10^9 cfu/day. (This EDI, of course, would be reached only if all target foods indeed contained *B. coagulans* at the maximum addition level). The intended use of *B. coagulans* strain BCP92 is identical to the use of *Bacillus coagulans* strains previously determined to be GRAS [GRN 399 (2011) GRN 526 (2014); GRN 597 (2015); GRN 601(2016); GRN 691 (2017); GRN 949 (2020)] and therefore would provide an alternate source of the microorganism in the spore preparation added to these foods but would not result in any change in exposure to the species.

B. coagulans BCP92 is not intended for use in foods that are targeted toward infants, such as infant formulas or foods formulated for infants, nor in meat and poultry products that come under USDA jurisdiction.

1.5. Statutory Basis for GRAS Determination

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6. Exclusion from Premarket Approval

Pellucid Lifesciences has concluded that the use of *Bacillus coagulans* BCP92 spores preparation is Generally Recognized As Safe, under the conditions of its intended use consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This GRAS conclusion has been reached in accordance with requirements in 21 CFR 170.220. Therefore, the use of *Bacillus coagulans* BCP92 spores preparation is exempt from the premarket approval requirements of the FD&C Act.

1.7. Availability of Data & Information

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Mr. Anis Malik at the below address. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225I(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Mr. Anis Malik
Executive Director
Pellucid Lifesciences Pvt. Ltd.
Plot No. 3538, Phase – 4, GIDC Industrial Estate
Chhatral – 382729, Gandhinagar, Gujarat, India
Phone: +91 9924124304
E-mail: malikanis@pellucidlifesciences.com

1.8. Data Exemption from Disclosure

Parts II through VII of this GRAS notification does not contain any data or information that is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document and the information contained in this dossier can be made publicly available.

1.9. Certification

Pellucid Lifesciences certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Pellucid Lifesciences, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of *Bacillus coagulans* BCP92 spores preparation. Pellucid Lifesciences accepts responsibility for the GRAS conclusion that has been made for *Bacillus coagulans* BCP92 spores as described in this dossier.

1.10. Name, Position/Title of Responsible Person who Signs the Dossier and Signature

Mr. Anis Malik
Executive Director
Pellucid Lifesciences Pvt. Ltd.
Plot No. 3538, Phase – 4, GIDC Industrial Estate
Chhatral – 382729, Gandhinagar, Gujarat, India
Phone: +91 9924124304
E-mail: malikanis@pellucislifesciences.com

Signature: 

1.11. FSIS/USDA – Use in Meat and/or Poultry

Pellucid Lifesciences does not intend to add *Bacillus coagulans* BCP92 spores to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

2. Part II – IDENTITY, SPECIFICATION AND MANUFACTURING

2.1. Identity

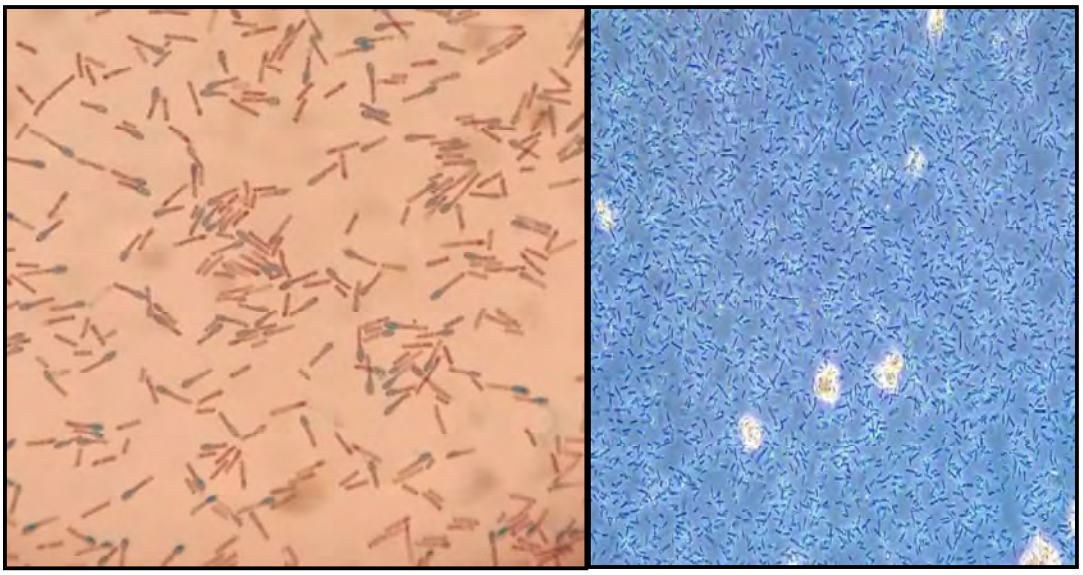
2.1.1. Name and Source of GRAS Organism

The specific bacterial strain which is the subject of this Generally Recognized As Safe (GRAS) assessment is *Bacillus coagulans* BCP92. It is a member of a subgroup of *Bacillus* spp. and is isolated from soil sample in Gujarat (India). *Bacillus coagulans* was recently named as *Weizmannia coagulans* (Gupta et al., 2020).

2.1.2. Description of GRAS Organism

General descriptive characteristics and properties of the *B. coagulans* BCP92 spore preparations manufactured by Pellucid Lifesciences are summarized in Table 1. *B. coagulans* BCP92 is a unique strain of spore forming *Bacillus* species. It is a gram-positive, spore forming, rod-shaped, slightly acidophilic, thermotolerant, aerobic to microaerophilic, highly resilient bacteria. *B. coagulans* strain (BCP92), the subject of the present GRAS determination, has been deposited with the Microbial Type Culture Collection (MTCC) - assigned number MTCC 25460. *B. coagulans* BCP92, originally isolated from soil, is a nonpathogenic, non-toxicogenic, naturally encapsulated spore-forming bacterium, pale brown colored powder with characteristic odor having total viable count not less than 150 x 10^9 cfu/g. Thermostable spores and a phase contrast image of cells is shown in Figure 1 below. *B. coagulans* strain BCP92 is not genetically engineered.

Figure 1. Phase contrast image of *B. coagulans* BCP92 spores and cells



(a) **(b)**

(a) Morphology of *B. coagulans* cells colored in red and associated endospores colored in green, when stained by Endospore stain. **(b)** Phase contrast image of cells at the time of harvest showing head and tail like structure which represent terminal spores.

The spores of *B. coagulans* can withstand temperatures in excess of 100°C, while the vegetative cells can grow at temperatures as high as 65°C. *B. coagulans* is a highly resilient bacteria commonly found in the soil, air and dust. It can grow in a highly alkaline environment

and the spores can also withstand the acidic environment of the stomach. The hierarchical classification of *B. coagulans* BCP92 is presented in Table 2.

