

ORIGINAL SUBMISSION

**Inactivated *Bacillus coagulans* GBI-30, 6086
is Generally Recognized as Safe for use in
foods**

Submitted by the Notifier:

Ganeden Biotech, Inc.
5800 Landerbrook Drive, Suite 300
Mayfield Heights, Ohio 44124

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc.
2800 East Madison Street, Suite 202
Seattle, Washington 98112

September 16, 2016

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GRN 000670

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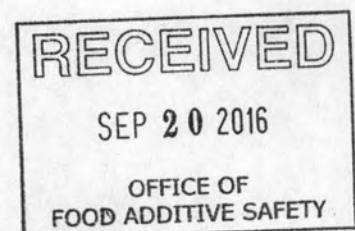
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Antonia Mattia, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Mattia:

Following our most recent meeting with FDA with Dr. David Keller (in person) and me by teleconference on Wednesday, September 7, 2016, pursuant to proposed regulation 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)), on behalf of Geneden Biotech, Inc. (the notifier), we are submitting, for FDA review, the enclosed notice that Inactivated *Bacillus coagulans* GBI-30, 6086 is GRAS for use in foods.

Please find one printed copy of the notification enclosed as well as a CD.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or john@aibmr.com.

Sincerely,

(b) (6)



John R. Endres, ND (Agent of the Notifier)
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1. GRAS Exemption Claim

Ganeden, Inc. (the notifier) has determined that Inactivated *Bacillus coagulans* GBI-30, 6086 is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. The determination has been made based on scientific procedures. Therefore the use of Inactivated *Bacillus coagulans* GBI-30, 6086 for its intended purpose is exempt from the requirement of pre-market approval.

(b) (6)

September 16, 2016

David Keller, DPM, MBA
Notifier

Date

1.1 Name and Address of the Notifier and Agent of the Notifier

Notifier

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Vice President of Scientific Operations
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1.2 Common or Usual Name

Inactivated *Bacillus coagulans* GBI-30, 6086

Synonyms: Thermally killed *Bacillus coagulans* GBI-30, 6086

Heat killed *Bacillus coagulans* GBI-30, 6086

1.3 Conditions of Use

Inactivated *Bacillus coagulans* GBI-30, 6086 is intended to be added at levels up to at a maximum of approximately 2×10^9 colony forming units (CFU) inactivated cells per serving. The food categories are the same as those identified for *Bacillus coagulans* GBI-30, 6086 as described in GRN 000399 under the subheading Conditions of Use.

*Inactivated *B. coagulans* GBI-30, 6086 is not intended for use in infant formula or any product that would require additional review by USDA.

1.4 Basis for GRAS determination

Scientific procedures are the basis for this GRAS determination. An independent and critical evaluation of the safety and GRAS status of the intended use of Inactivated *B. coagulans* GBI-30, 6086 was conducted by an expert panel qualified by training and experience to evaluate the safety of food ingredients. The expert panel consisted of Judith Hauswirth, PhD (Hauswirth Consulting, Inc.; Former Branch Chief, Office of Pesticides Program, U.S. Environmental Protection Agency; Former Acting Director, Division of Drugs and Environmental Toxicology, U.S. Food and Drug Administration) and John Endres, ND (Chief Scientific Officer, AIBMR Life Sciences, Inc.). The panel critically evaluated the scientific research available with regard to Inactivated *B. coagulans* GBI-30, 6086 and collectively determined that the ingredient is GRAS for its intended use in foods. The expert panel believes that other similarly qualified scientists reviewing the same publicly available data would come to the same conclusion.

1.5 Data/Information Availability Statement

The data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of David Keller, DPM, MBA, Vice President of Scientific Operations, Geneden, Inc., 5800 Landerbrook Drive, Suite 300, Mayfield Heights, Ohio 44124, Telephone: (440) 229-5204, email: keller@genedenprobiotics.com or will be sent to the FDA upon request.

