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Office of Food Safety Additive Safety (HFS-200) 5/9/2022
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

RE: GRAS Notification for ***Lactobacillus acidophilus* CBT LA1**
II961.1-CBI.1.2

To Whom It Concerns:

In accordance with 21 CFR, Part 170, Subpart E, we as the agent [REJIMUS, INC., 600 W. Santa Ana Blvd. Ste 1100, Santa Ana, CA 92701], respectfully provides notice of a claim that the addition of the microorganism ***Lactobacillus acidophilus* CBT LA1** to the foods identified in this notice at the specified levels is exempt from the premarket approval requirement of the Federal Food, Drug and Cosmetic Act because the notifier [**Cell Biotech Co. Ltd**., 50, Agibong-ro, 409 Beon-gil, Wolgot-myeon, Gimpo, Republic of Korea] has determined that the intended uses are generally recognized as safe (GRAS). The attached documents contain the specific information and data that address the safety of the substance for use in human food applications.

Respectfully,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

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PART 1 – SIGNED STATEMENTS AND CERTIFICATION

Cell Biotech Co. Ltd. submits this notification of a conclusion of GRAS through its agent, REJIMUS, INC. in accordance with 21 CFR §170.30.

Name and Address of Notifier and Agent

Agent:

Jim Lassiter
President/COO
REJIMUS, INC.
600 W. Santa Ana Blvd., Suite 1100
Santa Ana, CA 92701
Tel: +1 (949) 485-2112
www.rejimus.com

Notifier:

Cell Biotech Co. Ltd.
50 Agibong-ro, 409 Beon-gil
Wolgot-myeon, Gimpo
Republic of Korea
Tel: +82 31 987 6205

Name and Address of Manufacturer:

Cell Biotech Co. Ltd.
397 Aegibong-ro
Wolgot-myeon, Gimpo-si, Gyeonggi-do 415-872,
Republic of Korea
Tel: +82 31 987 8107

Name of the GRAS Substance

Cell Biotech Co. Ltd. (herein referred to as CBI) has undertaken an independent safety evaluation of the substance in this notification:

***Lactobacillus acidophilus* CBT LA1**

Intended Conditions of Use in Food

The intended use of *Lactobacillus acidophilus* CBT LA1 is a food ingredient for inclusion in dairy products where standards of identity do not preclude such use. The intended addition level to these foods is up to 1×10^{11} CFU per serving.

Lactobacillus acidophilus CBT LA1 will not be added to meat and poultry products (including soups and soup mixes containing meat or poultry), and will not be included in foods that are marketed towards infants and young children, inclusive of infant formula. *Lactobacillus acidophilus* CBT LA1 is not intended for addition to standardized foods unless it is permitted by the applicable standard of identity.

Basis for GRAS Determination

The statutory basis for conclusion of GRAS status is through scientific procedures in accordance with 21 CFR §170.30(a) and (b).

Premarket Approval Exemption

We have concluded that the intended use of *Lactobacillus acidophilus* CBT LA1 is GRAS for its intended conditions of use as stated in this notification and, such use of *Lactobacillus acidophilus* CBT LA1 is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act.*

Availability of Information

The data and information that serve as the basis of GRAS conclusion are available for review and copying at reasonable times at the offices of the Agent.

Should FDA have any questions of additional requests for information regarding this notification, the Agent shall provide further clarification and/or information at:

Attn: Jim Lassiter
REJIMUS, INC.
600 W. Santa Ana Blvd., Suite 1100
Santa Ana, CA 92701
Email: jim@rejimus.com

Trade Secrets

The notification does not contain trade secrets and the data are not exempt from disclosure under the Freedom of Information Act, 5 U.S.C. Part 552.

Authorization for FDA to share information with FSIS

As Agent for the Notifier, we authorize FDA to send any information deemed necessary to FSIS. The notice does not contain trade secrets and the data are not exempt from disclosure under the *Freedom of Information Act*, 5 U.S.C. 552.

Certification

Cell Biotech Co. Ltd. has concluded that *Lactobacillus acidophilus* CBT LA1 is generally recognized as safe for use in dairy products based on scientific procedures and supported by a history of use in accordance with 21 CFR Part 170, Subpart E. As their Agent, REJIMUS, INC. takes responsibility for all communications on this matter. To the best of our knowledge, this GRAS Notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of *Lactobacillus acidophilus* LA1.

Respectfully submitted,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

PART 2 – IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

Common name: *Lactobacillus acidophilus* CBT LA1 (KCTC 11906BP)

Taxonomic Lineage (Accessed from the Integrated Taxonomic Information System [http:www.itis.gov]):

Kingdom: Bacteria
Subkingdom: Posibacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Lactobacilliaceae
Genus: *Lactobacillus*
Species: *acidophilus*
Strain: CBT LA1

The *Lactobacillus* genus contains over 220 species and is the major genus of the lactic acid bacteria (LAB) group, which produce lactic acid as the major end-product of hexose sugar fermentation (Makarova et. al., 2006). LAB are generally gram-positive, non-sporeforming, facultative anaerobic or microaerophilic, cocci or rod shaped bacteria which occur naturally in and are utilized in fermented dairy and non-dairy product such as fermented vegetables, meats and beverages. They are found wherever substances rich in carbohydrates are available and are generally considered to be non-toxic and non-pathogenic (Bernardeau 2006; Douillard, 2014; Spano 2010).

The genus of LAB are diverse, but commonly include *Lactobacillus, Enterococcus, and Lactococcus,* amongst many others (Lahtinen, 2012). Some *Lactobacillus* species are exclusively found naturally in specific habitats (e.g., *L. helveticus* and *L. delbrueckii* ssp. *bulgaricus* in dairy products, *L. johnsonii* and *L. gasseri* in vertebrate gastrointestinal tracts) whereas other species, such as *L. plantarum* and *L. casei,* may be found in a variety of different environments. In healthy humans, *Lactobacilli* are normally present at a population density of approximately $10^3 – 10^7$ CFU/g in the oral cavity, $10^3 – 10^7$ CFU/g in the ileum, $10^4 – 10^8$ CFU/g the colon, and are the dominant microorganism in the vagina (Bernardeau 2006).

Identification

The organism that is the subject of notified substance, originally isolated from cheese, is identified as *Lactobacillus acidophilus* and has been uniquely characterized as a distinct strain known as CBT LA1 by means of genomic typing. The strain was deposited in the Korean Collection for Type Cultures (KCTC), accession number KCTC 11906BP.

Carbohydrate Utilization

Fermentative characteristics of *Lactobacillus acidophilus* CBT LA1 were analyzed using API 50 CHL kit. Results are shown in Table 1.

Table 1. Fermentative characteristics of *Lactobacillus acidophilus* CBT LA1 obtained with an API 50 CHL Kit. (Cellbiotech R&D Center (2018)).

No	Carbohydrates	Utilized	No	Carbohydrates	Utilized
0	Control	-	25	Esculine	-
1	Glycerol	-	26	Salicine	-
2	Erythritol	-	27	Cellobiose	-
3	D-Arabinose	-	28	Maltose	+
4	L-Arabinose	-	29	Lactose	-
5	Ribose	-	30	Melibiose	-
6	D-Xylose	-	31	Saccharose	+
7	L-Xylose	-	32	Trehalose	-
8	Adonitol	-	33	Inuline	-
9	β-Methyl-xyloside	-	34	Melezitose	-
10	Galactose	+	35	D-Raffinose	-
11	D-Glucose	+	36	Amidon	-
12	D-Fructose	+	37	Glycogene	-
13	D-Mannose	-	38	Xylitol	-
14	L-Sorbose	-	39	β-Gentiobiose	-
15	Rhamnose	-	40	D-Turanose	-
16	Dulcitol	-	41	D-Lyxose	-
17	Inositol	-	42	D-Tagatose	-
18	Mannitol	-	43	D-Fucose	-
19	Sorbitol	-	44	L-Fucose	-
20	α-Methyl-D-mannoside	-	45	D-Arabitol	-
21	α-Methyl-D-glucoside	-	46	L-Arabitol	-
22	N-Acetyl glucosamine	+	47	Gluconate	-
23	Amygdaline	-	48	2-Ceto-gluconate	-
24	Arbutine	-	49	5-Ceto-gluconate	-

Genomic Classification, Sequence and Profile

The 16S rRNA gene sequence were aligned and compared with different *Lactobacillus* strains: *L. acidophilus* (KCTC 11906BP), *L. acidophilus* (ATCC 4356), *L. delbrueckii* (ATCC 9649), *L. rhamnosus* (ATCC 7469), *L. reuteri* (DSM 20016), and *L. plantarum* (ATCC 14917). Percent identity and divergence were compared between *Lactobacillus* species and strains in Table 2. Distinctive sequences of 16S rRNA genes were used to generate the phylogenic tree shown in Figure 1 (Cellbiotech R&D Center 2018).

Random Amplified Polymorphic DNA (RAPD) is a method used to obtain a molecular "fingerprint" from random DNA segments of genomic DNA that have been amplified using a single primer of an arbitrary nucleotide sequence. *L. acidophilus* DNA was compared using RAPD with *Lactobacillus acidophilus* ATCC 4356 strain. Both strains were amplified through PCR, ribotyping and pulsed-field gel electrophoresis (PFGE) in order to compare the RAPD patterns and genotypes between both species (Figure 2). Fragment yields presented difference between strains. DNA fragments were amplified with (GTG) primer (5' – GTGGTGGTGGTGGTG – 3') using genomic DNA as a template and analyzed in 0.8% agarose gel (Syngene, UK).

Pulse Field Gel Electrophoresis (PFGE) digests the genomic DNA with rare-cutting restriction enzymes. Separation of the macrofragments occurs via a continuously reorienting electric field. *Lactobacillus acidophilus* CBT LA1 (KCTC 11906BP) and *L. acidophilus* (ATCC 4356) strains were cultivated to OD_{600}=4 and treated with proteinase K and multiple restriction enzymes. DNA fragments from digestion were analyzed on agarose gel.

Table 2. Percent identity of *Lactobacillus acidophilus* CBT LA1 with some closely related species based on 16S rRNA gene sequences. (Cellbiotech R&D Center 2018).

Percent Identity

Divergence

	1	2	3	4	5	6
1		99.0	90.7	84.3	86.4	83.0
2	0.4		91.6	85.1	87.1	83.8
3	7.3	6.9		84.8	85.3	84.2
4	13.1	12.8	12.8		87.1	90.1
5	12.5	12.1	13.0	9.1		86.3
6	14.7	14.4	13.9	8.3	10.5	

1. *L. acidophilus* (KCTC 11906BP)
2. *L. acidophilus*^{T} (ATCC 4356)
3. *L. delbrueckii*^{T} (ATCC 9649)
4. *L. rhamnosus*^{T} (ATCC 7469)
5. *L. reuteri*^{T} (DSM 20016)
6. *L. plantarum*^{T} (ATCC 14917)

Figure 1. Phylogenetic tree between *Lactobacillus acidophilus* CBT LA1 and other closely related Lactobacillus spp. based on 16S rRNA gene sequence (Cell Biotech Co. Ltd. 2018).