Table 1. General Descriptive Characteristics of *Bacillus coagulans* BCP92

Parameter	Description *
Organism	*Bacillus coagulans* BCP92
Origin	Isolated from soil
Physical characteristics	A pale brownish powder
Odor	Characteristic
Shelf life	36 months

*Based on information provided by Pellucid Lifesciences

Table 2. Classification of *Bacillus coagulans* BCP92

Taxonomy	Taxonomic Assignment
Kingdom	Bacteria
Division	Endospore-Forming Bacteria
Phyllum	Bacillota
Class	Bacilli; Gram-Positive Endospore-Forming Bacteria
Order	Bacillales; Gram-Positive Endospore-Forming Rods
Family	Bacillaceae
Genus	Bacillus
Species	*Bacillus coagulans / Weizmannia coagulans*
Strain	*Bacillus coagulans* BCP92

2.1.3. Identification and Characterization

2.1.3.1. Phenotypic Identification

Bacillus coagulans is a well characterized microorganism that was first described in 1915 at the Iowa Agricultural Experiment Station associated with the coagulation of evaporated milk (Sarles & Hammer, 1932). In 1935, *Bacillus coagulans* was identified as *Lactobacillus sporogenes* in the Fifth Edition of Bergey's Manual, since it shared characteristics of both genera *Lactobacillus* and *Bacillus.* In 1957, it was finally transferred to the genus *Bacillus* in the seventh edition of Bergey's Manual (Breed et al., 1957).

Researchers (Losada & Olleros, 2002) compared the differential characteristics between *Lactobacillus* and *Bacillus* species, including *B. coagulans*. These investigators suggested that the capacity of *B. coagulans* to form spores is a differential characteristic compared to other strains of *Lactobacillus*. The spore formation is a microencapsulation process in which a covering of calcium-dipicolinic acid-peptidoglycan complex is generated. This allows a high degree of stability in unfavorable conditions such as changes in humidity and temperature during storage or alterations in the gastrointestinal tract.

Recently, *Bacillus coagulans* was transferred into the novel genus *Weizmannia*, as it shares the two O-methyltransferase and the acetate kinase genes which are characteristics of this clade (Gupta et al., 2020).

2.1.3.2. Phenotypic Biochemical Characterization

B.coagulans BCP92 was positive for catalase, and also showed a positive result in the Methyl Red test. The biochemical characteristics of *B. coagulans* BCP2 are summarized in Table 3. The strain was characterized as a member of the genus of *Bacillus* and species *coagulans*.

Table 3. Biochemical Characterization of *Bacillus coagulans* strain BCP92

Test	Observation	Test	Observation
Gram staining	+	Nitrate Reduction	-
Adonitol	-	Malonate	-
Arabinose	-	Maltose	+
Arabitol	-	Mannitol	+
Catalase	+	Mannose	+
Cellobiose	+	Melibiose	+
Citrate utilization	-	Methyl red	+
Dextrose	+	Malonate	-
Dulcitol	-	Raffinose	-
Erythritol	-	Rhamnose	+
Esculin hydrolysis	+	Salicin	-
Fructose	+	Sorbitol	+
Galactose	+	Sorbose	-
Glycerol	+	Sucrose	+
Indole	-	Trehalose	+
Inulin	+	L-Arabinose	+
Sodium gluconate	-	Xylitol	-
Lactose	+	Xylose	+
Voges Proskauer's	-		

2.1.3.3. Hemolytic Activity of *B. coagulans* BCP92

The ability to rupture red blood cells is a general characteristic of pathogenic bacteria (Bang et al., 2021). Therefore, non-hemolytic activity is primary criteria to consider microorganism as safe. To determine the hemolytic activity, overnight culture of *Bacillus coagulans* BCP92 was streaked on 5% sheep blood agar plates (TM Media: TMP017) and incubated at 37°C for 48 h. After incubation, the plates were observed for α-hemolysis (dark and greenish zones), β-hemolysis (lightened – yellow or transparent zones), and γ-hemolysis (no change or no zones).

Bacillus coagulans BCP92 showed no zone of hemolysis when streaked onto sheep blood agar plates, which was indicative of γ-hemolytic activity (non-hemolytic). This is corroborative with results from studies described by researchers for other strains of *Bacillus coagulans* (Bang et al., 2021; Konuray Altun & Erginkaya, 2021; Sreenadh et al., 2022; Styková et al., 2022).

2.1.3.4. Antimicrobial Activity

In an *in vitro* study (unpublished, Pellucid Lifesciences, 2022), *Bacillus coagulans* BCP92 was evaluated for its antimicrobial activity against some pathogenic bacteria such as *Salmonella enteritidis*, *Klebsiella aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* and against the pathogenic fungus *Candida albicans.* In this study, none of the test cultures were inhibited, indicating that *B. coagulans* BCP92 cultures do not produce detectable levels of inhibitory substances against the test cultures. Similar results were noted with *B. coagulans* ATCC 7050.

The available information indicates that *B. coagulans* BCP92 does not produce antibiotics. FDA has listed the use of *B. coagulans* in the production of glucose isomerase enzyme. In its list of enzyme preparations used in food, FDA has stated that, “Insoluble glucose isomerase enzyme

preparations are derived from recognized species of precisely classified, nonpathogenic, and nontoxigenic microorganisms, including *Streptomyces rubiginosus*, *Actinoplane missouriensis*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes* and *Bacillus coagulans* grown in a pure culture fermentation that produces no antibiotic."[1]

2.1.3.5. Acid and Bile Tolerance of *B. coagulans* BCP92

The spore preparation of *B. coagulans* BCP92 was tested for its ability to survive under different gastrointestinal conditions through an *in vitro* study. In the acid tolerance assay, *B. coagulans* BCP92 was transferred into 9 mL of phosphate buffer saline (PBS) at pH 2.5 (adjusted with HCl) and then subjected for incubation in an incubator (30°C). The number of viable cells in PBS was determined every hour, up to 4 hours of incubation on Glucose Yeast Extract agar (GYEA) plates in triplicates. Similarly, for the bile salt tolerance assay, *B. coagulans* BCP92 was transferred into 9 mL of PBS buffer containing bile salt (0.3% w/v), and then subjected for incubation in an incubator (37^0C). The number of viable cells was enumerated after 0,1, 2, 3 and 4 h of incubation periods on GYEA plates in triplicates.