2. Characterization

B. coagulans was first described in 1915 at the Iowa Agricultural Experiment Station, with regard to the coagulation of canned evaporated milk.¹ GenedenBC³⁰ is the trade name for a proprietary preparation of a *B. coagulans* strain designated

as *B. coagulans* GBI-30, 6086. *B. coagulans* GBI-30, 6086 is a patented probiotic organism. It is L+ lactic acid producing, non-toxicogenic, and non-pathogenic. The organism is a gram-positive spore-forming rod that is aerobic to microaerophilic in nature. Its size is 0.9 µm x3.0 µm x5.0 µm. *B. coagulans* GBI-30, 6086 is manufactured as a pure cell mass consisting solely of *B. coagulans*. This Notification is for inactivated thermally killed cells of this identical strain.

B. coagulans GBI-30, 6086 was the subject of GRN 000399 submitted to FDA by Ganeden Biotech, Inc. and filed by the Agency on August 23, 2011. On July 31, 2012, GRN 000399 received the Agency's response letter with no questions pertaining to Ganeden's claim that *B. coagulans* GBI-30, 6086 is GRAS for its intended use as an ingredient in baked goods and baking mixes; beverages and beverage bases; breakfast cereals; chewing gum; coffee and tea; condiments and relishes; confections and frostings; dairy product analogs; fruit juices; frozen dairy desserts and mixes; fruit and water ices; gelatins, puddings, and fillings; grain products and pastas; hard candy; herbs, seeds, spices, seasonings, blends, extracts, and flavorings; jams and jellies; milk; milk products; nuts and nut products; plant protein products; processed fruits; processed vegetables and vegetable juices; snack foods; soft candy; soups and soup mixes; sugar; and sweet sauces, toppings, and syrups at a maximum level of approximately 2×10^9 colony forming units (CFU) per serving.

For the purpose of the toxicological studies described in GRN 000399 and discussed below, pure *B. coagulans* GBI-30, 6086 was used.

2.1 Identification

B. coagulans GBI-30, 6086 was identified to the genus level and then confirmed to be a pure strain of *B. coagulans* Hammer as reported in GRN 000399 under the subheading DNA Ribotyping Analysis on page 5, which is incorporated here by reference, where it is also noted that each lot of *B. coagulans* GBI-30, 6086 is subjected to 16S Ribosomal DNA base pair analysis as part of Ganeden's ongoing QC program.

To further characterize *B. coagulans* GBI-30, 6086, whole-genome sequencing was performed using the Illumina GAIIx platform at CRA-Genomics Research Centre (Piacenza, Italy) with a paired-end library.² This sequencing project has been deposited in DDBJ/EMBL/GenBank under the accession number JPSK00000000. The genome consists of 3,458,655 base pairs with a GC content of 46.38%. From the total prediction of 3,373 genes, 3,197 coding sequences (CDS), 18 rRNAs, 82 tRNAs, 79 pseudogenes, 1 ncRNA, and 3 clustered regularly interspaced short palindromic region (CRISPR) arrays were identified. CDS genes were predicted to encode approximately 500 proteins related to energy metabolism and 80 related to dormancy and sporulation. Genes involved in adhesion (i.e., fibronectin- and mucus-binding proteins) and active metabolism (e.g., biotin biosynthesis) were also identified. The complete 16S rRNA gene

sequence was searched against the ExTaxon database and was aligned with those of *B. coagulans* DSM 1^T, related taxa, and other representatives of the *Bacillus* genus using Clustal Omega^{3,4}. A phylogenetic tree was constructed using the number of differences algorithm as substitution model and neighbor-joining as tree inference method as implemented in MEGA v.6 software package.⁵ The 16S rRNA analysis demonstrated 99.92% identity compared to *B. coagulans* DSM 1^T, confirming that *B. coagulans* GBI-30, 6086 be allotted to species *B. coagulans*.

2.2 Other Ingredients

The other ingredients in the final product, Inactivated *B. coagulans* GBI-30, 6086, consist of maltodextrin, microcrystalline cellulose, and/or sodium bicarbonate, which are used for bulking purposes, and milk powder and organic inulin, which can be used as diluents. Pursuant to 21 CFR 184.1444 (maltodextrin) and 184.1736 (sodium bicarbonate) these bulking agents are GRAS. The GRAS status of microcrystalline cellulose is included in the Select Committee on GRAS Substances (SCOGS) review of ethyl cellulose. Milk powder is GRAS due to common use pursuant to 21 CFR 182.1 and inulin is GRAS for use in foods pursuant to GRN Nos. 000477 and 000576. All corn-derived products used in the production of *Bacillus coagulans* GBI-30, 6086 are manufactured to meet European Union quality standards (EC 1829, EC 1830), which does not allow for GMO products.