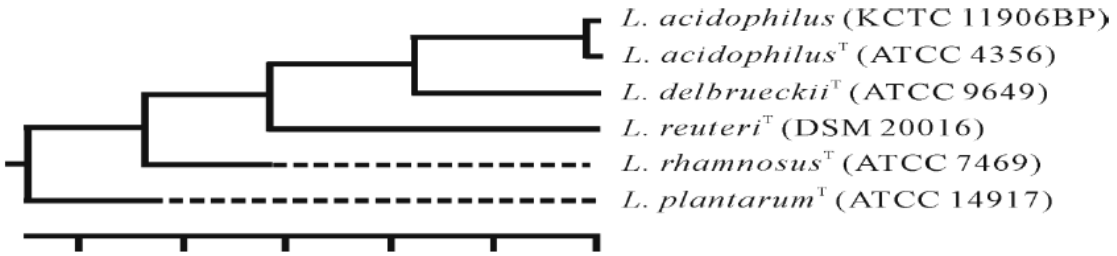
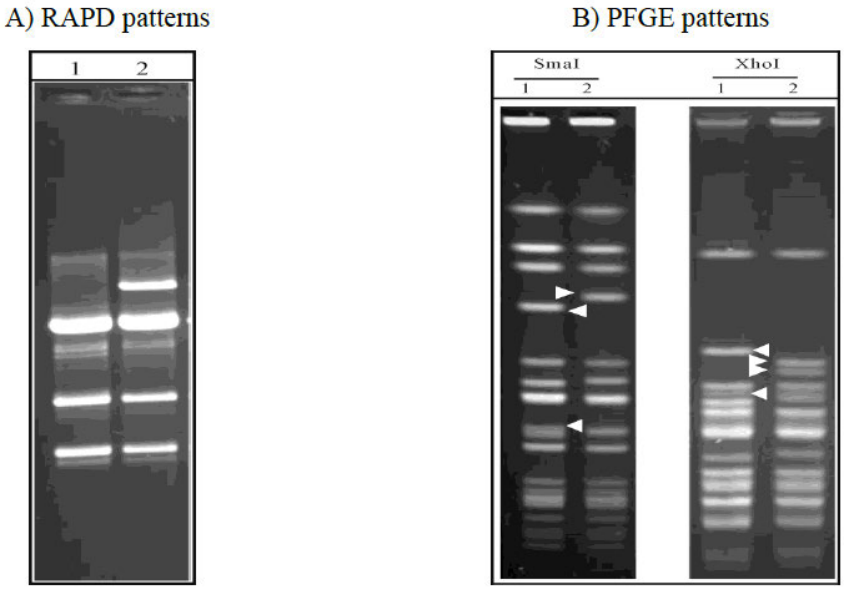


Figure 2. RAPD and PFGE results between *Lactobacillus acidophilus* CBT LA1 - Lane 2 and *Lactobacillus acidophilus* ATCC 4356 – lane 1 (Cellbiotech R&D Center 2018).



Manufacturing

Components

All components employed in the manufacture of *Lactobacillus acidophilus* CBT LA1 are suitably used for one or more effects described within FDA's Substances Added to Food Inventory as identified in Table 3.

Table 3. Identification of the ingredients used in the manufacturing process.

Fermentation Medium Ingredient	**CAS No.**	**Reference**
Fructose	[57-48-7]	21 CFR §184.1866
Soy Protein Isolate	[977076-84-8]	21 CFR §184.1553
Yeast Extract Powder	[8013-01-1]	21 CFR §184.1983
Potassium Phosphate, Dibasic	[7758-11-4]	21 CFR §182.6285
Sodium acetate	[977127-84-6]	21 CFR §184.1721
Magnesium Sulfate	[10034-99-8]	21 CFR §184.1443
Manganese Sulfate	[15244-36-7]	21 CFR §182.5461
L-Cysteine Monohydrate	[7048-04-6]	21 CFR §184.1272
L-Ascorbic acid	[50-81-7]	21 CFR §182.8013
Polysorbate 80	[9005-65-6]	21 CFR §178.3400
Sodium Chloride	[7647-14-5]	21 CFR §182.1
Protease	[9001-92-7]	21 CFR §182.1027
Coating Ingredient	**CAS No.**	**Reference**
Trehalose	[6138-23-4]	FEMA No. 4600 (FEMA GRAS Publication No. 24)
L-Arginine	[74-79-3]	21 CFR §172.320
Potassium Phosphate, Dibasic	[7758-11-4]	21 CFR §182.6285
Potassium Phosphate, Monobasic	[7778-7-0]	21 CFR §175.105
Xanthan Gum	[11138-66-2]	21 CFR §172.695
Cornstarch	[977050-21-3]	21 CFR §182.70 / 21 CFR §182.90
Sodium Carboxymethylcellulose	[9004-32-4]	21 CFR §182.1745
Sodium Chloride	[7647-14-5]	21 CFR §182.1
Excipient	**CAS No.**	**Reference**
Cornstarch	[977050-21-3]	21 CFR §182.70 / 21 CFR §182.90

Process Description and Flow Chart

The flowchart for the manufacturing process through packaging is shown at Figure 3 below.

Preparation of culture medium

All fermentation medium ingredients are blended together. The mixture is then sterilized with saturated steam.

Cultivation

Stock organism is prepared and tested for microbiological contaminants. The stock organism is then inoculated into the prepared medium where it is allowed to propagate. During fermentation, the process is monitored by testing for pH and for change in optical density approximately every two hours. Once the endpoint is reached, bacterial morphology is inspected by microscopy and the organisms are separated via filtration from the culture medium.

Preparation of coating materials

Coating ingredients are added to water, mixed, and sterilized with saturated steam.

Blending

The concentrated organisms, coating mixture, and cornstarch are blended together and then dispensed into trays for freezing.

Drying

Trays containing the blended product are initially quick-frozen and then freeze dried.

Milling

Freeze-dried material is removed from the drying trays, milled, placed in polyethylene bags, passed through a metal detector, and stored as semi-finished product.

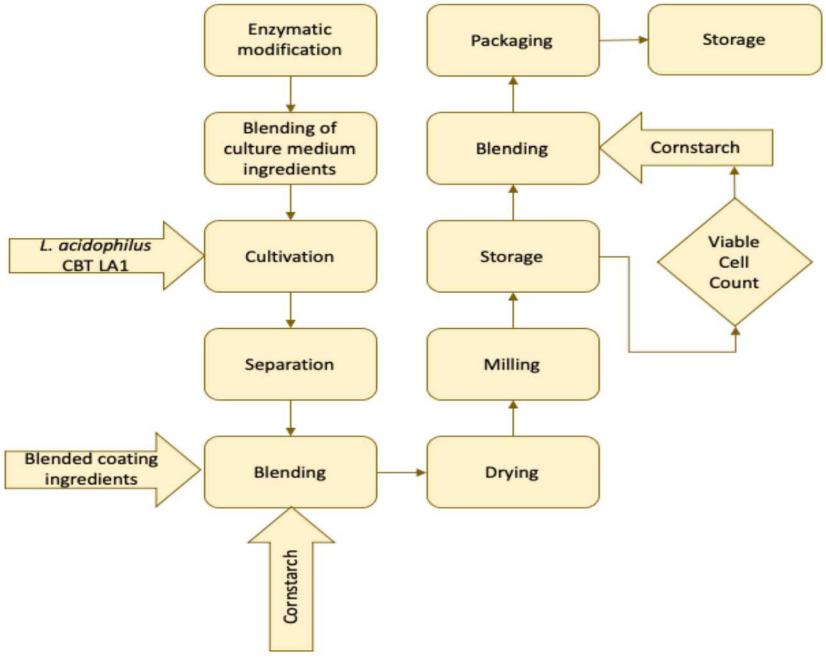
Standardization

The semi-finished product is tested for viable cell count and blended with a corresponding amount of cornstarch to ensure standardized potency.

Packaging

The standardized product is then packaged, passed through a metal detector again, sampled by QC for testing, and stored in a low -temperature warehouse.

Figure 3. Manufacturing process flow chart.



Specifications

Food grade specifications for *Lactobacillus acidophilus* CBT LA1 have been established as shown in Table 4. Test results of three production batches are additionally presented in demonstration of the ability to consistently produce the notified substance in conformance with these specifications. Consistency of conformance to specifications is further evidenced by stability study results.

Table 4. *Lactobacillus acidophilus* CBT LA1 food grade specifications and conforming test results.

Parameter	Limits	Method	Batch 57R	Batch 65R	Batch 80R
Appearance	Light brown powder	Visual	Light brown powder	Light brown powder	Light brown powder
Viable Cell Count	$\geq 1.0 \times 10^{11}$ CFU/g	USP <2022> or equivalent	Conforms	Conforms	Conforms
Coliforms	Absent in 10g	USP <2023> or equivalent	Conforms	Conforms	Conforms

Stability Data

In order to determine the stability of *Lactobacillus acidophilus* CBT LA1, the food ingredient was placed in a stability study by Cell Biotech Co. Ltd.

A 12-month stability study was conducted at 5 ± 3 °C using 3 different batches of *Lactobacillus acidophilus* CBT LA1. At each time point, samples were analyzed in triplicate using 3 different analysts; the results of viable cell count assays are averaged and summarized in Table 5. Coliform testing was additionally performed by each analyst at all time points, the results of which are negative for all samples. Appearance test was performed by each analyst at all time points, the results of which were of a light brown powder.

Table 5. Viable cell count and percent survival rate of *Lactobacillus acidophilus* CBT LA1 at 5 ± 3 °C.

Strain	Batch No.	Test	Time Point				
			Initial	3 Months	6 Months	9 Months	12 Months
***Lactobacillus acidophilus* CBT LA1**	57R	VCC (CFU/g)	3.75×10^{11}	3.40×10^{11}	3.04×10^{11}	2.74×10^{11}	2.52×10^{11}
		Survival Rate (%)	100.0	90.8	81.2	73.1	67.2
	22R	VCC (CFU/g)	5.10×10^{11}	4.73×10^{11}	4.34×10^{11}	4.13×10^{11}	3.67×10^{11}
		Survival Rate (%)	100.0	92.8	85.2	81.1	72.1
	26R	VCC (CFU/g)	4.77×10^{11}	4.36×10^{11}	4.12×10^{11}	3.69×10^{11}	3.24×10^{11}
		Survival Rate (%)	100.0	91.5	86.4	77.4	67.9
	Average Survival Rate (%)		100.0	91.7	84.3	77.2	69.1

Technical Effects

This substance will be used to provide as a dietary source of *Lactobacillus acidophilus* CBT LA1 as a food ingredient to dairy products.