The results of the study indicated that *Bacillus coagulans* BCP92 is stable in acidic pH (pH-2.5) and at a bile salt concentration of 0.3% (w/v), as only a 13% and 26% loss of viable counts were observed in these conditions, respectively. These results correspond to the data obtained by researchers with other strains of *Bacillus coagulans* (Chaudhari et al., 2022a; Majeed et al., 2016). Thus *in vitro* studies revealed *B. coagulans* BCP92 maintained its survivability under different gastrointestinal conditions.

2.1.3.6. Antibiotic Susceptibility Testing of *B. coagulans* BCP92

The antibiotic susceptibility of *B. coagulans* BCP92 was determined based on disc diffusion assay as per the method described by (Sui et al., 2020) with some modification. Himedia antibiotic sensitivity disc (Himedia, Mumbai, India) were used and the antibiotics tested included Amikacin (30µg), Amoxyclav (30µg), Ampicillin (10µg), Ampicillin/Sulbactam (10/10µg), Cefazolin (30µg), Cefepime (30µg), Cefotaxime (Cephotaxime) (30µg), Cefoxitin (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Co-Trimoxazole (25µg), Doxycycline HCl (30µg), Erythromycin (15µg), Gentamicin (10µg), Imipenem (10µg), Levofloxacin (5µg), Lenezolid (30µg), Netillin (30µg), Norfloxacin (10µg), Ofloxacin (5µg), Oxacillin (1µg), Penicllin-G (10 Unit), Rifampicin (5µg), Streptomycin (10µg), Teicoplanin (30µg), Tetracycline (30µg), and Vancomycin (30µg).

Approximately 1×10^8 CFU/ml of *B. coagulans* BCP92 was seeded onto each Mueller Hinton agar (MHA) plate. Then, antibiotic-impregnated discs were placed on the plates. Following incubation of the plates at 37°C for 24-48 h, the zones of growth inhibition were measured. The level of susceptibility to antibiotics was classified as Susceptible (S) if the zone diameter was ≥20 mm; Intermediate (I) if the zone diameter was 14-20 mm; and Resistant (R) if the zone diameter was ≤14 mm. Zones of growth inhibition ≥ 14 mm were observed for all the antibiotics at the tested concentration, as indicated in Table 4. Hence *Bacillus coagulans* BCP92 was concluded to be sensitive (complete or intermediate sensitivity) to the aforementioned antibiotics.

[1] Available at: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm

Table 4. Antibiotics susceptibility of *Bacillus coagulans* strain BCP92

Antibiotic	Symbol	Concentration	Zone of inhibition (mm)	Result
Amikacin	AK	30µg	30	S
Amoxyclav	AMC	30µg	36	S
Ampicillin	AMP	10µg	26	S
Ampicillin/ Sulbactam	A/S	10/10µg	26	S
Cefazolin	CZ	30µg	14	I
Cefepime	CPM	30µg	14	I
Cefotaxime	CTX	30µg	38	S
Cefoxitin	CX	30µg	24	S
Ceftriaxone	CTR	30µg	24	S
Cefuroxime	CXM	30µg	26	S
Chloramphenicol	C	30µg	30	S
Ciprofloxacin	CIP	5µg	38	S
Clindamycin	CD	2µg	36	S
Co-Trimoxazole (Sulphamethoxazole/Trimethoprim)	COT	25µg	28	S
Doxycycline HCl	DO	30µg	40	S
Erythromycin	E	15µg	28	S
Gentamicin	GEN	10µg	26	S
Imipenem	IPM	10µg	28	S
Levofloxacin	LE	5µg	34	S
Lenezolid	LZ	30µg	33	S
Netillin	NET	30µg	20	S
Norfloxacin	NX	10µg	33	S
Ofloxacin	OF	5µg	35	S
Oxacillin	OX	1µg	20	S
Penicillin-G	P	10Unit	36	S
Rifampicin	RIF	5µg	38	S
Streptomycin	S	10µg	40	S
Teicoplanin	TEI	30µg	30	S
Tetracycline	TE	30µg	40	S
Vancomycin	VA	30µg	33	S

Susceptible (S): zone diameter ≥20 mm; Intermediate (I): zone diameter 14-20 mm; and Resistant (R): zone diameter ≤14 mm.

In summary, *B. coagulans* BCP92 has been thoroughly analyzed for risk-associated factors following phenotypic/biochemical studies. Various studies/analyses carried out on this strain showed no safety concern and was concluded safe for human consumption.

2.1.4 Genotypic Characterization

In an attempt to genetically characterize *B. coagulans* BCP92, 16S rRNA sequencing was carried out. A fragment of 1295 bp was amplified, corresponding to almost the entire 16S rRNA gene. This amplicon was then sequenced using Sanger sequencing method. The partial sequence is presented in Appendix I. In order to fully characterize *Bacillus coagulans* BCP92, whole genome sequencing (WGS) was also carried out in parallel (Pellucid, 2022).

2.1.4.1 Genomic DNA isolation and quality assessment

For genomic identification, *B. coagulans* strains, BCP92 (MTCC 25460)[2] cultures were grown on sterile Luria Bertani broth for 24 hours at 37^{0}C in a temperature controlled incubator. The cells were then resuspended in 0.9% saline and pelleted for further processing and isolation of genomic DNA using the DNeasy UltraClean Microbial kit (Qiagen, USA). For nucleic acid isolation, the steps indicated in the kit was followed, with the initial resuspension of the cell pellet in a PowerBead Solution. Quality assessment of genomic DNA was performed by 0.8% agarose gel electrophoresis as well as DNA was quantified using Qubit dsDNA HS Assay Kit (Life Technologies, USA) DNA concentration was measured using Qubit® Fluorometer (Invitrogen, USA)