3. Manufacturing and Production

The production process for Inactivated *B. coagulans* GBI-30, 6086 consists of fermentation followed by recovery, followed by a kill step followed by spray-drying or freeze-drying. The purpose of the recovery process (centrifugation) is to retrieve and concentrate the Inactivated *B. coagulans* cells post-fermentation.

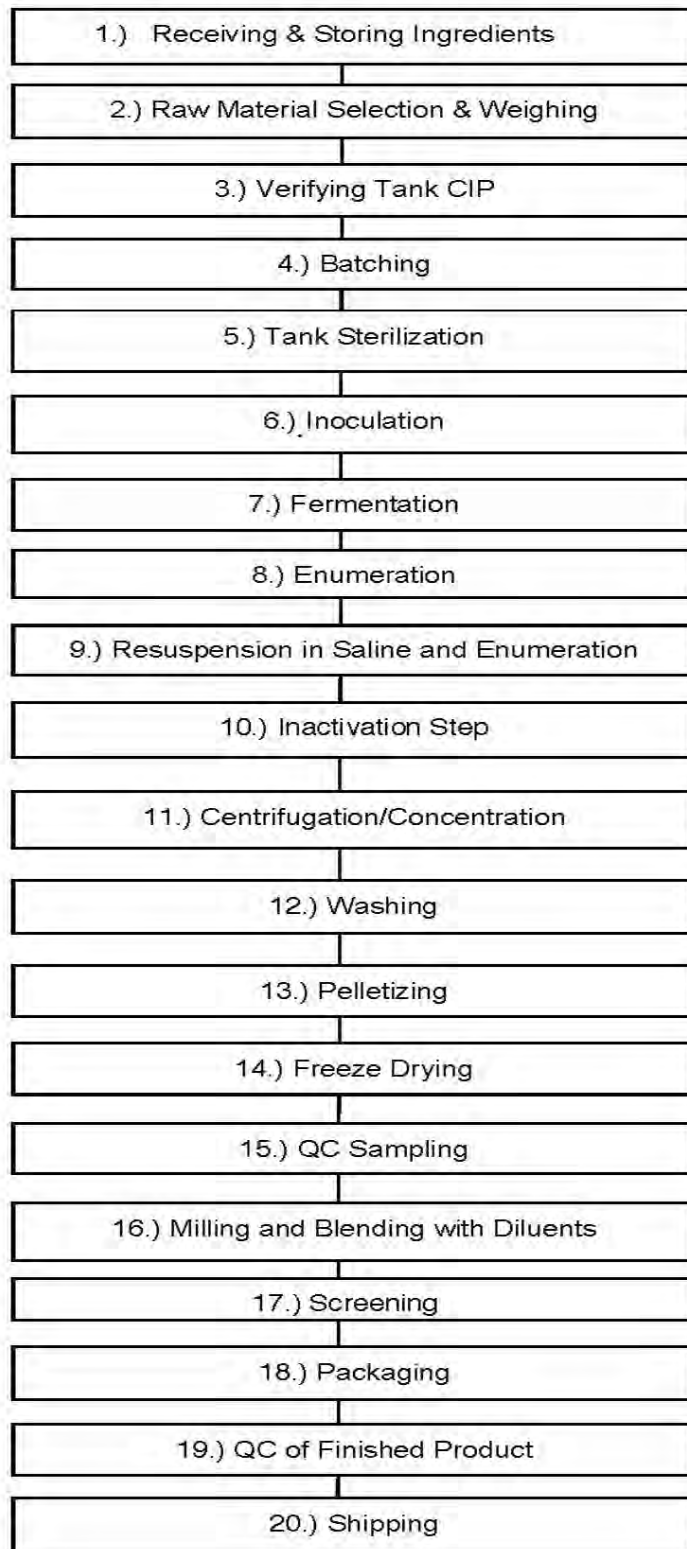


Figure 1. Manufacturing Flow Chart

3.1 Product Specifications

The product specifications for Inactivated *B. coagulans* GBI-30, 6086, along with the specification methods, are listed in Table 1 below.