PART 3 - DIETARY EXPOSURE

Intended Use and All Sources in Diet

The intended use of *Lactobacillus acidophilus* CBT LA1 is as a food ingredient for inclusion in dairy products to provide at least 1×10^{11} CFU per serving.

The consensus of an international scientific expert panel categorized live microorganisms for human use as defined in Table 6. The panel suggested a minimum level of 1×10^9 CFU of LAB per serving to be the minimum criteria in support a claim of "contains live and active cultures." (Hill 2014)

Table 6. Categories of live microorganisms for human use (Hill et al. 2014).

Description	Claim	Criteria*	Minimum level of evidence required to make claim	Comments
Not probiotic				
Live or active cultures	"Contains live and active cultures"	Any food fermentation microbe(s) Proof of viability at a minimum level reflective of typical levels seen in fermented foods, suggested to be 1×10^9 CFU per serving[73]	No product-specific efficacy studies needed	The terms 'live' or 'active' do not imply probiotic activity Fermented foods containing live cultures might also qualify as a 'probiotic' if they meet the criteria for that category (e.g. evidence that yogurt can improve lactose digestion in lactose maldigesters would qualify it as a 'probiotic'[74,75])
Probiotic				
Probiotic in food or supplement without health claim	"Contains probiotics"	A member(s) of a safe[76,77] species, which is supported by sufficient evidence of a general beneficial effect in humans OR a safe microbe(s) with a property (e.g. a structure, activity or end product) for which there is sufficient evidence for a general beneficial effect in humans Proof of viability at the appropriate level used in supporting human studies[73]	Well-conducted human studies (e.g. these could involve RCT(s), observational studies, systematic reviews or meta-analyses supporting the observed general beneficial effect for the taxonomical category concerned) The evidence does not have to be generated for the specific strain included in the product	Extrapolation of evidence must be based on reasonable expectations that the strain(s) incorporated in the product would have similar general beneficial effects in humans This evidence could be based on taxonomical or functional comparisons
Probiotic in food or supplement with a specific health claim	Specific health claim, such as "helps to reinforce the body's natural defences in children" or "helps reduce the risk of antibiotic-associated diarrhoea"	Defined probiotic strain(s) Proof of delivery of viable strain(s) at efficacious dose at end of shelf-life[73]	Convincing evidence needed for specific strain(s) or strain combination in the specified health indication Such evidence includes well-conducted studies in humans, including: positive meta-analyses on specific strain(s) or strain combinations, as per principles outlined by Cochrane,[78] PASSCLAIM,[79] or GRADE;[80] well-conducted RCT(s) OR strong evidence from large observational studies[81]	Well-designed observational studies are useful to detect the effect of foods on health in 'real life', that is, outside the controlled environment of an RCT (e.g. data on health benefits by dietary fibre are mostly observational) Sample sizes must be large enough to manage confounding factors
Probiotic drug	Specific indication for treatment or prevention of disease, such as "useful for the prevention of relapse of ulcerative colitis"	A defined strain(s) of live microbe Proof of delivery of viable probiotic at efficacious dose at end of shelf-life Risk–benefit assessment justifies use	Appropriate trials to meet regulatory standards for drugs	What constitutes a drug claim varies among countries

*Unless otherwise indicated, all criteria indicated must be met. Abbreviations: CFU, colony forming unit; GRADE, Grades of Recommendation Assessment, Development and Evaluation; PASSCLAIM, Process for the Assessment of Scientific Support for Claims on Food; RCT, randomized controlled trial.

Consumption Data

Based on the food consumption data reported in the most recent National Health and Nutrition Examination Survey (NHANES 2017-2018) dataset compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics, and the Nutrition Coordinating Center, the EDIs of dairy products were determined by several age groups.

The intended use of at least 1.0×10^{11} CFU per serving in dairy products would result in intakes in all users of 8.94×10^{10} CFU and 1.85×10^{11} CFU per person per day in the mean and 90th percentile, respectively (Table 7). A maximum exposure would occur in male adults with a 90th percentile EDI of 2.05×10^{11} per person per day.

Table 7. EDIs of *Lactobacillus acidophilus* CBT LA1 from proposed uses in dairy products across all users based on 2017-2018 NHANES.

Group	% (n)	Dairy intake g/day		Dairy, serving/day		*Lactobacillus acidophilus* CBT LA1, cfu/day	
		Mean	90th percentile	Mean	90th percentile	Mean	90th percentile
Children, 3-11	74.04 (739)	360.44	456.85	0.97	1.87	9.74×10^{10}	1.87×10^{11}
Females, 12-19	42.44 (191)	186.02	362.90	0.76	1.49	7.62×10^{10}	1.49×10^{11}
Males, 12-19	54.73 (243)	265.10	477.28	1.09	1.96	1.09×10^{11}	1.96×10^{11}
Females, 20 and up	38.21(826)	179.05	360.87	0.73	1.48	7.34×10^{10}	1.48×10^{11}
Males, 20 and up	44.06(871)	222.93	499.63	0.91	2.05	9.13×10^{10}	2.05×10^{11}
All users	47.61(3161)	218.16	452.44	0.89	1.85	8.94×10^{10}	1.85×10^{11}

Assuming all servings of the intended dairy products consumed contain *Lactobacillus acidophilus* CBT LA1, the suggested three daily servings would result in a cumulative exposure of 2.68×10^{11} CFU per day ($8.94\times10^{10} \times 3$). The estimated 90th percentile of consumers of dairy products at this level of recommended consumption adjusted for the findings of the per capita data would potentially be exposed to up to 5.55×10^{11} CFU per day *Lactobacillus acidophilus* CBT LA1. The LD_{50} identified is the uppermost safety point that has been studied to date. The study presented by CBI R&D Center (2018) demonstrated that > 10^{11} CFU/kg was still safe for the rats at that dosage. In point of fact, no true LD_{50} nor NOAEL has ever been determined for this organism. This is due to the fact that an amount of organism greater than this cannot feasibly be administered to the rats.

The LD_{50} of greater than 10^{11} CFU/kg from the animal studies from the Cell Biotech R&D Center corresponds to the human equivalent dose of 9.6×10^{11} CFU in a 60 kg human (using the animal-specific body surface area-based conversion factor presented in the Center for Drug Evaluation and Research's Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers 2005). Therefore, even if the general population consumers of dairy products were to meet these guidelines, the recommended levels of the cumulative exposure of 2.68×10^{11} CFU per day and the cumulative exposure at an estimated 90th percentile of 5.55×10^{11} CFU per day is less than the LD_{50} levels of greater than 10^{11} CFU/kg (or 9.6×10^{11}) of *Lactobacillus acidophilus* CBT LA1.

Substances Expected to Be Formed in Food

Under the intended conditions of use, there are no substances expected to be formed in the foods in which *Lactobacillus acidophilus* CBT LA1 is included. The metabolic by-products from *Lactobacillus acidophilus* CBT LA1 do not go beyond the expected fermentation products from any of the other LAB microorganisms. These include lactic acid, carbon dioxide and the ATP necessary for the cell. *Lactobacillus acidophilus* CBT LA1 is not known to secrete any exotoxins or any other substances that are classified as harmful to humans. Additionally, the number of viable organisms will decline during a product's shelf life to further minimize the exposure to any of the metabolic by-products.

Substances Naturally Present or Due to Manufacturing

Any remaining ingredients used to produce the fermentation media should have little to no presence in the overall finished output and, therefore, the EDIs for these ingredients were not determined or calculated.

The coating ingredients and excipients used in the manufacturing process are listed in FDA's Substances Added to Food Inventory for various uses:

- Trehalose is listed as a flavoring agent or adjuvant.
- L-arginine is listed as a nutrient supplement.
- Potassium phosphate, dibasic is listed as an emulsifier or emulsifier salt, nutrient supplement, pH control agent, sequestrant, or stabilizer or thickener.
- Potassium phosphate, monobasic is listed as malting or fermenting aid, nutrient supplement, pH control agent, or stabilizer or thickener.
- Xanthan gum is listed as an anticaking agent or free-flow agent, color or coloring adjunct, drying agent, emulsifier or emulsifier salt, formulation aid, processing aid, solvent or vehicle, stabilizer or thickener, surface-finishing agent, or texturizer.
- Cornstarch is listed as an anticaking agent or free-flow agent, drying agent, flavoring agent or adjuvant, formulation aid, humectant, non-nutritive sweetener, nutritive sweetener, solvent or vehicle, stabilizer or thickener, or texturizer.
- Sodium carboxymethylcellulose is listed as an anticaking agent or free-flow agent, drying agent, emulsifier or emulsifier salt, formulation aid, processing aid, humectant, stabilizer or thickener, or texturizer.
- Sodium chloride is listed as an anticaking agent or free-flow agent, antimicrobial agent, color or coloring adjunct, emulsifier or emulsifier salt, firming agent, flavoring agent or adjuvant, formulation aid, nutrient supplement, solvent or vehicle, stabilizer or thickener.

PART 4 – SELF-LIMITING LEVELS OF USE

There is no recognized self-limiting level of use for this organism. Issues of palatability of the substance are not present at the levels of inclusion identified.

PART 5 – EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

As the conclusion of general recognition of safety is through scientific procedures, this Part is not applicable. Information about the current international marketplace availability of products containing *Lactobacillus acidophilus* CBT LA1 as an ingredient is discussed as part of the scientific procedures upon which the general recognition of safety is based. Nevertheless, the historical use of foods fermented with *Lactobacilli* and specifically *Lactobacillus acidophilus* is discussed in Part 6.

PART 6 – NARRATIVE

Introduction

Fermented foods have a long history of consumption in the human population, with some of the earliest records of such in Southeast Asia and Africa (Nout 1992). Prevalence of fermented foods is much higher in some parts of the world outside the U.S., such as in Sudan where it seems the majority of foods are prepared and preserved by fermentation (Dirar 1992).

Used as an inexpensive means throughout the world, lactic acid-producing bacteria (LAB) are one major group of microorganisms used to process milk, meat, and various plant material like vegetables, cereals, and legumes into fermented foods that undergo flavor and nutritive profile changes from their original forms as well as gain the benefit of improved stability (Steinkraus 1992). By preventing the formation of pathogenic and spoilage organisms, fermented foods have an increased shelf life and decreased potential for causing food poisoning (Hesseltine 1981).

In the United States, LAB in general are permitted for use in several standardized foods. A variety of cheeses, whose requirements are found within 21 CFR Part 133—Cheeses and Related Cheese Products, include the use of these and other types of bacterial cultures. LAB are also used in the production of Sour Cream [§131.160], are optional ingredients for use in Bread, Rolls, and Buns [§136.110(c)(10)], and may be used as characterizing microbial organisms or as microbial cultures to produce aroma and flavor in the production of Acidified Milk [§131.111] and Cultured Milk [§131.112].

