7. Part VII – SUPPORTING DATA AND INFORMATION

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8. APPENDIX I

Bacillus Coagulans BCP92 16S rRNA Sequence

>16s_rRNA_NODE_120_405bp_to_1700bp_NCBI
AAGGAGGTGATCCAGCCGCACCTTCCGATACGGCTACCTTGTTACGACTTCACCCCAATCATCTGTCCCACCTT
CGGCGGCTGGCTCCGTAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTG
TACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCGGCTTCATGCAGGCGG
GTTGCAGCCTGCAATCCGAACTGGGAATGGTTTTCTGGGATTGGCTTAACCTCGCGGTCTCGCAGCCCTTTGTA
CCATCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCG
GTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGGTCAAGGGTTGCGCTCGTTGCG
GGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCCGAAGGGG
AAGGCCCTGTCTCCAGGGAGGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCA
CATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGA
GTGCTTAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGG
ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGCCGC
CTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCA
CTCAAGCCTCCCAGTTTCCAATGACCGCTTGCGGTTGAGCCGCAAGATTTCACATCAGACTTAAGAAGCCGCCT
GCGCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT
AGCCGTGGCTTTCTGGCCGGGTACCGTCAAGGCGCCGCCCTGTTCGAACGGCACTTGTTCTTCCCCGGCAACAG
AGTTTTACGACCCGAAGGCCTTCTTCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCT
ACGCATCGTTGCCTTGGTGAGCCGTTACCCCACCAAC

9. APPENDIX II

Certificate of analysis from three (non-consecutive) batches

CERTIFICATE OF ANALYSIS

- Name of the Product : ***Bacillus coagulans* BCP92**
- Batch Number : B06200061607
- Manufacturing Date : JUN - 2023
- Expiry Date : MAY - 2026
- Result of analysis

TEST	SPECIFICATION	METHOD	RESULT
Appearance	Free-flowing, white to pale brownish powder.	Visual	Complies
Description	Aerobic, Gram-positive endospores as identified by red-colored rods with green-colored endospores when stained by Schaeffer-Fullten & observed under the microscope.	Microscopy	Complies
Bacillus coagulans viable spore count (cfu/g)	Not Less Than 150 billion cfu/g	PELL/STP/001	**260 billion cfu/g**
Loss on drying (at 105 °C for 1 hour)	Not More Than 7.0 % W/W	PELL/STP/004	6.3 %
Lactic acid-producing capacity	Not Less Than 10 ml of 0.05 M NaOH consumed	PELL/STP/002	13.5 ml
Microbial Analysis			
Yeast and Mould count	Not More Than 100 cfu/g	USP <2021> USP <2022>	Less Than 10 cfu/g
Escherichia coli	Shall be absent in 10 g		Absent
Salmonella	Shall be absent in 10 g		Absent
Pseudomonas aeruginosa	Shall be absent in 1 g		Absent
Staphylococcus aureus	Shall be absent in 1 g		Absent
Bacillus cereus	Not More Than 10^3 cfu/g	FDA BAM chapter 14	Less Than 10 cfu/g
Heavy Metals			
Arsenic	Not More Than 0.5 mg per kg (0.5 ppm)	AOAC	BLQ (LOQ:0.1)
Lead	Not More Than 0.5 mg per kg (0.5 ppm)		0.113 ppm
Mercury	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)
Cadmium	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)

Document Number: PELL/COA/001_3.1

Declaration: This product complies with all the applicable product standards as prescribed by the Quality Department at Pellucid Lifesciences Pvt. Ltd.

CERTIFICATE OF ANALYSIS

- Name of the Product : ***Bacillus coagulans* BCP92**
- Batch Number : B06202061607
- Manufacturing Date : JUN - 2023
- Expiry Date : MAY - 2026
- Result of analysis