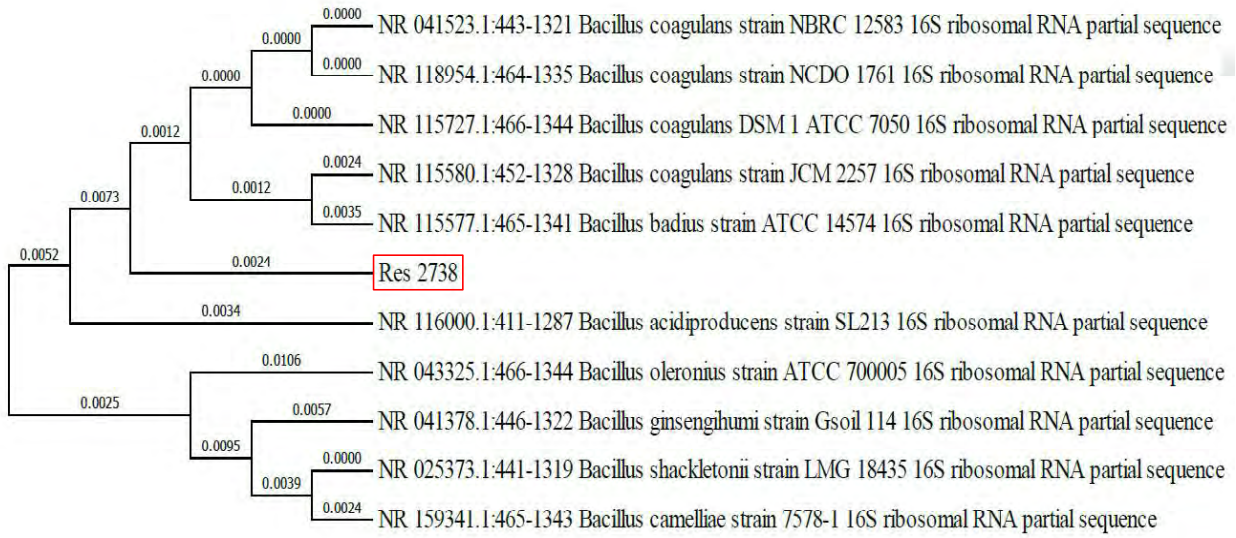
2.1.4.1.1 16S rRNA gene amplification and sequence analysis

The phylogenetic characterization of *B. coagulans* BCP92 strain was based on 16S rRNA sequence analysis. Polymerase chain reaction (PCR)-based amplification of the 16S rRNA gene was carried out using two different primer sets. These two sets of primers allowed the amplification of two overlapping DNA fragments that span across more than 1300 bases of the 16S rRNA gene of bacteria. Amplification was carried out in a Gene Amp PCR System (Applied Biosystems, USA). The amplified DNA fragments were separated on a 2% agarose gel and purified by using PCR purification Kit. DNA sequencing reaction of the PCR amplicons was carried out using two primers (binding to position 357 and 1391 on the 16S rRNA gene, for the forward and reverse primers, respectively), using BDT v3.1 Cycle Sequencing Kit and ABI 3500xl Genetic Analyzer.

The 16S rRNA sequence obtained was used to carry out BLAST analysis with the database of NCBI GenBank. Based on the maximum identity score, the first fifteen sequences were selected and aligned using multiple sequence alignment. The finding from this analysis is presented in Figure 2 and indicate that *B. coagulans* BCP92 (labeled as Res 2738, see phylogenetic tree below) is closely related to several strains of *B. coagulans,* and more particularly to the *B. coagulans* type strain (DSM 1, ATCC 7050). The partial sequence is presented in Appendix I.

[2] *B. coagulans* strain BCP92 deposited with the Microbial Type Culture Collection (MTCC) facility as *B. coagulans*, and was given the designation number 25460.

Figure 2. The phylogenetic tree of *B. coagulans* BCP92 Res 2738 with different *Bacillus* reference strains.



This phylogenetic tree was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.06122534 is shown (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Nei & Kumar, 2001)and are in the units of the number of base substitutions per site.

The number of base differences per site from between sequences in comparison to *B. coagulans* BCP92 Res 2738 is shown in Figure 3. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were total of 843 positions in the final dataset. Evolutionary analyses were conducted using MEGA7 (Kumar et al., 2016).

Figure 3. Description of phylogeny and distance matrix for the strains analyzed

	1	2	3	4	5	6	7	8	9	10
1. Res 2738										
2. NR 115727.1:466-1344 Bacillus coagulans DSM 1 ATCC 7050 16S ribosomal RNA partial sequence	0.003570									
3. NR 041523.1:443-1321 Bacillus coagulans strain NBRC 12583 16S ribosomal RNA partial sequenc	0.003570	0.000000								
4. NR 115580.1:452-1328 Bacillus coagulans strain JCM 2257 16S ribosomal RNA partial sequence	0.007163	0.003570	0.003570							
5. NR 115577.1:465-1341 Bacillus badius strain ATCC 14574 16S ribosomal RNA partial sequence	0.008368	0.004766	0.004766	0.005957						
6. NR 116000.1:411-1287 Bacillus acidiproducens strain SL213 16S ribosomal RNA partial sequence	0.013186	0.011989	0.011989	0.015636	0.015646					
7. NR 118954.1:464-1335 Bacillus coagulans strain NCDO 1761 16S ribosomal RNA partial sequence	0.003570	0.000000	0.000000	0.003570	0.004766	0.011989				
8. NR 043325.1:466-1344 Bacillus oleronius strain ATCC 700005 16S ribosomal RNA partial sequenc	0.027866	0.026658	0.026658	0.030392	0.031648	0.021719	0.026658			
9. NR 025373.1:441-1319 Bacillus shackletonii strain LMG 18435 16S ribosomal RNA partial sequen	0.030392	0.029180	0.029180	0.032935	0.034199	0.026688	0.029180	0.023020		
10. NR 041378.1:446-1322 Bacillus ginsengihumi strain Gsoil 114 16S ribosomal RNA partial sequer	0.032777	0.031568	0.031568	0.035325	0.036590	0.024153	0.031568	0.026681	0.009572	
11. NR 159341.1:465-1343 Bacillus camelliae strain 7578-1 16S ribosomal RNA partial sequence	0.032898	0.031684	0.031684	0.035455	0.036725	0.029180	0.031684	0.025500	0.002378	0.011992

2.1.4.1.2 Whole Genome Sequencing

2.1.4.1.2 DNA Library Preparation

Preparation of libraries were conducted via Ion Xpress™ Plus Fragment Library Kit (Thermo Fisher Scientific, USA) according to manufacturing instruction (including fragmentation, purification of fragments, ligation, amplification and quantification steps). For quantitation of DNA library, Ion Library Taqman Quantitation kit was used. The fragment size was checked (QC Step) for purified fragmented DNA. This was performed according to the instructions of Agilent™ High Sensitivity DNA Kit by Agilent™ 2100 Bioanalyzer.

2.1.4.1.3 Template Preparation and Sequencing

After Library preparation, the template was prepared according to manufacturer instructions of Ion 540™ Kit (Thermo scientific, USA) by using Ion OneTouch™ 2 System. The library was loaded on a chip using Ion 540™ Chip Kit and sequenced by Ion GeneStudio S5 Plus System (Ion Torrent, Thermo scientific, USA). Raw Sequencing reads were subjected to QC and pre-processing by Torrent Suite Software. Trimming of low-quality 3' ends, removal of adaptor sequences and quality parameter was set to ≥Q20 for filtering out low quality bases was performed by the Ion Torrent Suite. The NCBI accession number for the Whole Genome Sequence (WGS) of *B. coagulans* BCP92 is PRJNA926375 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA926375).