History of GRAS Notifications

There is a history of successfully notified GRAS substances intended for inclusion in foods dating back to 2002 (GRAS No. 49).

GRAS notices of food ingredient substances containing the same species as *Lactobacillus acidophilus* CBT LA1 to which FDA has no questions are presented below in Table 8. These GRAS notices reference and

address a large body of established scientific procedures evidencing the safe and common use of various strains of *Lactobacillus acidophilus* and its subspecies. GRAS notices of *Lactobacillus* organisms of species other than acidophilus which FDA has no questions are presented below in Table 9.

Table 8. GRAS notices containing *Lactobacillus acidophilus* receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).

GRAS No.	Date of Closure	Substance
502	19-Aug-14	*Lactobacillus acidophilus* strain La-14
378	26-Mar-12	Cultured dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources fermented by *Streptococcus thermophilus, Bacillus coagulans, Lactobacillus acidophilus, L. paracasei* subsp. *paracasei, L. plantarum, L. sakei, L. bulgaricus and Proprionibacterium freudenreichii* subsp. *shermanii* or mixtures of these strains.
357	19-Apr-11	*Lactobacillus acidophilus* strain NCFM
171	7-Dec-05	*Lactobacillus acidophilus, L. lactis*, and *Pediococcus acidilactici*

Table 9. GRAS notices of *Lactobacillus* organisms of species other than *acidophilus* receiving reply from FDA of no questions (GRAS Notices Inventory Database).

GRAS No.	Date of Closure	Substance
847	30-Sep-2019	*Lactobacillus plantarum* ECGC 13110402
840	27-Aug-2019	*Lactobacillus paracasei* strain F19
810	05-Apr-2019	*Lactobacillus paracasei* subsp. *paracasei* strain F-19e
758	20-Aug-2018	*Lactobacillus helveticus* R0052
722	16-Feb-2018	*Lactobacillus plantarum* Lp-115
685	31-Oct-2017	*Lactobacillus plantarum* strain 299v
531	14-Aug-2014	*Lactobacillus fermentum* CECT5716
502	27-Feb-2014	*Lactobacillus acidophilus* La-14
440	16-Aug-2012	*Lactobacillus reuteri* strain NCIMB 30242
410	16-Nov-2011	*Lactobacillus reuteri* strain DSM 17938
357	19-Apr-2011	*Lactobacillus acidophilus* NCFM
288	27-Mar-2009	*Lactobacillus rhamnosus* strain HN001
281	31-Aug-2009	*Lactobacillus rhamnosus* strain HN001 produced in a milk-based medium
254	18-Nov-2008	*Lactobacillus reuteri* strain DSM 17938

Approved Use

The status of *Lactobacillus acidophilus* in Canada involves the accepted use of the microorganism in food products. Specific claims may be made about these products when the level of use is a minimum of 1×10^9 CFU per serving.

In Europe, *Lactobacillus acidophilus* is commonly used to ferment dairy products producing foods with improved flavor and texture (Hill, 2018). The addition is typically as a non-primary LAB for commercial purposes in producing such foods.

In a December 12th, 2019 update to their Qualified Presumption of Safety list, the European Food Safety Authority confirmed *Lactobacillus* spp. (including *L. acidophilus*) presence in and inventory of recommended biological agents intentionally added to food or feed based on review of latest applicable literature.

Antibiotic Resistance

Determination of the minimal inhibitory concentration (MIC) of select antibiotics [ampicillin (AMP), gentamycin (GEN), kanamycin (KAN), streptomycin (STM), erythromycin (ERM), clindamycin (CLM), tetracycline (TET), and chloramphenicol (CP)] was performed in accordance with ISO 10932:2010 using *Lactobacillus acidophilus* CBT LA1 as the test strain. Observed MIC values for *Lactobacillus acidophilus* CBT LA1 were determined to be lower than the cut-off values prescribed by 2012 Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance published by the European Food Safety Authority (EFSA), as shown in Table 10 and this strain is therefore susceptible to AMP, VAN, GEN, KAN, STM, ERM, CLM, TET, and CP.

Table 10. Antibiotic sensitivity of *Lactobacillus acidophilus* CBT LA1.

Strain	**Minimum Inhibitory Concentrations (µg/mL) of Antibiotics**								
	AMP	VAN	GEN	KAN	STM	ERM	CLM	TET	CP
***L. acidophilus* CBT LA1**	< 0.5	< 2	< 16	< 0.5	< 16	< 0.5	< 1	< 4	< 2
EFSA Cut-off Value	1	2	16	64	16	1	1	4	4

Current Marketplace Availability of *Lactobacillus acidophilus* CBT LA1

While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of Lactobacillus acidophilus CBT LA1 in foreign countries and in multiple food products.

In vitro Toxicity Studies

Hemolysis Assay

The Cell Biotech R&D Center tested *Lactobacillus acidophilus* CBT LA1 for its hemolytic activity by inoculating microorganism in MRS agar supplemented with 5% horse blood and incubated under anaerobic conditions. The test showed no hemolytic activity.

Animal Studies

The pathogenicity and acute toxicity of *Lactobacillus acidophilus* CBT LA1 were investigated using male and female Sprague-Dawley rats (5 of each sex in each group). The animals were intragastrically administered either 0.85% saline solution or 1×10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 and observed for the ensuing 14 days. The net body weight gain, gross pathological findings, feed and water consumption, organ weight, and body temperature were monitored and recorded for two (2) weeks.

This investigation revealed no mortalities or obvious adverse clinical signs in rats administered with the live bacterial cells at the investigated dose level as shown on Table 11. In addition, results indicate no significant differences in net body weight gain (Figure 4), gross pathological findings (Table 12), feed and water consumption (Figure 5), organ weight (Table 13), and body temperature (Table 14) among the different treatment groups and between the treated and control rats (Cell Biotech R&D Center 2018).

Table 11. Mortality of male and female rats orally administered with 1×10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 (Cellbiotech R&D Center 2018).

Sex	LAB Strain	Days After Administration														Final Mortality (%)	LD_{50}
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Male	CBT LA1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$> 1 \times 10^{11}$ CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Female	CBT LA1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$> 1 \times 10^{11}$ CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Figure 4. Body weight curves for male and female rats given 10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 and control for 14 days. Values are mean ± SE. (Cellbiotech R&D Center 2018).

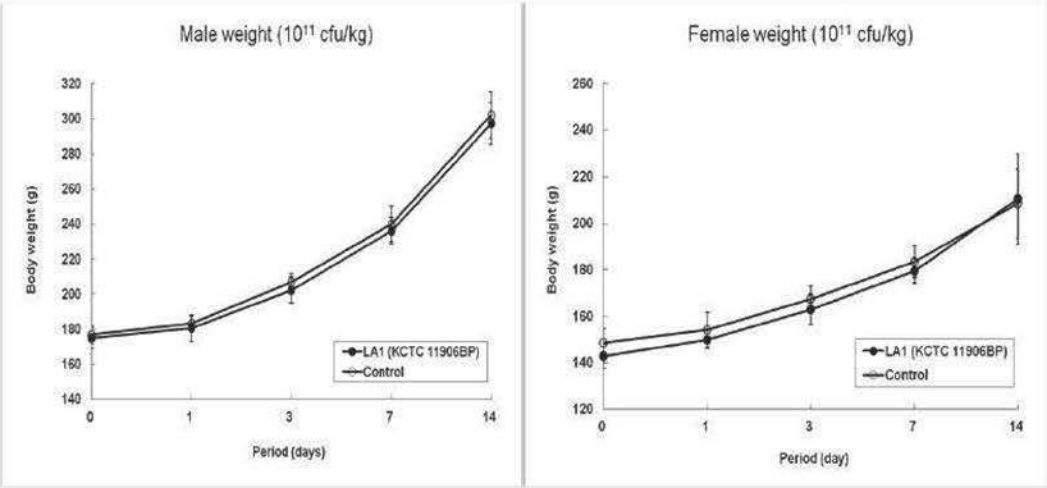


Table 12. Clinical findings of male and female rats orally administered with 10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 ((Cell Biotech R&D Center 2018)).

Sex	LAB Strain	Clinical Signs	Hours after treatment				Days after treatment				
			1	2	5	6	1	3	5	7	14
Male	CBT LC5	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Control	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Female	CBT LC5	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Control	NAD	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

NAD: No abnormality detected

Figure 5. Food and water consumption of male and female rats given 10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 and control for 14 days. ((Cell Biotech R&D Center 2018)).

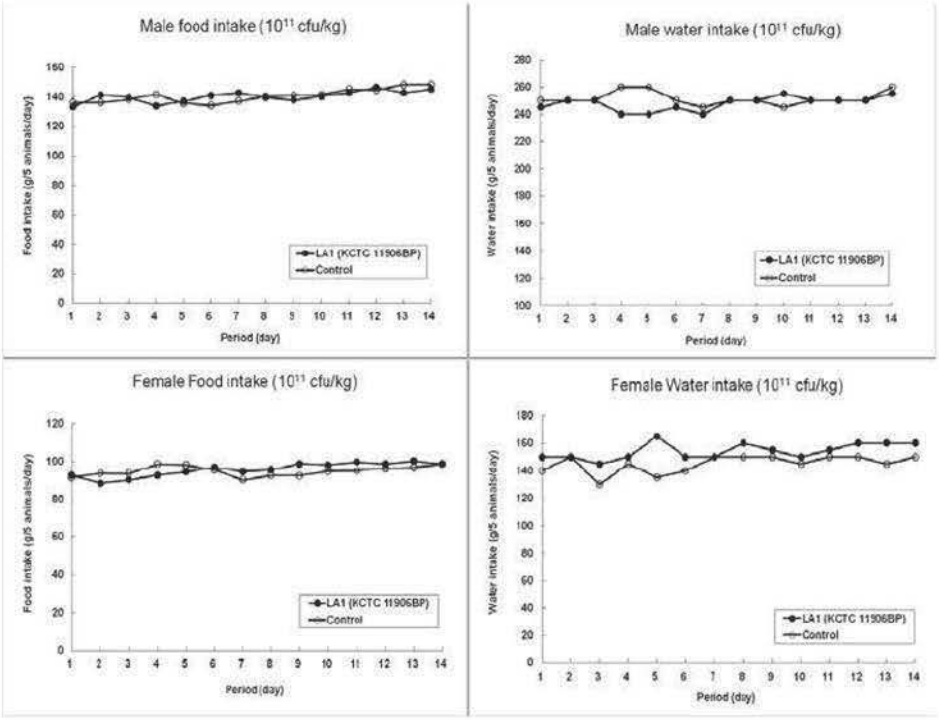


Table 13. Absolute organ weights (g) of male and female orally administered with 10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 ((Cell Biotech R&D Center 2018)).