TEST	SPECIFICATION	METHOD	RESULT
Appearance	Free-flowing, white to pale brownish powder.	Visual	Complies
Description	Aerobic, Gram-positive endospores as identified by red-colored rods with green-colored endospores when stained by Schaeffer-Fullten & observed under the microscope.	Microscopy	Complies
Bacillus coagulans viable spore count (cfu/g)	Not Less Than 150 billion cfu/g	PELL/STP/001	**250 billion cfu/g**
Loss on drying (at 105 °C for 1 hour)	Not More Than 7.0 % W/W	PELL/STP/004	6.2 %
Lactic acid-producing capacity	Not Less Than 10 ml of 0.05 M NaOH consumed	PELL/STP/002	12.6 ml
Microbial Analysis			
Yeast and Mould count	Not More Than 100 cfu/g	USP <2021> USP <2022>	Less Than 10 cfu/g
Escherichia coli	Shall be absent in 10 g		Absent
Salmonella	Shall be absent in 10 g		Absent
Pseudomonas aeruginosa	Shall be absent in 1 g		Absent
Staphylococcus aureus	Shall be absent in 1 g		Absent
Bacillus cereus	Not More Than 10^3 cfu/g	FDA BAM chapter 14	Less Than 10 cfu/g
Heavy Metals			
Arsenic	Not More Than 0.5 mg per kg (0.5 ppm)	AOAC	BLQ (LOQ:0.1)
Lead	Not More Than 0.5 mg per kg (0.5 ppm)		0.144 ppm
Mercury	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)
Cadmium	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)

Document Number: PELL/COA/001_3.1

Declaration: This product complies with all the applicable product standards as prescribed by the Quality Department at Pellucid Lifesciences Pvt. Ltd.

CERTIFICATE OF ANALYSIS

- Name of the Product : ***Bacillus coagulans* BCP92**
- Batch Number : B06204061607
- Manufacturing Date : JUN - 2023
- Expiry Date : MAY - 2026
- Result of analysis

TEST	SPECIFICATION	METHOD	RESULT
Appearance	Free-flowing, white to pale brownish powder.	Visual	Complies
Description	Aerobic, Gram-positive endospores as identified by red-colored rods with green-colored endospores when stained by Schaeffer-Fullten & observed under the microscope.	Microscopy	Complies
Bacillus coagulans viable spore count (cfu/g)	Not Less Than 150 billion cfu/g	PELL/STP/001	**270 billion cfu/g**
Loss on drying (at 105 °C for 1 hour)	Not More Than 7.0 % W/W	PELL/STP/004	6.3 %
Lactic acid-producing capacity	Not Less Than 10 ml of 0.05 M NaOH consumed	PELL/STP/002	12.9 ml
Microbial Analysis			
Yeast and Mould count	Not More Than 100 cfu/g	USP <2021> USP <2022>	Less Than 10 cfu/g
Escherichia coli	Shall be absent in 10 g		Absent
Salmonella	Shall be absent in 10 g		Absent
Pseudomonas aeruginosa	Shall be absent in 1 g		Absent
Staphylococcus aureus	Shall be absent in 1 g		Absent
Bacillus cereus	Not More Than 10^3 cfu/g	FDA BAM chapter 14	Less Than 10 cfu/g
Heavy Metals			
Arsenic	Not More Than 0.5 mg per kg (0.5 ppm)	AOAC	BLQ (LOQ:0.1)
Lead	Not More Than 0.5 mg per kg (0.5 ppm)		0.133 ppm
Mercury	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)
Cadmium	Not More Than 0.5 mg per kg (0.5 ppm)		BLQ (LOQ:0.1)

Document Number: PELL/COA/001_3.1

Declaration: This product complies with all the applicable product standards as prescribed by the Quality Department at Pellucid Lifesciences Pvt. Ltd.

6.4.1 Conclusion of the Expert Panel

The intended use of *B. coagulans* BCP92 spore preparation has been determined to be safe through scientific procedures and the safety was shown by genomic analysis of the strain, a record of safe ingestion of numerous strains of *B. coagulans*, phenotypic characterization of *B. coagulans* BCP92, toxicity of other strains, and research in humans, concluding that the expected exposure to *B. coagulans* BCP92 spore preparation is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of *B. coagulans* BCP92 has been made through the deliberations of an Expert Panel consisting of Madhusudan G. Soni, Ph.D., FACN, FATS and David Ribet, Ph.D., who reviewed a monograph prepared by Brincor Associates, LLC for Pellucid Lifesciences, as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to *B. coagulans* BCP92 spore preparation anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on *B. coagulans* BCP92 that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of *B. coagulans* BCP92 spore preparation. It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion regarding the safety of *B. coagulans* BCP92 under its intended conditions of use.