2.1.4.1.4 Assembly & Annotation

Sequence reads were de-novo assembled using SPAdes assembler 3.1.0 with the default settings. Resulted scaffolds were filtered based on the scaffolds length (≥500bp). The final assembled genome has 151 Scaffolds with the total length of 3,476,489 bp and an average G+C content of 46.35%.

B. coagulans BCP92 was annotated using RAST tool kit (RASTtk). Annotation of the genome predicted a total of 3461 genes, including 3192 protein-coding genes, 28 rRNAs, 82 tRNAs, 5 ncRNAs, 154 pseudogenes and 1 CRISPR Array. There were no plasmid sequences identified in the genome.

2.1.4.1.5 Identification and Reference Genomes

Annotated sequences of marker genes 16s rRNA and gyrB were aligned with the National Centre of Biotechnology Information (NCBI) database, using BLAST algorithm for species identification. *B. coagulans* BCP92 was identified using gyrB and 16S rRNA genes as phylogenetic markers. Closely related *Bacillus* species cannot be distinguished by 16S rRNA sequence analysis alone. Therefore, the *gyrB* gene, which encodes the subunit B protein of DNA gyrase, was selected as an additional phylogenetic marker. The 16S rRNA sequence and *gyrB* gene sequences showed >99% homology to *Bacillus coagulans* reference strains including the *Bacillus coagulans* type strain ATCC 7050.

Average nucleotide identity (ANI) calculated using FastANI toolkit v1.33 revealed 99.93%, 99.97%, 99.96%, 99.95% and 94.01% similarity of *B. coagulans* BCP92 with *B. coagulans* SNZ-1969, *B. coagulans* LSBC-1, *B. coagulans* Unique IS-2, *B. coagulans* GBI-30 and *B. coagulans* ATCC-7050, respectively.

The de novo assembled genome of *B. coagulans* BCP92, resulted in 151 scaffolds of 3,476,489 bp with 3346 coding sequences and G+C content of 46.35%. The genomic DNA G+C content, defined as the proportion of guanines and cytosines within the overall number of nucleotides in the genome, is one of the features in taxonomic descriptions of microorganisms (Meier-Kolthoff et al., 2014). The mol % G+Cs estimated for *B. coagulans* based on the whole genome sequence was 46.35%, which is in agreement to the values reported for *B. coagulans* (44 to 50%) by (Sudha et al., 2010).

2.1.4.1.6 Genome Comparison

Orthologous Average Nucleotide Identity Tool (OAT) was also used to predict orthoANI between genomes, and heatmap showing OrthoANI for *Bacillus coagulans* BCP92 with other genomes. Most similar genome was *Bacillus coagulans* GBI-30 (99.99%). Similarity with representative genome of *Bacillus coagulans* strain DSM 1_ATCC 7050 was 94.60%. Thus, *Bacillus coagulans* BCP92 was identified as a member of *B. coagulans* species on the basis of ~95% or higher ANI with the reference strains of the same species. Thus, the whole genome sequence confirmed the strain identity as *Bacillus coagulans* and a general phylogenetic tree is depicted in Figure 4.

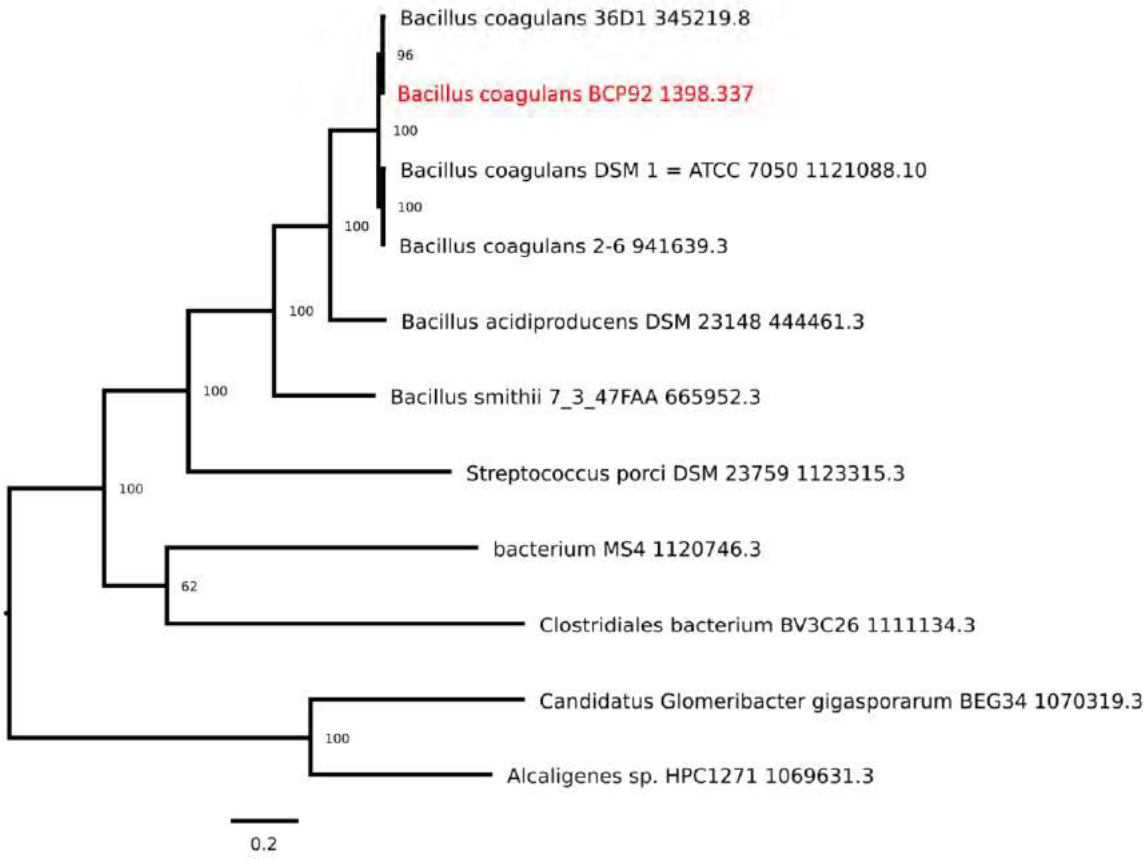
2.1.4.1.7 Identification of genes related to Antibiotic Resistance, Virulence factors, and Toxins

Antibiotic resistance genes were investigated in the *B. coagulans* BCP92 whole genome sequence by three methods (1) BLASTp search against Comprehensive Antibiotic Resistance Database (CARD), (2) Resistance Gene Identifier (RGI) with loose and strict parameters, and (3) One Codex analysis, and was found to be absent. A total of 17 different antibiotic class marker genes were checked for its presence in *B. coagulans* BCP92. Almost all the marker genes were found to be absent with 0.0% of gene coverage, 0.0x depth and 0.0% identity with antimicrobial resistance marker genes. A negative report resulted with a screen of 28 antibiotic classes of the One Codex Antimicrobial Resistance Research Panel.