Table 1. Product specifications

Test Items	Specification	Method
Physical Characteristics		
Enumeration	Equivalent to 1.5×10^{10} (15 Billion per gram colony forming units)- Live <i>Bacillus coagulans</i> is not detected	Internal Method
Appearance	Light Tan to Beige Powder	Visual inspection
Moisture	NMT 9%	AOAC 926.08
Sieve Test	100% through 40 mesh	Internal Method
Sieve Test	80% through 80 mesh	Internal Method
Heavy Metals		
Arsenic	NMT 5 ppm	EPA 3050/6020 USP 730
Cadmium	NMT 5 ppm	EPA 3050/6020 USP 730
Lead	NMT 5 ppm	EPA 3050/6020 USP 730
Mercury	NMT 5 ppm	EPA 3050/6020 USP 730
Microbiological Tests		
Standard Plate Count	NMT 5000 CFU/g	BAM CH.3
Yeast and Mold	NMT 100 CFU/g	BAM CH. 18
Total Coliforms	NMT 10 CFU/g	BAM CH. 4
<i>E. coli</i>	None Detected	BAM CH. 4
<i>Staphylococci</i> (Coag. Pos.)	None Detected	AOAC 975.55
<i>Salmonella</i>	None Detected	AOAC 999.08
<i>Listeria</i>	None Detected	AOAC 996.14
<i>P. aeruginosa</i>	None Detected	Internal Method

3.2 Method for Enumeration

Enumeration is run at the end of fermentation. Based on R&D work, a known quantity of powder is produced once dried. The CFU per gram (pre inactivation step) is calculated. Based on these results, it can be calculated how much diluent needs to be added to have a final product equivalent to 15 billion CFU cells inactivated.

3.3 Shelf-life Stability

A three-year shelf-life from the time of manufacture has been recommended as an appropriate expiration period for the live *B. coagulans* GBI-30, 6086. This recommendation is based upon accelerated and real-time shelf-life stability studies performed to assess the stability of *B. coagulans* GBI-30, 6086. As these products are Inactivated cells of the same strain, shelf life would be at least as long as the live cells.

4. Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

5. Intended Use and Exposure

This product is intended to be included in the same categories of foods and at the same inclusion rates as the live *B. coagulans* GBI-30, 6086 as stated in GRN 000399 under subheading Conditions of Use for its intended use in food up to levels equivalent to approximately 2×10^9 CFU inactivated cells per serving.

5.1 Estimated Daily Intake (EDI)—Exposure

In GRN 000399 under the subheading Intended Use, the EDI of the live *B. coagulans* GBI-30, 6086 was 36.4×10^9 CFU per day, which is significantly less than the ADI derived from the NOAEL from the 1-year chronic reproduction toxicology study of 93.8×10^9 CFU per day. As this product would be added at the same level and would be substitutive with respect to exposure to the live cells and not used in conjunction, the estimated exposure would be identical.

6. Safety Assessment

When live cells are consumed, the body is exposed to the same cell wall components as present in the inactivated form. While live cells will multiply once germinated in the body and therefore increase in number, the number of cells consumed will remain constant following consumption of the inactivated form. As the addition level per serving of food is the same level as the live cells described in GRN 000399, all of the toxicological studies incorporated by reference from GRN 000399 support the safety of the inactivated cells.

6.1 Toxicological Studies on *Bacillus coagulans* GBI-30, 6086

The following toxicological studies were described in GRN 000399 under the subheading Toxicology Studies of the major heading Safety Assessment on pages 8–17, and are incorporated herein by reference:

- a bacterial reverse mutation assay conducted according to OECD 471⁶;
- an in vivo peripheral blood micronucleus assay in mice conducted according to OECD 474 and US FDA Redbook 2000 IV.C.1.d⁶;
- an in vitro chromosomal aberration assay conducted according to OECD 473⁶;
- an acute oral toxicity study in rats conducted according to OECD 423 and US FDA Redbook II—Draft, Acute oral Toxicity Tests (1993)⁶;