Sex	Parameters	Lab	CBT LA1	Control
		No. of Animals	5	5
Male	Body weight (g)		297.42 ± 12.02	302.15 ± 13.24
	Liver (g)		10.94 ± 0.56	12.21 ± 0.56
	Spleen (g)		0.66 ± 0.07	0.70 ± 0.11
	Kidney (g)	Right	1.13 ± 0.10	1.12 × 0.05
		Left	1.17 ± 0.08	1.18 ± 0.12
Female	Body weight (g)		210.54 ± 19.43	208.47 ± 14.94
	Liver (g)		7.94 ± 0.82	7.65 ± 1.15
	Spleen (g)		0.42 ± 0.10	0.41 ± 0.08
	Kidney (g)	Right	0.83 ± 0.04	0.78 ± 0.12
		Left	0.86 ± 0.07	0.77 ± 0.10

Table 14. Body temperature changes in male and female orally treated with 10^{11} CFU/kg *Lactobacillus acidophilus* CBT LA1 ((Cell Biotech R&D Center 2018)).

Day	No.	Male body temperature		Female body temperature	
		CBT LA1 (°C)	Control (°C)	CBT LA1 (°C)	Control (°C)
Pre-treatment	Ave	34.34	34.82	35.16	35.40
	SEM	0.48	0.39	0.32	0.24
Day 1	Ave	34.94	34.62	34.98	35.30
	SEM	0.64	0.38	0.38	0.80
Day 2	Ave	34.80	34.52	35.60	35.44
	SEM	0.32	0.68	0.62	0.45
Day 3	Ave	34.80	35.86	34.50	35.36
	SEM	0.45	0.34	0.63	0.48
Day 4	Ave	35.32	35.26	35.06	35.26
	SEM	0.50	0.33	0.27	0.33

Human studies

Study 1

Kwak et al. (2014) studied the effects of short-term microbial therapy in alleviating small intestine bacterial overgrowth (SIBO) and intestinal permeability in chronic liver disease. Fifty-three patients with chronic liver disease were given either microbial therapy, which included *Lactobacillus acidophilus* CBT LA1, or a placebo. Results show that the 4-week microbial treatment was effective in alleviating SIBO symptoms but did not improve intestinal permeability and liver function. Monitoring of adverse events was not included in the study design, and so none were reported in the published article.

Study 2

Forty participants aged 60 years or older were randomly assigned to take a capsule containing multiple bacterial strains (2.5 × 108 viable cells), which included *Lactobacillus acidophilus* CBT LA1, to assess efficacy in functional constipation. Following a 2-week period, results indicate there were no overall changes in body mass index, weight, or overall health. The study conducted by Yeun et al. (2014) demonstrated that the multi-organism treatment has a positive effect in alleviating constipation. There were no adverse effects noted as a result of consuming the microorganism mixture.

Study 3

A randomized, double-blind, placebo-controlled trial enrolled forty-nine patients suffering from irritable bowel syndrome (IBS) to determine the effect of multispecies microorganisms on this condition. Twenty-five patients were given a capsule containing 5×10^9 viable cells of 6 microbial strains in equal measure, including *Lactobacillus acidophilus* CBT LA1, twice daily for four weeks.

All of the active arm participants in the study appeared to tolerate the capsules well with no adverse events reported. Significantly more patients experienced global relief of IBS symptoms in the microbial study group than in the placebo group (Yoon et al. 2014).

Study 4

Lee et al. (2014) studied the effects of co-administration of specific microorganisms with herbal medicine compared to an herbal medicine alone on obesity, metabolic endotoxemia, and dysbiosis in a randomized, double-blind, placebo-controlled clinical trial. A total of 50 female patients from 19 to 65 years of age were enrolled in the study with the active group given a capsule containing 5×10^9 CFU of 7 microorganisms, including *Lactobacillus acidophilus* CBT LA1, twice daily for eight weeks. A significant difference in HDL cholesterol levels were noted between the groups, with the microorganisms apparently well tolerated.

Study 5

Yoon et al. (2015) conducted a trial on the effect of administering a 6-species microbial mixture, including *Lactobacillus acidophilus* CBT LA1, on changes in fecal microbiota and symptoms of irritable bowel syndrome. 81 patients completed the study with 39 of them having ingested 2 capsules containing 5×10^9 viable cells daily for 4 weeks. The study concluded that most of the intestinal flora strain fecal concentrations increased in patients and that the diarrhea symptom score was improved for the active group with no adverse effects noted.

Study 6

Ipar et al. (2015) reported the effects of a synbiotic on anthropometric measurements, lipid profile, and oxidative stress in obese children in an open-label, randomized, controlled study. 77 children ranging in ages from 4 to 17 years were enrolled in the study. Each of the children in the study were given a sachet containing 4.3×10^8 CFU of *Lactobacillus acidophilus* in a 5-species synbiotic mixture, with vitamins and fructooligosaccharide, to be ingested once daily for one month. No adverse effects were reported from the 35 patients in the active group who completed the study.

Study 7

Dinleyici et al. (2012) conducted a single-blind, randomized clinical trial on the effect of a five-species synbiotic mixture with vitamins and fructooligosaccharide, on the duration of diarrhea and length of hospital stay in children with acute diarrhea. The patients included 43 girls age 3 months to 10 years and 70 boys in the same age bracket.

The study material contained a dose of 2.5×10^9 CFU bacteria, including *Lactobacillus acidophilus*, given once daily for five days in addition to oral rehydration salts and/or intravenous therapy. The 113 patients given the microbial therapy tolerated it well with no adverse events reported.

Conclusion

The scientific data, information, methods, and principles described in this notification provide the basis for conclusion that *Lactobacillus acidophilus* CBT LA1 is generally recognized among qualified experts to be safe for inclusion in the food types described in the amounts noted. The historic safe use of *Bifidobacterium longum* in the food supply along with the evaluation of the consumption data serve as the foundation on which the safety of this uniquely identified strain is established.

Inclusion of *Lactobacillus acidophilus* and other lactic acid-producing bacteria is identified and sometimes mandated in FDA regulations surrounding standards of identity for select food types. FDA has also responded with no questions to numerous GRAS notices submitted for other strains of *Lactobacillus acidophilus*, other species of *Lactobacillus*, as well as members of other genera of lactic acid-producing bacteria, intended for inclusion as food ingredients. The applicable GRAS notices, referenced in Table 8 and Table 9 within Part 6 of this notice, incorporate myriad studies demonstrating the safety of ingestion of substances closely related to *Lactobacillus acidophilus* CBT LA1.

Lactobacillus acidophilus CBT LA1 is well characterized genetically, taxonomically known as an organism lacking potential for harm, and supported by analyses conducted by Cell Biotech R&D Center (2018) in demonstration of its safety and elucidation of its genotypic and phenotypic traits. The substance's potential for pathogenicity and acute toxicity tested negative. *Lactobacillus acidophilus* CBT LA1's potential for antibiotic resistance was tested in accordance with EFSA guidelines.

Additional efficacy studies in humans and animals have been performed without the occurrence of observation of *adverse* events. An LD_{50} of greater than 10^{11} CFU/kg was established in rats which corresponds to a human equivalent amount of 9.6×10^{11} CFU in a 60kg human (using the animal-specific body surface area-based conversion factor presented in the Center for Drug Evaluation and Research's Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers [2005]). The estimated level of cumulative daily intake of *Bifidobacterium longum* CBT BG7 at the 90th percentile of high-level consumers of products of the intended inclusion food is 5.55×10^{11} CFU per day of *Lactobacillus acidophilus* CBT LA1. The 90th percentile for actual consumption of 5.55×10^{11} CFU/day is below the maximum safe starting dose of 9.6×10^{11} CFU/serving.

All data and information pertaining to the studies performed on the material, in-house documentation, and additional information were made available to the Expert Panel, and their findings reflect review of the totality of the information used in the preparation of this notice as shown on the Expert Panel Endorsement pages.

PART 7 – SUPPORTING DATA AND INFORMATION

Generally Unavailable

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Cell Biotech Co. Ltd. *Lactobacillus acidophilus* CBT LA1

February 25, 2021

Cell Biotech Co. Ltd. intends to market ***Lactobacillus acidophilus* CBT LA1** as an ingredient in dairy products. ***Lactobacillus acidophilus* CBT LA1** is produced by growth of a certified source strain of the organism in an appropriate medium. The strain is verified prior to inoculation of the medium. The resultant microorganism is freeze-dried for use in dairy products.

The use of this microorganism in the production of food products is historic. The application of the specific strain ***Lactobacillus acidophilus* CBT LA1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Lactobacillus acidophilus* CBT LA1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. (Foodwise), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY STEVEN DENTALI, PH.D.

I, Steven Dentali, hereby affirm that ***Lactobacillus acidophilus*** **CBT LA1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature:__  Date: 17 March 2021

Steven Dentali, Ph.D.
Dentali Botanical Sciences

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Cell Biotech Co. Ltd. *Lactobacillus acidophilus* CBT LA1

February 25, 2021

Cell Biotech Co. Ltd. intends to market ***Lactobacillus acidophilus* CBT LA1** as an ingredient in dairy products. ***Lactobacillus acidophilus* CBT LA1** is produced by growth of a certified source strain of the organism in an appropriate medium. The strain is verified prior to inoculation of the medium. The resultant microorganism is freeze-dried for use in dairy products.

The use of this microorganism in the production of food products is historic. The application of the specific strain ***Lactobacillus acidophilus* CBT LA1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Lactobacillus acidophilus* CBT LA1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. (Foodwise), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY JEANNE MOLDENHAUER, M. SC.

I, Jeanne Moldenhauer, hereby affirm that ***Lactobacillus acidophilus* CBT LA1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature: 

Date: 6 APR 21

Jeanne Moldenhauer, M. Sc.
Excellent Pharma Consulting

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The use of this microorganism in the production of food products is historic. The application of the specific strain ***Lactobacillus acidophilus* CBT LA1** identified in this dossier is further demonstrated in this submission as Generally Recognized as Safe through support from the application of scientific procedures evaluating the safety of the item.

At the request of Cell Biotech Co. Ltd., a panel of independent scientists (the "Expert Panel"), qualified by their relevant national experience, education and training, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of ***Lactobacillus acidophilus* CBT LA1** as an ingredient in dairy products is safe, suitable, and would be Generally Recognized as Safe (GRAS) based on a combination of historic use and scientific procedures. The Expert Panel consisted of following experts: Steven Dentali, Ph.D. (Dentali Botanical Sciences), Mary C. Mulry, Ph.D. CFS (FoodWise One LLC), and Ms. Jeanne Moldenhauer, M.Sc. (Excellent Pharma Consulting).