Virulence factor genes/proteins were downloaded from the Virulence Factor Database (VFDB). The total number of sequences in the core database was 4195. A BLAST homology search between the protein sequences of *B. coagulans* BCP92 and virulence factor proteins revealed no significant hits. In summary, *Bacillus coagulans* strain BCP92 does not contain any

sequences/genes related to antibiotic resistance or virulence factor genes, thus confirming the safety of the strain through the genome-based approach.

Figure 4. The general phylogenetic tree of *B. coagulans* BCP92



2.1.4.1.8 Identification of CRISPR−Cas Sequences within the Genome

Coding sequences for Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated genes (Cas) were identified using manual searches through the functional annotation generated from NCBI PGAP and RAST toolkit. The presence of a CRISPR system indicates an advantage in promoting genome stability by acting as a barrier to entry of foreign DNA elements.

B. coagulans BCP92 genome harbors 33 spacers, 34 repeats organized in at least 1 CRISPR arrays with the same direct repeat sequence. The exact number of arrays cannot be determined due to the fragmented nature of the BCP92 genomic assembly that includes small contigs containing partial, truncated CRISPR arrays. Additionally, this array is linked to several unidirectionally arranged Cas genes (cas1, cas2, cas3, cas4, cas5, cas7, and cas8). Such genomic association is typical of Class I-C, characterized by the lack of cas6, encoding for an endoribonuclease employed by most Type-I systems for pre-crRNA processing and by the presence of a single protein encoded by the Cas8c gene. The comparative genomic analysis revealed that eight strains of *Bacillus coagulans* possess cas operons of type I-B (2–6, 36D1, H-1, P-38, and XZL9), type I-C (CSIL1,

DSM1, H-1, MA-13), or type IV (DSM1), mostly located in the vicinity of CRISPR arrays as observed by Aulitto et al., (2022).

The CRISPR-Cas system is considered a defense mechanism against mobile genetic elements. This system can protect against re-invasion by capturing and integrating foreign nucleic acid fragments from the initial invasion. The CRISPR locus, with associated Cas-genes, equally provides the host strain with the potential to defend itself against any incoming extra-chromosomal DNA molecules. This is an indication of stability of the genome of *B. coagulans* BCP92 and a very low possibility of the strain to acquire antimicrobial resistant genes since resistance genes are mostly introduced by mobile genetic elements.

2.1.4.1.9 Identification of Biogenic Amines Producing Genes

Functional annotation of the entire genome revealed that the *B. coagulans* BCP92 does not possess any protein-encoding gene involved in the production of biogenic amines such as histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, and spermidine; with the exception of CDS putative for arginine decarboxylase, which is for the production of agmatine and no other toxin was identified. Rather than, it possesses gene-encoding putrescine synthesis and putrescine importer, which can be used in putrescine usage pathways and gene-encoding putrescine aminotransferase, which is responsible for degradation of it. The rest of the bioamines were not identified in *Bacillus coagulans* BCP92.

2.1.4.1.10 Assessment of safety and stability of the strain

Genes involved in the production of lipopeptides (*fenA*, *fenB*, *fenD*, *fenE*, *srfAA*, *srfAB*, *srfAC*, *lchA*, *lchAB*, *lchAC*) were manually searched through NCBI PGAP annotation. Genes as encoded above for surfactins, cyclic lipopeptides, fengycin and lychenisin were not found on the genome. Genes involved in the production of toxin were also screened. The genome of *B. coagulans* BCP92 did not harbor the genes encoding for haemolysin BL (Hbl), the non-haemolytic enterotoxin (Nhe), enterotoxin (cytotoxin K) and emetic toxin gene (cereulide - ces). Similar results were observed for *B. coagulans* GBI 30, 6086 as determined by Salvetti et al., (2016).

Mobile Genetic Elements (MGE), such as prophage, transposase and other mobile elements were identified within the genomes of the *Bacillus coagulans* BCP92. Only one intact prophage region was predicted within the genome of the strain. The intact prophage was found on contig 1 (genomic coordinate: 77335-130113 [52.7Kb]). The putative intact prophage region identified, however, lacked essential genes such as topoisomerase, replisome (Primase) and DNA polymerase required for replication. The results were further confirmed with Phage-web and Virosorter2 as it predicts partial phage in the contig 1 region. These suggests that this prophage sequence present in assembled genome is defective and non-functional.

Stability testing was performed for *B. coagulans* BCP92 to study its shelf life. In a real-time stability study, the samples were stored in a stability chamber at 25°C ± 2°C and 60% ± 5% relative humidity for 36 months. In an accelerated stability study, samples were stored in stability chamber at accelerated storage conditions (40°C ± 2°C and 75% ± 5% relative humidity) for a period of six months. *B. coagulans* BCP92 was found stable for 36 months under real-time storage conditions as only a 13% loss of viable count was observed. *B. coagulans* BCP92 was also found to be stable for a period of 6 months under accelerated storage conditions, as only a ~10% loss of viable count was observed. The shelf-life storage stability results obtained in the present studies corroborate the results presented in other GRAS notices for *B. coagulans* [GRN 949 (2020); GRN

597 (2015); GRN 601(2015); GRN 526 (2014); GRN 399 (2011)]. These results suggest shelf life *of B. coagulans* BCP92 is at least three years under real-time storage conditions.

In summary, the *de novo* assembled genome of *B. coagulans* BCP92, indicated that no significant mobile elements were identified with respect to the loci which have significant homology against antibiotic resistance genes, virulence factor genes, or biogenic amine producing genes. The presence of a CRISPR sequence in the assembled genome indicates an advantage in promoting genome stability by acting as a barrier to the entry of foreign DNA elements. In conclusion, *B. coagulans* BCP92 does not contain any sequences/genes in the genome that are risk associated, thus confirming the safety of the strain through the genome-based approach.

This approach is similar to a recent study wherein the safety of *Bacillus coagulans* IDCC 1201 isolated from green malt was analyzed by its genomic and phenotypic characteristics and determining its toxicity. The presence of antibiotic resistance and toxigenic genes and gene transferability were investigated using whole-genome analysis. The strain's hemolytic and enzyme activities, minimum inhibitory concentrations of antibiotics, and biogenic amine and d-lactate production were also examined. The whole-genome analysis demonstrated that *B. coagulans* IDCC 1201 had no antibiotic resistance or toxigenic genes; the strain was susceptible to the nine antibiotics. The strain exhibited functional potential such as bile tolerance and intestinal adhesion in *in vitro* experiments. In conclusion, *B. coagulans* IDCC 1201 was considered safe with regard to human health (Bang et al., 2021).