In summary, on the basis of scientific procedures[1], including reports of exposure to other *B. coagulans* strains, resulting in a maximum estimated daily intake of 36.4×10^9 cfu/day or 2×10^9 cfu/serving of *B. coagulans* BCP92 spores is considered safe. The intended uses are compatible with current regulations and is produced according to current good manufacturing practices (cGMP).

Signatures

[redacted]
Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

August, 07, 2023
Date

[redacted]
David Ribet, Ph.D.

August 7th 2023
Date

[1] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

February 15, 2024

Questions/Comments for GRN 1159:

FDA Query: 1. In Table 5 on page 21, yeast and mold count is specified as ≤ 10 cfu/g. However, yeast and mold count is specified as ≤ 100 cfu/g in the Certificates of Analysis on pages 65, 66, and 67. Please clarify the discrepancy.

Response: *Thank you for pointing out the discrepancy and we apologize for the typographical error. In Table 5 on page 21, we request that the mold count needs to be corrected to match with the certificate of analysis ≤ 100 cfu/g.*

FDA Query: 2. On page 19, the notifier states, "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". Please clarify whether the specifications provided in Table 5 (page 21) are for the ingredient prior to or after the standardization with maltodextrin (or other formulating agents).

Response: *We confirm that the specifications provided in Table 5 (page 21) are for the ingredient prior to the standardization with maltodextrin.*

FDA Query: 3. The descriptions of the intended food uses in Part 1.4 (pages 4-5) and Part 3.1 (page 23) differ: (a) The following food categories are listed in Part 1.4 but not in Part 3.1: soft candy; 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. (b) Two food categories (soft candy; fats and oils) are not listed in Part 1.4 but are listed in Part 3.1. In addition, on page 23, the notifier states that the intended use levels and food categories to which their ingredient will be added are identical to those in GRN 000949. We note that the food categories listed above in (a) as well as soft candy were included in the intended food uses in GRN 000949, as described in our response letter (see GRN 000949 response letter). Please also note that alcoholic beverages were not among the intended food uses listed in our response letter for GRN 000949. Please confirm that you intend to use your ingredient only in the food categories that are listed in our response letter for GRN 000949.

Response: *We apologize for inadvertently not listing certain food categories in Part 1.4 versus Part 3.1. However, we do agree and confirm that we intend to use our ingredient (Bacillus coagulans BCP92) only in the food categories that are listed in the response letter for GRN 000949*

FDA Query: 4. In Table 5 on page 21, the specification limits for arsenic, lead, mercury, and cadmium are specified as ≤ 0.5 mg/kg. We note that the results from the analyses of three batches show that the levels of arsenic, mercury and cadmium are all consistently below the limit of

quantitation (LOQ) of 0.1 mg/kg and the levels of lead are below 0.2 mg/kg. In line with FDA's "Closer to Zero" initiative that focuses on reducing dietary exposure to heavy metals we would recommend that you consider lowering the specification limits for heavy metals to be reflective of batch analyses and as low as possible.

Response: *Thank you for pointing out the FDA's "Closer to Zero" initiative to us. In the case of mercury and arsenic, we agree that the value in the specification table (Table 5, page 21) could be corrected as ≤ 0.1 mg/kg. However, due to the potential for inherent seasonal variation of the raw materials used in manufacturing, we request to retain the specification limit of ≤0.5 mg/kg for cadmium and lead. We assure you that we are already in progress with a quality initiative to carefully select and source the raw materials to achieve a specification limit of ≤ 0.3 mg/kg for lead and cadmium.*

FDA Query: 5. Please state that the ingredient is not intended for use in foods where standards of identity preclude its use.

Response: *Thank you for bringing this to our attention and as suggested, we agree that "Ingredient is not intended for use in foods where standards of identity preclude its use" could be stated in section 1.4.*

FDA Query: 6. On page 18, the notifier discusses the materials used in the fermentation process and states "There are no ingredients based on milk, soy, or any of the top eight allergens. Sesame is now considered a major food allergen under the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act (https://www.fda.gov/food/cfsan-constituent-updates/faster-act-video-foodindustry-and-other-stakeholders). For the administrative record, please state whether sesame or substances derived from sesame are used in your manufacturing process and whether this poses a safety concern.