The Expert Panel, independently and collectively, evaluated the dossier inclusive of the following:

Basis for GRAS Determination	Narrative Summary
Claim Regarding GRAS Status	Determination of the Expert Panel
Manufacturing Process	Summary and Diagrams
Stability Data	Data and Presentation
Dietary Exposure	Summary of intended exposure
Basis for Determination	Discussion of studies
Public and Private Studies	Supporting studies included

In addition, the Expert Panel evaluated all other information deemed necessary and/or sufficient in order to arrive at its independent, critical evaluation of these data and information. The Expert Panel has attained a unanimous conclusion that the intended uses described herein for Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1**, meeting appropriate food-grade specifications as described in the supporting dossier, as a dairy ingredient is identified as Generally Recognized as Safe (GRAS) by Self-determination for use as a food ingredient across a range of food categories identified in the dossier. Such dairy products that include Cell Biotech Co. Ltd. ***Lactobacillus acidophilus* CBT LA1** in accordance with the described applications and levels specified in the dossier, manufactured according to current Good

Manufacturing Practice (cGMP), are safe for human consumption. These determinations are made based on a combination of historic use of the microorganism in food products with support from scientific procedures.

The individual endorsement pages follow hereunder.

ENDORSEMENT BY MARY C. MULRY, PH.D. CFS

I, Mary Mulry, hereby affirm that ***Lactobacillus acidophilus* CBT LA1** is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature: [redacted] Date: 3/18/21

Mary C. Mulry, Ph.D. CFS
FoodWise One LLC

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: 2021-12-06

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Myung-jun Chung	CEO

Organization *(if applicable)*: Cell Biotech Co. Ltd.

Mailing Address *(number and street)*: 50 Agibong-ro, 409 Beon-gil

City	State or Province	Zip Code/Postal Code	Country
Wolgot-myeon, Gimpo	Gyeonggi-do		Korea, Republic of

Telephone Number	Fax Number	E-Mail Address
+82 31 987 6205		ceo@cellbiotech.com

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Jim Lassiter	COO

Organization *(if applicable)*: REJIMUS, INC.

Mailing Address *(number and street)*: 600 W Santa Ana Blvd Suite 1100

City	State or Province	Zip Code/Postal Code	Country
Santa Ana	California	92701	United States of America

Telephone Number	Fax Number	E-Mail Address
9492290072		jim@rejimus.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Lactobacillus acidophilus CBT LA1

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

1 DVD+R

3. For paper submissions only:

Number of volumes 1

Total number of pages 34

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

The intended use of Lactobacillus acidophilus CBT LA1 is a food ingredient for inclusion in dairy products where standards of identity do not preclude such use. The intended addition level to these foods is up to 1 × 10^11 CFU per serving.

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☒ Yes ☐ No

Did you include this other information in the list of attachments?

☒ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Cell Biotech Co. Ltd.
(name of notifier)

has concluded that the intended use*(s)* of Lactobacillus acidophilus CBT LA1
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Cell Biotech Co. Ltd. agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them;
(name of notifier)

agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

50 Agibong-ro, 409 Beon-gil
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
Jim Lassiter Digitally signed by Jim Lassiter Date: 2022.05.09 12:14:52 -07'00'	Jim Lassiter, President/COO	05/09/2022

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	Cell_Biotech_Co_Ltd_L_acidophilus_CBT_LA1_2018.pdf	GRAS Notice
	Bernardeau_2006.pdf	GRAS Notice
	Dinleyici_2014.pdf	GRAS Notice
	Dirar_1992.pdf	GRAS Notice
	Douillard_deVos_2014.pdf	GRAS Notice
	EFSA_2012.pdf	GRAS Notice
	Hill_2014.pdf	GRAS Notice
	Ipar_2015.pdf	GRAS Notice

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Kwak_2014.pdf	GRAS Notice
	Lee_2014.pdf	GRAS Notice
	Makarova_2006.pdf	GRAS Notice
	National_Dairy_Council_NHANES_2010.pdf	GRAS Notice
	National_Research_Council_1992.pdf	GRAS Notice
	Nout_1992.pdf	GRAS Notice
	Spano_2010.pdf	GRAS Notice
	Steinkraus_1992.pdf	GRAS Notice
	WHO_2011.pdf	GRAS Notice

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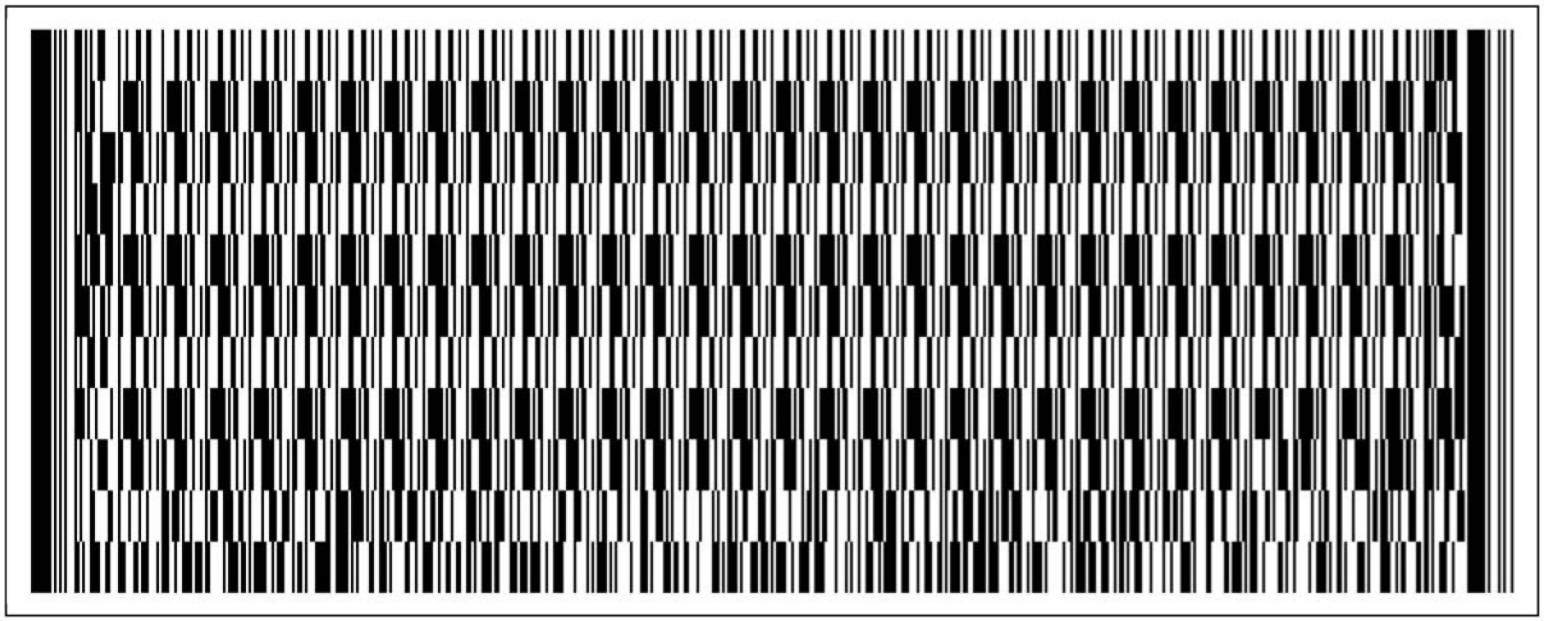
Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Yeun_2014.pdf	GRAS Notice
	Yoon_2014.pdf	GRAS Notice
	Yoon_2015.pdf	GRAS Notice
	Hill_2018.pdf	GRAS Notice
	GRASNotice_II961.1-CBI.1.2_Lactobacillus_acidophilus_CBT_LA1_2022-05-09.pdf	Administrative



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5/16/2023

Katie Overbey, PhD
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
katie.overbey@fda.hhs.gov

RE: First Response to FDA Questions/Comments Regarding GRN 001083
II961.1-CBI.3

Dear Dr. Overbey,

REJIMUS, INC. received your email dated 4/24/23 regarding additional FDA questions/comments to GRN 001083. This is a first response to address the majority of the questions presented. Additional questions from FDA related to GRNs 001078 – 001082 are intended to be provided by the Agency as a result of the meeting held between FDA and REJIMUS on 5/10/23. We have not yet received these questions and the answers to these are likely identical to questions raised concerning GRN 001083. Unfortunately, a follow-up response will be necessary and is expected to be provided to you by 5/23/23 to address the additional questions surrounding the intended maximum use level as well as the target level over the shelf life of food. The follow-up response will also include the updated literature search.

Should you have any questions or concerns with this additional information or have additional requests based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001083

Question 1

On page 7, the notifier states "LAB (lactic acid bacteria) are generally gram-positive, non-spore forming, facultative anaerobic or microaerophilic, cocci or rod-shaped bacteria," however, the notifier does not describe the morphology of *L. acidophilus* strain KCTC 11906BP (*L. acidophilus* strain "CBT LA1"). Please provide a brief description of the morphology of *L. acidophilus* strain KCTC 11906BP.

Response

Lactobacillus acidophilus strain KCTC 11906BP is a gram-positive non-spore forming rod. The morphology of the colony is a short chain rod shape.

Question 2

Please provide a brief description of *Lactobacillus acidophilus* strain KCTC 11906BP including phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites), and whether this poses a safety concern. For example, on page 17, the notifier states, "*Lactobacillus acidophilus* CBT LA1 is not known to secrete any exotoxins or any other substances that are classified as harmful to humans" but does not describe how this was confirmed.

Response

Lactobacillus acidophilus CBT LA1 is a lactic acid bacterium (LAB). LAB produce bacteriocins, small peptides 3-6 kDA in size that help protect against pathogenic invasion (Savadogo et al. 2006). Most bacteriocins produced by LAB are membrane active compounds that increase permeability of the cytoplasmic membrane and show a spectrum of bactericidal activity that falls within two broad groups as shown in the Table below (Savadogo et al. 2006). Therefore, the phenotypic characteristics of Lactobacillus acidophilus strain KCTC 11906BP do not pose a safety concern.

Antimicrobial peptides produced by lactic acid bacteria (Savadogo et al. 2006).

Group I: Modified bacteriocins (the lantibiotics)		Group II: Unmodified bacteriocins	
Type A	Type B	One peptide bacteriocins	Two peptide bacteriocins
Nisin Lactocin S Lacticin 481 Carnocin UI 49	NK [a]	Pediocin-like bacteriocins [b]: Pediocin PA1, Leucocin A, Sakacin P, Curvacin A, Mesentericin Y105,	Lactococcin G Lactacin F Plantaricin E/F Plantaricin J/K
Cytolysin		Carnobacteriocin BM1, Carnobacteriocin B2, Enterocin A, Piscicolin 126, Bavaricin MN, Piscicocin V1a	Lactobin A Plantaricin S[c] Pediocin L50[d] Thermophilin 13
		Nonpediocin- like bacteriocins: Lactococcin A and B, Crispacin A, Divergicin 750, Lactococcin 972, AS-48[e], Enterocin B, Carnobacteriocin A	

[a] Not known: lantibiotics of type B produced by lactic acid bacteria are presently not known
[b] References for the pediocin like bacteriocins are: Pediocin PA1 (Henderson et al.,1992 ; Marug et al., 1992), leucocin A (Hastings et al., 1991), sakacin P (Tichaczek et al., 1992), curvacin A (Tichaczek et al., 1992 ; Holck et al., 1992), mesentericin Y105 (Hechard et al., 1992), carnobacterioin BM1 and B2 (Quadri et al., 1994), enterocin A (Aymerich et al., 1996), piscicolin 126 (Jack et al. , 1996), bavaricin MN (Kaiser , Montville ,1996), piscicocin V1a (20).
[c] Reference for plantaricin S: (Tichaczek et al., 1993).
[d] originally published as a modified ine peptide bacteriocin (Cintas et al. , 1995), but recent results indicate that is an unmodified two-peptide bacteriocin (Cintas et al.unpublished results)
[e] As-48 is a cyclic antimicrobial peptide produced by *Enterococcus faecalis* (Martinez-Bueno et al. , 1994).