2.1.5 Overview of the Manufacturing Process

Bacillus coagulans BCP92 spores are produced after growing the BCP92 strain by fermentation in accordance with current Good Manufacturing Practice (cGMP). Fermentation is a well-known process that occurs in food and has been used for the cultivation of microorganisms for decades. The manufacturing facility is ISO 22000 and GMP/HACCP certified. The typical fermentation batch size is about 15,000 L. Standard Operating Procedures (SOPs) are followed and verification methods are in place. Materials used in the fermentation process (inoculum, seed, and main fermentation) are all food-grade substances approved for this use. There are no ingredients based on milk, soy, or any of the top eight allergens. *Bacillus coagulans* BCP92 spore production involves four stages and they are as follows and further elaborated in sections below: (1) Inoculum preparation, (2) Fermentation of *Bacillus coagulans* BCP92, (3) Concentration and spray drying, and (4) Blending. The manufacturing process is schematically presented in Figure 5. The fermentation process is conducted in a contained and sterile environment in a closed vessel.

2.1.5.1 Inoculum preparation

Inoculum development is a multi-step process in order to obtain sufficient growth at every stage. A suspension of a pure culture of *B. coagulans* BCP92 is aseptically transferred to an inoculum flask (pre-seed 1) containing fermentation medium and incubated on a shaking incubator at 37°C for 24±4 h. Then pre-seed 1 is transferred to a second flask (pre-seed 2) and again incubated on a shaking incubator at 37°C for 24±4 h. The pre-seeded (flask 2) is then transferred to a seed fermenter where optimum conditions are provided (pH, temperature, agitation, air, etc.) for growth to occur for 12-16 h.

2.1.5.2 Fermentation of *Bacillus coagulans* BCP92

Growth achieved in a seed fermenter is then transferred to another fermenter for the main fermentation process to be initiated and proceeded for 48±4 h to obtain sufficient growth of

Bacillus coagulans BCP92. During the fermentation process temperature, dissolved oxygen and pH are monitored to ensure and maintain product quality. At this stage, microscopy checks are performed to confirm the morphological parameters of *Bacillus coagulans* BCP92.

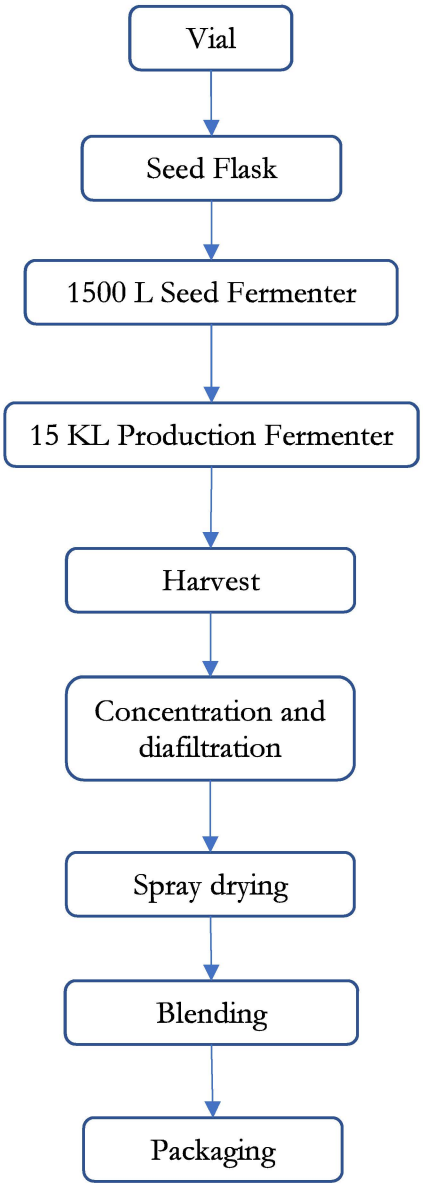
2.1.5.3 Concentration and Spray drying

After the fermentation process is completed, the fermentation broth is subjected to centrifugation to separate the spores (biomass) from the soluble media components. The spore biomass is collected as a thick slurry and subjected to further processing. Temperature and pH are controlled during this step. As the next step, diafiltration is performed using phosphate buffered saline (PBS), the biomass is collected in a spray dryer feeding tank and subjected to spray drying to obtain the product in powder form. At each stage, samples are withdrawn to check the quality growth and seed culture via microscopy.

2.1.5.4 Blending

For the manufacturing of the dry spore preparation of *Bacillus coagulans* BCP92, the spray-dried intermediate product obtained from different batches is further formulated with approved food-grade formulating agents (optional) such as maltodextrin in a hexagonal blender. Throughout the process quality is controlled and all operations are performed according to SOPs. *Bacillus coagulans* BCP92 final product in the form of spore is tested for quality as per specification and the final product is released upon quality assurance.

Figure 6. Flow Chart for the Manufacturing of *B. coagulans* BCP92



2.2 Specifications

Food grade specifications of *B. coagulans* BCP92 spore preparations have been established by Pellucid Lifesciences and are presented in Table 5. Analytical results from three non-consecutive lots (Appendix II) demonstrate that *B. coagulans* BCP92 is consistently manufactured to meet these specifications and is presented in Table 6. The *B. coagulans* BCP92 the subject of the present GRAS assessment was found, through 16s rDNA Analysis, to be over 99% similar to *B. coagulans* type strain ATCC 7050. The major allergens like gluten, nuts, milk and dairy products are not present in the final spore preparation. All assay methods used for establishing specification are current and have been validated for this purpose.

Table 5. Specification of *B. coagulans* BCP92 Preparation

Parameter	Characteristics (Pellucid, 2018)*	Assay Method
Appearance	Free flowing, white to pale brownish powder	Visual & Organoleptic evaluation
Identification	Aerobic, gram positive thermostable spores	Microscopy
Loss on drying (105° for 1 hour)	NMT 7% w/w	Pellucid Lifesciences: Internal Method
Viable spore count cfu/g	NLT 150 billion	Pellucid Lifesciences: Internal Method
Lactic acid producing capacity	NLT 10 ml of 0.05 N NaOH consumed	Pellucid Lifesciences: Internal Method
Heavy metals		
Arsenic	NMT 0.5 ppm	AOAC 21st Edition 2015.01 (Briscoe, 2015)
Lead	NMT 0.5 ppm	
Mercury	NMT 0.5 ppm	
Cadmium	NMT 0.5 ppm	
Microbiological assays		
Yeast and Mold	NMT 10 cfu/g	USP General Chapter, 2021, USP Dietary Supplement Chapter, 2022
Escherichia coli	Negative/10 g	
Salmonella	Negative/10 g	
Pseudomonas aeruginosa	Negative/1 g	
Staphylococcus aureus	Negative/1 g	
Bacillus cereus	NMT 10 cfu/g	FDA BAM Chapter 14 (Tallent et al., 2012)

*Based on information provided by Pellucid Lifesciences; NMT = Not more than; NLT = Not less than; cfu = colony forming units; ppm = parts per million.