- a 90-day repeated-dose oral toxicity study in rats conducted according to OECD 408 and US FDA Redbook 2000 IV.C.4.a⁶;
- a one-year chronic repeated-dose oral toxicity study combined with a one-generation reproduction toxicity study in rats conducted according to OECD 452 and US FDA Redbook 2000 IV.C.5a and OECD 415 and US FDA Redbook 2000 IV.C.9a., respectively⁷;
- an eye irritation study in rabbits conducted according to OECD 405⁶;
- a skin irritation study in rabbits conducted according to OECD 404.⁶

These studies showed no evidence of genotoxic potential, oral toxicity in acute, subchronic, and chronic studies in rats, reproductive toxicity in rats, or eye and skin irritation in rabbits of *B. coagulans* GBI-30, 6086. Based on the results observed on the parameters evaluated in these studies, there was no indication of any toxicological effects on the organ systems evaluated.

6.2 Virulence and Toxicity

As shown in GRN 000399 under the subheading Additional Support of Safety on pages 18–19 and incorporated herein by reference, US FDA, Health Canada, and EFSA recognize *B. coagulans* as a non-pathogenic, non-toxicogenic organism. Furthermore, EFSA’s granting of qualified presumption of safety (QPS) status to *B. coagulans* beginning in 2007 and maintained through the current 2015 publication, in addition to affirming non-pathogenicity, provides that the agency has no current safety concerns for the organism’s intended uses in foods so long as the strain satisfies the qualifications of absence of toxigenic potential (e.g., toxins that can be produced in food and enterotoxins) and acquired antibiotic resistance genes.⁸⁻¹⁶

6.2.1 *Bacillus coagulans* GBI-30, 6086

An unpublished PCR assay, as reported in GRN 000399 under the subheading Additional Support of Safety and incorporated herein by reference, showed no evidence that *B. coagulans* GBI-30, 6086 contains genes that encode for enterotoxins or hemolysins.

Since the submission of GRN 000399, additional work, based on the complete genomic sequence of *B. coagulans* GBI-30, 6086 has been carried out confirming and adding to this evidence.⁴ In this study, putative virulence factors (VF) were identified according to the method of Chen et al.¹⁷ using a local Protein-protein Basic Local Search Tool (BLASTP) with protein sequences derived from the *B. coagulans* GBI-30, 6086 annotated genes to query the Virulence Factor Database (VFDB). Basic Local Alignment Search Tool (BLAST) results were considered in the study if they demonstrated greater than 30% identity and 70% coverage.

Genes encoding for the hemolysin BL (HBL complex; hblC, hblD, hblA and hblB: AJ007794), the non-hemolytic enterotoxin NHE (NHE complex; nheA, nheB and nheC: Y19005), the enterotoxin T (bceT; D17312), the cytotoxin K (cytK; AJ277962), and the cereulide (cesA, cesH, cesP, cesT, cesB, cesC, cesD; DQ360825) were searched for on the genome of *B. coagulans* GBI-30, 6086 using BLASTX in order to further evaluate the potential for the presence of enterotoxin genes. Additionally, searches were conducted to evaluate the presence of genes involved in the synthesis of lipopeptides such as fengycin (fenA, AF023464; fenB, Appl Microbiol Biotechnol BACFENB; fenD, CAA09819; fenE, AF023465), surfactins (srfAA, D13262; srfAB, AF233756; srfAC, CAB12145), and lychenisin (lchAA, lchAB, lchAC; AJ005061).

BLASTX was also searched against the genome of *B. coagulans* GBI-30, 6086 for the presence of genes related to biogenic amines.

B. coagulans GBI-30, 6086 was grown in the presence of arginine, histidine, lysine, ornithine, putrescine and tyrosine, and supernatants were obtained for HPLC determination and quantification of biogenic amine production according to the methods of Martuscelli et al., Tabanelli et al., and Tabanelli et al.,¹⁸⁻²⁰ respectively.

The majority of the 200 putative VFs identified in the genome of *B. coagulans* GBI-30, 6086 using VFDB were defensive (multidrug transports and resistance proteins, a peroxidase, and an alkyl hydroperoxide reductase) or non-classical VFs related to normal cellular activities (Clusters of Orthologous Groups (COG) database (<http://www.ncbi.nlm.nih.gov/COG/>)). The majority of putative VFs were related to extracellular structures and may represent beneficial traits for adhesion to host cells or the sporulation mechanism (VFDB). Furthermore, possible VFs with relatively low similarity to known VFs can be detected by BLAST similarity. Therefore, the putative VFs identified in the genome of *B. coagulans* GBI-30, 6086 were not considered to be harmful.