Attachment II961.1-CBI.3-A1

Question 3

On page 26, the notifier states "The substance's potential for pathogenicity and acute toxicity tested negative." Please provide a statement affirming that *L. acidophilus* strain KCTC 11906BP is non-pathogenic and non-toxigenic.

Response

Based on the results of the toxicity studies, there were no signs of the mortality or adverse effects of the animals at levels of 1 x 10^{11} CFU/kg. In addition, according to the Pathogenicity Island Database (http://www.paidb.re.kr/about_paidb.php?m=h), there are no pathogenicity islands (PAI) observed in the genome of this strain. Therefore, it can be affirmed that Lactobacillus acidophilus strain KCTC 11906BP is non-pathogenic and non-toxigenic.

Question 4

Please state whether L. acidophilus strain KCTC 11906BP is genetically engineered.

Response

Lactobacillus acidophilus strain KCTC 11906BP is not genetically engineered.

Question 5

On page 9, the notifier describes how pulsed field gel electrophoresis was performed on *L. acidophilus* strain KCTC 11906BP and *L. acidophilus* strain ATCC 4356; however, the notifier does not provide a discussion regarding the results obtained. Please briefly summarize the results from this analysis.

Response

The presented method for pulse field gel electrophoresis in the notification demonstrated that the DNA fragments of Lactobacillus acidophilus strain KCTC 11906BP are different from the reference Lactobacillus acidophilus strain ATCC 4356. Therefore, it can be indicated that Lactobacillus acidophilus strain KCTC 11906BP is a new strain of Lactobacillus acidophilus species.

Question 6

On page 12, the notifier states "stock organism is prepared and tested for microbiological contaminants." Please briefly describe which contaminants are tested at this stage and the methods of analysis used for each one.

Response

The stock organism is analyzed for i) aerobic microbial count and ii) total yeast and mold count.

Question 7

Please briefly specify how the purity of *L. acidophilus* strain KCTC 11906BP is ensured during manufacturing, and state whether the fermentation process is conducted in a contained, sterile environment.

Response

Prior to inoculation of the organism into the prepared sterilized medium, the stock of the strain is checked for purity. As a process inspection in the cultivation of the organism, a bacterial morphology under microscopy is performed.

The fermentation process is conducted in a contained, sterile environment. The broth storage tank and its components used in the fermentation process is steam sterilized prior to use. During the fermentation process, the bottom valve of the broth storage tank is opened, and the cultivated broth is transferred to a separator that is cleaned via Clean-in-place (CIP).

Question 8

In Table 3 (page 11), the notifier provides a list of raw materials used during the manufacturing process. The CAS numbers provided for soy protein isolate, yeast extract powder, sodium acetate, and corn starch do not appear to correspond to the correct substances. For the administrative record, please provide the correct CAS numbers for these four substances. In addition, we note that the correct names for the substances designated by CAS No. 10034-99-8 and CAS No. 048-04-6 are magnesium sulfate heptahydrate and L-cysteine hydrochloride, respectively. Please confirm.

Response

The CAS numbers for the following raw materials have been corrected.

Ingredient	CAS No.
Soy Protein Isolate	[977076-84-8]
Yeast Extract Powder	[8013-01-2]
Sodium acetate	[127-09-3]
Corn Starch	[977050-51-3]

According to the U.S. Food and Drug Administration Substances Added to Food database (screenshot below), Magnesium sulfate has an identified CAS Number of 10034-99-8 as shown in the screenshot below. It is acknowledged that Magnesium sulfate heptahydrate does have the same CAS number.

MAGNESIUM SULFATE

CAS Reg. No. (or other ID)*:	10034-99-8
Substance*:	MAGNESIUM SULFATE
Other Names:	♦ MAGNESIUM SULFATE ♦ EPSOM SALT ♦ MAGNESIUM SULFATE HEPTAHYDRATE ♦ SULFURIC ACID MAGNESIUM SALT (1:1), HEPTAHYDRATE ♦ MAGNESIUM SULFATE (1:1), HEPTAHYDRATE
Used for*† (Technical Effect):	ANTICAKING AGENT OR FREE-FLOW AGENT, EMULSIFIER OR EMULSIFIER SALT, FORMULATION AID, LUBRICANT OR RELEASE AGENT, MALTING OR FERMENTING AID, NUTRIENT SUPPLEMENT, PH CONTROL AGENT, PROCESSING AID, STABILIZER OR THICKENER
Food additive and GRAS regulations (21 CFR Parts 170-186)*:	184.1443

CAS No. 7048-04-6 is confirmed as L-cysteine hydrochloride monohydrate.

Question 9

In Table 3 (page 11), the notifier provides a list of raw materials used during the manufacturing process. The reference provided for manganese sulfate (21 CFR 182.5461) does not correspond to a regulation in the CFR. Please provide a clarified or appropriate reference for manganese sulfate. Further, the references provided for trehalose (FEMA No. 4600), monobasic potassium phosphate (21 CFR 175.105), and corn starch (21 CFR 182.70/ 21 CFR 182.90), either do not appear to be applicable references for these substances based on the intended use or correspond to different substances than those listed in the table. Please provide corrected references for these substances.

Response

The regulatory references for the following raw materials have been corrected and are affirmed.

Ingredient	Reference
Manganese sulfate	21 CFR§184.1461
Trehalose	GRN 000045
Potassium Phosphate, Monobasic	SCOGS Report No. 32
Corn starch	SCOGS Report No. 115

Question 10

In Table 3 (page 11), the notifier cites 21 CFR 172.320 to support the regulatory status of the use of L-arginine as a "coating ingredient." We note that 21 CFR 172.320 authorizes the use of L-arginine as a nutrient added to food and is not applicable to its use as a coating ingredient. FDA has not evaluated the use of L-arginine as a coating ingredient. Therefore, we respectfully request that you provide a statement that you will remove the use of L-arginine as a coating ingredient.

Response

The formulation of the microorganism delivered in dry form will not use L-arginine as a coating ingredient. The coating system for the microorganism consists of the remaining ingredients shown in the original Table 3.

Coating Ingredient	CAS No.	Reference
Trehalose	[6138-23-4]	GRN 000045
Potassium Phosphate, Dibasic	[7758-11-4]	SCOGS Report No. 32
Potassium Phosphate, Monobasic	[7778-7-0]	SCOGS Report No. 32
Xanthan Gum	[11138-66-2]	21 CFR §172.695
Corn starch	[977050-21-3]	SCOGS Report No. 115
Sodium Carboxymethylcellulose	[9004-32-4]	21 CFR §182.1745
Sodium Chloride	[7647-14-5]	21 CFR §182.1

Question 11

In Table 3 (page 11), the notifier lists the components of the fermentation media, and other raw materials, including soy protein isolate. Per the FDA's Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and Food Allergy Safety, Treatment, Education, and Research Act of 2021 (FASTER), soy is identified as one of the nine major food allergens in the U.S. Aside from this substance, please state whether any of the remaining raw materials used in the manufacturing process are major allergens or are derived from any of these allergens. For any of the raw materials used that are major allergens or are derived from them, please discuss why these materials do not pose a safety concern.

Response

Aside from the noted soy protein isolate used only in the fermentation medium, the product that is the subject of this GRAS determination does not have any other raw materials used in the manufacturing process that represent any of the major food allergens required to be listed in accordance with the Food Allergen Labeling and Consumer Protection Act, identified as milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans and sesame.

Question 12

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (page 11). Please clarify what "coating ingredient" means in this context.

Response

The inclusion of these materials occurs toward the end of the fermentation process. The intent of the inclusion is to encapsulate the microorganism comprising the finished ingredient for delivery in its dried and final form.

Question 13

Please clarify whether all raw materials used during the manufacturing process are food grade.

Response

All raw materials used during the manufacturing process are food grade. The raw materials used have regulatory statuses that are safe for inclusion in food.

Question 14

Figure 3 (page 13) includes an "enzymatic modification" as the first step in the flow chart for the manufacturing process. However, this step is not described in any detail in the notice. Table 3 (page 11) lists "protease" as the enzyme but does not specify the type of enzyme or its source. The reference cited in Table 3 is 21 CFR 182.1027 which does not correspond to a regulation in the CFR.1 Please clarify the following:

a. The identity of the enzyme(s) used in the stated "enzymatic modification" step, including the enzyme commission number(s)

b. The intended use of the enzyme(s) during the manufacturing process

c. The source of the enzyme(s) (e.g., microbial-derived)

d. If the enzyme is produced by a microorganism, please provide clarification regarding the strain's phenotype (i.e., pathogenicity, toxicity) and genotype (i.e., genetic modifications)

e. How the notifier ensures that the enzyme(s) are inactivated and/or removed in the final product

Response

a. *The enzyme used in the enzymatic modification step is a protease (Alcalase) with the enzyme commission number 3.4.21.62.*

b. The intended use of the enzyme during the manufacturing process is for protein hydrolysis.

c. The source of the enzyme is from the microorganism, Bacillus licheniformis.

d. The microorganism, Bacillus licheniformis, where the enzyme is produced is a non-pathogenic strain and is not genetically engineered. In addition, protease enzymes using the non-pathogenic strain of Bacillus licheniformis are considered GRAS according to 21 CFR§184.1027 "Mixed carbohydrase and protease enzyme product."

e. After fermentation is complete, all components of the fermentation media, including the enzyme, are removed from the strain through the separator.

Question 15

The method for measuring viable cell count is listed on page 13 as USP <2022>. We note that this method is intended to be used to measure the absence of *Clostridium species*, *Escherichia coli*, *Salmonella* species, and/or *Staphylococcus aureus* and not for measure of viable cell count. Please clarify this discrepancy in type of use.

Response

The method referenced in the GRAS notification was misidentified. As a clarification, the viable cell count is performed as an in-house method. The method for viable cell count is attached.

Attachment: II961.1-CBI.3-A2

Question 16

The method for measuring coliforms is listed on page 13 as USP <2023>; we note that this is not a validated USP method per se, but rather refers to the USP document titled "Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements." Please provide the correct method used to analyze for the presence of coliforms.