Table 6. Comparison of the Specifications for the Three Batches (non-consecutive) of *Bacillus coagulans* BCP92

Parameters	Specification	Method	Batch No.		
			B06200061607	B06202061607	B06204061607
Appearance	Free-flowing, white to pale brownish powder.	Visual & Organoleptic evaluation	Complies	Complies	Complies
Description	Aerobic, Gram-positive, thermostable spores	Microscopy	Complies	Complies	Complies
Viable spore count (cfu/g)	NLT 150 billion	Pellucid Lifesciences: Internal Method	260 Billion	250 Billion	270 Billion
Loss on drying (at 105 °C for 1 hour)	NMT 7.0 % w/w	Pellucid Lifesciences: Internal Method	6.3 %	6.2%	6.3%

Lactic acid-producing capacity	NLT 10 ml of 0.05 M NaOH consumed	Pellucid Lifesciences: Internal Method	13.5 ml	12.6 ml	12.9 ml
Microbial Analysis					
Yeast and Mould count	NMT 10 cfu/g	USP <2021> USP <2022>	<10 cfu/g	< 10 cfu/g	< 10 cfu/g
Escherichia coli	Negative/10 g		Absent	Absent	Absent
Salmonella	Negative/10 g		Absent	Absent	Absent
Pseudomonas aeruginosa	Negative/1 g		Absent	Absent	Absent
Staphylococcus aureus	Negative/1 g		Absent	Absent	Absent
Bacillus cereus	NMT 10 cfu/g	FDA BAM Chapter 14 (Tallent et al., 2012)	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Heavy Metal Analysis					
Arsenic	NMT 0.5 ppm	AOAC 21st Edition 2015.01 (Briscoe, 2015)	BLQ	BLQ	BLQ
Lead	NMT 0.5 ppm		0.113	0.144	0.133
Mercury	NMT 0.5 ppm		BLQ	BLQ	BLQ
Cadmium	NMT 0.5 ppm		BLQ	BLQ	BLQ

* Based on information provided by Pellucid Lifesciences; NMT = Not more than; NLT = Not less than; cfu = colony forming units; ppm = parts per million. BLQ - Below Limit of Quantification (LOQ - Limit of Quantification-0.1 mg/kg)

3 Part III – DIETARY EXPOSURE

3.1 Intended Use Levels and Food Categories

Pellucid Lifesciences intends to use a *B. coagulans* BCP92 spore preparation as a food ingredient in a wide variety of foods at a level not exceeding $2x10^9$ cfu/serving. Intended uses do not include infant formula or any foods under the jurisdiction of the U.S. Department of Agriculture. The food categories as defined in 21 CFR §170.3(n) to which *B. coagulans* BCP92 is to be added are very similar to those mentioned in GRN 949 and are listed below:

(1) Baked goods and baking mixes, including all ready-to-eat and ready-to-bake products, flours and mixes, requiring preparation before serving. (2) Beverages, alcoholic, including malt beverages, wines, distilled liquors, and cocktail mix. (3) Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks, drinking water, sport drinks. (4) Breakfast cereals, including ready-to-eat and instant and regular hot cereals. (5) Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip, and miscellaneous cheeses. (6) Chewing gum, including all forms. (7) Coffee and tea, including regular, decaffeinated, and instant types. (8) Condiments and relishes, including plain seasoning sauces and spreads, olives, pickles, and relishes, but not spices or herbs. (9) Confections and frostings, including candy and flavored frostings, marshmallows, baking chocolate, and brown, lump, rock, maple, powdered, and raw sugars. (10) Dairy product analogs, including nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products. (12) Fats and oils, including margarine, dressings for salads, butter, salad oils, shortenings and cooking oils. (16) Fresh fruit juices, including only raw fruits, citrus, melons, and berries, and home prepared "ades" and punches made therefrom. (20) Frozen dairy desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties. (21) Fruit and water ices, including all frozen fruit and water ices. (22) Gelatins, puddings, and fillings, including flavored gelatin desserts, puddings, custards, parfaits, pie fillings, and gelatin base salads. (23) Grain products and pastas, including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables. (25) Hard candy and cough drops, including all hard type candies. (26) Herbs, seeds, spices, seasonings, blends, extracts, and flavorings, including all natural and artificial spices, blends, and flavors. (28) Jams and jellies, commercial, including only commercially processed jams, jellies, fruit butters, preserves, and sweet spreads. (30) Milk, whole and skim, including only whole, low-fat, and skim fluid milks. (31) Milk products, including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages, and other milk origin products.

3.1.4 Estimated Daily Intake from the Proposed Uses

The proposed use levels of *B. coagulans* BCP92 and the food categories to which it will be added are identical to those stated for *B. coagulans* LBSC in GRN No. 000949, filed on April 13, 2020, to which FDA had no questions. This same addition level and intended uses were also specified in GRN No. 000399 for *B. coagulans* GBI-30 6086, GRN No. 000526 for *B. coagulans* Unique IS2, GRN No. 000597 for *B. coagulans* SNZ 1969, and GRN No. 000691 for *B. coagulans* SANK 70258. FDA had no questions regarding any of these GRAS determinations.

Since the intended use of *B. coagulans* BCP92 corresponds to the already existing use for other strains of *B. coagulans*, it simply represents another strain with no increase in consumer exposure to the species. FDA accepted the Estimated Daily Intake (EDI) presented in GRN No. 000949, 36.4×10^9 cfu/day, which was also adopted in the preceding GRAS notices for other strains of *B. coagulans*. Therefore, that same EDI is appropriate for *B. coagulans* BCP92.

4 Part IV – SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the notified ingredient *B. coagulans* BCP92 spore preparation in food applications.

5. Part V – EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

Not applicable. The statutory basis for the conclusion of GRAS status of *B. coagulans* BCP92 spore preparation in this document is not based on common use in food prior to 1958. The GRAS conclusion for *B. coagulans* BCP92 spore preparation is based on scientific procedures.