B. coagulans GBI-30, 6086 does not carry any known enterotoxin or hemolysin genes as determined by the BLASTX analysis. Additionally, the genome of *B. coagulans* GBI-30, 6086 was determined not to contain genes encoding for the harmful cyclic lipopeptides—surfactins—or other lipopeptides with toxin activity, such as fengycin and lychenisin.

The HPLC analysis for biogenic amines demonstrated that *B. coagulans* GBI-30, 6086 did not produce tyramine, histamine, putrescine, cadaverine and phenylethylamine, and the polyamines, spermine and spermidine under the conditions of the assay. In the genomic analysis, with the exception of genes encoding for the metabolic pathways from arginine to putrescine and putrescine to spermidine, genes related to the production of biogenic amines were absent. Because the corresponding biogenic amines related to the identified pathways

were not produced, it is presumed the genes were either not functional or not expressed at a sufficient level for production of detectable amounts.

6.2.2 *Bacillus coagulans* in general

The D(-)-lactate isomer of lactic acid is not produced in human metabolism, but human exposure can occur from bacterial production²¹, however human cells do not efficiently metabolize and excrete D(-)-lactate. *B. coagulans* does not produce D(-)-lactate.^{22,23}

With the exception of the members of the *Bacillus cereus* group (e.g., *B. cereus*, *B. anthracis*, *B. thuringiensis*), the virulence of members of the *Bacillus* genus may be considered very low, and identified risk factors for *Bacillus* bacteremia include drug addiction, hemodialysis, and leukemia (all of which may contribute to immunosuppression).⁴¹ Based on two retrospective studies investigating *Bacillus* bacteremia, the presence of central venous catheters may increase risk of *Bacillus* bacteremia in immunocompromised patients.^{24,25}

Of 1038 bacteremias occurring in patients of the University of Maryland Cancer Center, Baltimore between January 1978 and June 1986, 24 episodes (2.3%) of *Bacillus* bacteremia were documented, yet only a single case (0.1%) was documented as *B. coagulans* bacteremia.⁴² The classification of the *B. coagulans* bacteremia as a definite clinical infection or possible infection was not reported nor were the specific patient details (e.g., source of infection, signs, symptoms, outcomes). Based on the reported results and previous reports cited and discussed by the authors, no other cases of infection or possible infection from *B. coagulans* have been documented in the English literature dating back to the 1950s, and the majority of cases of *Bacillus* bacteremias were due to *Bacillus cereus*. Thus, *B. coagulans* may be considered non-virulent.

6.3 Allergenicity

Inactivated *B. coagulans* GBI-30, 6086 is manufactured according to the above processes and is labeled as appropriate and required according to allergens.

No reports of allergic reactions to *B. coagulans* were found in our searches of the scientific literature and government databases, including FDA's Safety Information and Adverse Event Reporting Program, MedWatch, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine (accessed June 6, 2016).

7. Justification for Use in Foods

No studies reviewed for this submission, including those described in GRN 000399 and incorporated herein by reference, have revealed a toxicological adverse effect from consumption of *B. coagulans* GBI-30, 6086 or any other toxicologically relevant outcome measures. Toxicological studies have also failed

to identify any target organs. Furthermore, studies have established that *B. coagulans* GBI-30, 6086 is non-pathogenic, non-toxicogenic, and does not harbor transferable antibiotic resistance genes.

The scientific procedures forming the pivotal and corroborative data of the safety assessment (i.e., the technical element of the GRAS standard) for this intended use of Inactivated *B. coagulans* GBI-30, 6086 in foods are published and readily available in the public domain as open access. This published data and the consensus of the Expert Panel satisfy the common knowledge element of the GRAS standard and provide ample evidence that there is reasonable certainty that consumption of Inactivated *B. coagulans* GBI-30, 6086 for its intended use in food up to levels equivalent to approximately 2×10^9 CFU inactivated cells per serving.

8. References

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