Response: *We confirm that sesame or substances derived from sesame are not used in the manufacturing process and hence does not pose a safety concern.*

FDA Query: 7. On page 19, the notifier states that during the fermentation process, "microscopy checks are performed to confirm the morphological parameters of *Bacillus coagulans* BCP92." For the administrative record, please clarify what is meant by "morphological parameters." Additionally, please address the following: a) Please describe how the production of spores is ensured. b) Please provide an approximate ratio of spores to vegetative cells.

Response: *A wet mount and a well stained slide of Bacillus coagulans BCP92 is observed under the microscope for morphological parameters which are typical phenotypic characteristics of the strain. For example, the homogeneity of bacterial population with a validated growth pattern, uniformity in the size, shape, motility via microscopic evaluation and characteristic viable growth on the agar plates ensures strain purity/production of spores.*

a) Aseptically withdrawn samples of End of Fermentation broth are plated on Soyabean Casein Digest Agar (SCDA), Hicrome coliform agar (HCA), Violet Red Bile Agar (VRBA) which are then incubated for 24 to 48 hours for verifying the fermentation broth status in terms of strain purity. The plate data is used for batch clearance whereas microscopic observation for morphological parameters is used for intermediate/fermentation clearance.

b) The approximate ratio is 80:20 of spores to vegetative cells.

March 4, 2024

Follow-up Question for GRN 1159:

FDA Query: In response to our question 4 (amendment dated February 15, 2024), you requested to retain the specification limit of ≤ 0.5 mg/kg for cadmium and lead "due to the potential for inherent seasonal variation of the raw materials used in manufacturing".

Please explain what "seasonal variation" means in relation to the microorganism strain, fermentation medium components, and any other materials used in the manufacture of your ingredient. We note that we typically do not see levels of heavy metals higher than 0.1 mg/kg in ingredients produced by controlled fermentation and following current good manufacturing practices. We also note that batch analyses included in a GRAS notice should be representative for the notified substance. Therefore, we consider that the batch analyses provided in GRN 001159 are representative for your ingredient. Considering the consistency of the results for cadmium and lead (≤ 0.1 mg/kg and 0.113-0.144 mg/kg, respectively) across the three non-consecutive batches, we reiterate our request that you lower the specification limits for cadmium and lead to better reflect the results from the batch analyses.

Response: *Pellucid Lifesciences agrees to decrease specification of cadmium to* ≤ 0.1 *mg/kg to align with FDA's closer to zero initiative. In the case of lead we kindly request a limit of* ≤ 0.2 *mg/kg to not only adhere to FDA's closer to zero initiative but also to accommodate for the batch analysis data to be reflective of the notified substance.*

By seasonal variation we meant in relation to the raw materials used in fermentation. We assure you that we will update our procurement and storage plans to maintain the specification mentioned above in the article of commerce/GRAS ingredient.

March 21, 2024

Follow-up Question for GRN 1159:

FDA Query:

On page 17 of the notice, you state that "*B. coagulans* BCP92 does not possess any protein-encoding gene involved in the production of biogenic amines… with the exception of CDS putative for arginine decarboxylase, which is for the production of agmatine". Please provide a brief discussion on the presence of this CDS and discuss whether it, and the potential for agmatine synthesis, poses a safety concern.

Response: *Literature review indicates that the presence of CDS for arginine decarboxylase is intrinsic to the genome of B. coagulans and its existence shown for several B. coagulans strains (e.g., GRN 670 & GRN 949).*

To rule out the possibility of the CDS putative for arginine decarboxylase resulting in the production of agmatine, Pellucid Lifesciences performed a simplified rapid procedure (Goldschmidt and Lockhart, 1971) to determine the presence of agmatine in B. coagulans BCP92. This assay did not detect the production of agmatine in the conditions tested, suggesting that the gene is nonfunctional and B. coagulans BCP92 strain does not produce agmatine. Furthermore, the absence of plasmids in the B. coagulans BCP92 genome (Shaikh et al. 2024), render it non-transferable and non-toxic phenotypic properties confirm the non-functionality of the gene.

Taken together, the presence of CDS putative for arginine decarboxylase in B. coagulans BCP92 for the production of agmatine, poses no safety concern.

References:

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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. 2024 Jan 2;77(1):ovad145. doi: 10.1093/lambio/ovad145.