Response

Coliforms are tested according to KFDA Food Code VIII. Food Analytical Method, 4.7 Coliforms.

Question 17

In Table 4, the notifier lists specifications for microorganisms, including coliforms, but does not provide specifications for other common, notable foodborne pathogen analyses, such as *Salmonella* serovars (page 13). Please clarify if further analysis is performed to identify the genera or species of any presumptive positive result from analysis of coliforms. If further analysis is not performed, please describe why analysis for coliforms is sufficient. Additionally, please briefly describe how microbial contamination is controlled during the manufacturing process.

Response

Microbiological testing such as E. coli, S. aureus, Salmonella, L. monocytogenes is performed and meets specifications as shown in the Certificate of Analysis for each presented batch. Testing of presumptive positive coliform results are further conducted to confirm the genus and species of any presumptive coliforms identified during the initial testing.

The contamination control program utilized during the manufacturing process includes the testing for contamination of stock organism(s), and all equipment used in the fermentation as well as the manufacturing processes, which are conducted through controlled cleaning programs. The finished ingredient testing is performed to verify purity and potency in accordance with the approved specification.

Attachment: II961.1-CBI.3-A3

Question 18

The notifier does not provide specifications for heavy metals (Table 4, page 13). We note that we typically request that, at a minimum, a limit for lead be included in the specifications for fermentation-derived ingredients. Please include a limit for lead in the specifications for L. acidophilus strain KCTC 11906BP and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet this specification limit. Please note that the limit for lead should be as low as possible and be reflective of the results of the batch analyses. In addition, please specify the analytical method that is used to test for lead.

Response

Heavy metals are being performed as identified in the Certificate of Analysis. These include results for Lead, Arsenic, Cadmium, and Mercury in three non-consecutive batches. The limit for Lead is ≤ 1.0 mg/kg. Attached is the Certificate of Analysis of the three non-consecutive batches. The analytical method used for testing for lead is through ICP performed under Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal.

Attachment: II961.1-CBI.3-A3

Question 19

Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications (including lead) have been validated for that particular purpose.

Response

All analytical methods used in the testing of the batches (including lead) are tested based on the compendial methods and are, therefore, validated for their respective purpose.

Question 20

On page 14, the notifier states *L. acidophilus* strain KCTC 11906BP is intended to be added to dairy products at concentrations needed to provide at least 10^{11} CFU per serving. According to the stability study (Table 5, page 14), the survival rate decreases ~30% during 12-months of storage. Considering the loss during storage, please provide narrative how you ensure that 1×10^{11} CFU per serving remains viable over the product shelf life.

Response

In Progress

Additional questions related to GRN 1078 – 1082 will be provided from the Agency as a result of the FDA meeting on 5/10/23 regarding the clarification of the intended maximum use level as well as the target level over the shelf life of food. These additional questions will be applicable to this response and will be addressed in the follow-up response.

Question 21

Please provide food subcategories included in the estimation of consumption of "dairy products" in Table 7. In addition, please specify a serving size for each food subcategory and provide the reference that was used as the basis for determining the serving size.

Response

In Progress

Additional questions related to GRN 1078 – 1082 will be provided from the Agency as a result of the FDA meeting on 5/10/23 regarding the clarification of the intended maximum use level as well as the target level over the shelf life of food. These additional questions will be applicable to this response and will be addressed in the follow-up response.

Question 22

Please clarify what population is represented by "all users" in your dietary exposure estimate (Table 7). If the dietary exposure estimate is not for the U.S. population aged 2 years and older, please provide mean and 90th percentile eaters-only dietary exposure estimates for U.S. population aged 2 years and older.

Response

In Progress

Additional questions related to GRN 1078 – 1082 will be provided from the Agency as a result of the FDA meeting on 5/10/23 regarding the clarification of the intended maximum use level as well as the target level over the shelf life of food. These additional questions will be applicable to this response and will be addressed in the follow-up response.

Question 23

On page 16, the notifier states, "three daily servings would result in a cumulative exposure of 2.68×10^{11} CFU per day ($8.94 \times 10^{10} \times 3$)." Further, the notifier states, "the recommended levels of the cumulative exposure of 2.68×10^{11} CFU per day and the cumulative exposure at an estimated 90th percentile of 5.55×10^{11} CFU per day." Please note that the cumulative dietary exposure should consider background sources, and all current and proposed uses of *L. acidophilus* strain KCTC 11906BP. For the administrative record, please confirm that the term "cumulative" was incorrectly used in the statements mentioned above.

Further, the notifier states, "The estimated 90th percentile of consumers of dairy products at this level of recommended consumption adjusted for the findings of the per capita data" on page 16. We consider that data in Table 7 represent estimates for "users" (eaters) only, i.e., individuals consuming the proposed dairy products at least once during the survey period. Please note that "per capita" estimates would include eaters and non-eaters. For the administrative record, please confirm that the estimates in Table 7 are for the eaters-only population and explain what you mean by "the findings of the per capita data."

Response

Currently, Lactobacillus acidophilus strain KCTC 11906BP is considered a novel ingredient in food and there are no current uses of this strain. As dairy products are the only proposed food, the dietary exposure of the ingredient is only based on the dairy products. Therefore, the term "cumulative" was inappropriately used.

The estimates used in the Table 7 is confirmed as eaters-only population. Therefore, the appropriate term was "findings from the eaters-only population" and not "findings of the per capita data."

Question 24

Please provide an updated literature search that discusses the safety of *L. acidophilus*, including the safety of Lactobacilli, this strain, or closely related strains, as applicable. Please do not limit your discussion solely to studies in human populations and include a discussion on pathogenicity and toxigenicity. Further, any reports of bacteremia or foodborne illness involving Lactobacilli should also be discussed. Please include the date (month and year) the literature search was performed and the dates or years the search spanned (e.g., 1990-present). Also, discuss whether findings from any publications contradict your GRAS conclusion.

Response

In Progress

Additional literature search is currently being performed. The response to this question will be addressed in the follow-up response.

Question 25

In Tables 8 and 9, the notifier lists several GRAS notices where the subject of the notice was a strain of *Lactobacillus acidophilus* or Lactobacilli[2] that have been submitted to FDA and have received "no questions" letters (page 19). Additionally, on page 26, the notifier states "The applicable GRAS notices, referenced in Table 8 and Table 9 within Part 6 of this notice, incorporate myriad studies demonstrating the safety of ingestion of substances closely related to *Lactobacillus acidophilus* CBT LA1" but does not identify or summarize the relevant information from each GRAS notice. Reference to these and other previous Lactobacillus-related GRAS notices should note specific information or findings from the selected previous notices that support the basis of the notifier's argument for the safety of the intended use of their ingredient. As each GRAS notice stands on its own, please briefly summarize the information incorporated by reference from the GRAS notices listed in Tables 8 and 9 in the context of the notifier's safety conclusion.

Response

Table 8 and 9 has been updated to include a summary of each of the listed GRAS notices.

Table 8. *GRAS notices containing Lactobacillus acidophilus receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).*

GRAS No.	Date of Closure	Substance	Intended Use	Amount
502	8/19/14	*Lactobacillus acidophilus* strain La-14	Ready-to-eat breakfast cereals; bars (e.g., breakfast, energy, nutrition); milk, milk drinks (e.g., flavored milks), milk products (e.g., butter), fermented milks (e.g., Kefir, sour cream, buttermilk), yogurt, cheese (including cheese food, cheese spreads), and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit "ades," fruit drinks, jams and jellies; nut and peanut spreads; margarines; snack foods (e.g., cookies, crackers, chips, granola); meal replacements; sauces, condiments; confections (e.g., bars, candy, coatings, drops, cookie filling); chewing gum; and in medical foods	Minimum 1 x 10^9 colony forming units per 250 gram serving of food at the time of consumption.

GRAS No.	Date of Closure	Substance	Intended Use	Amount
378	3/26/12	Cultured dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources fermented by *Streptococcus thermophilus, Bacillus coagulans, Lactobacillus acidophilus, L. paracasei* subsp. *paracasei, L. plantarum, L. sakei, L. bulgaricus and Proprionibacterium freudenreichii* subsp. *shermanii* or mixtures of these strains.	As antimicrobial agents in a variety of food categories including meat and poultry, but excepting infant formula and infant foods	Levels of 0.1 to 4.5%
357	4/19/11	*Lactobacillus acidophilus* strain NCFM	Ready-to-eat breakfast cereals; bars; cheeses, milk drinks, and milk products; bottled water and teas; fruit juices, fruit nectars, fruit "ades," and fruit drinks; chewing gum; and confections	10^9 colony forming units per standard serving (cfu/serving)
171	12/7/05	*Lactobacillus acidophilus, L. lactis*, and *Pediococcus acidilactici*	For use to control growth of pathogenic bacteria in fresh chopped/ground, whole muscle cuts, and carcasses of meat and poultry	levels between 10^6 to 10^8 colony forming units of lactobacilli per gram of product

Table 9. *GRAS notices of Lactobacillus organisms of species other than lactis receiving reply from FDA of no questions (GRAS Notices Inventory Database)*

GRAS No.	Date of Closure	Substance	Intended Use	Amount
847	9/30/19	*Lactobacillus plantarum* ECGC 13110402	Conventional foods (excluding infant formula and foods under the jurisdiction of the United States Department of Agriculture (USDA))	Up to 1 x 10^{10} colony forming units (CFU) per serving
840	8/27/19	*Lactobacillus paracasei* strain F19	Dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and other food categories	At levels intended to provide a daily intake of 10^9 colony forming units (CFU)/serving
810	4/5/19	*Lactobacillus paracasei* subsp. *paracasei* strain F-19e	Non-exempt powdered infant formulas for term infants	Levels of 10^9 colony-forming units (CFU)/800 mL of reconstituted formula

GRAS No.	Date of Closure	Substance	Intended Use	Amount
758	8/20/18	*Lactobacillus helveticus* R0052	Non-exempt powdered infant formulas for term infants	Each individual bacterial culture is intended for use at a maximum level of 3 x 10^9 colony forming units (CFU)/800 mL of reconstituted formula. The combined bacterial culture is intended for use at a maximum level of 5 x 10^9 CFU/800 mL of reconstituted formula.
722	2/16/18	*Lactobacillus plantarum* Lp-115	Conventional foods, including yogurt and other dairy products, soy products, beverages, chewing gum, and confectionary snacks	At 1 × 10^{10} colony forming units (CFU)/serving
685	10/31/17	*Lactobacillus plantarum* strain 299v	Conventional foods	Up to 1 x 10^{10} CFU/serving
531	8/14/14	*Lactobacillus fermentum* CECT5716	Term infant formulas	Maximum level of 10^7 colony forming units per gram (cfu/g) of powdered non-exempt milk-based infant formula

GRAS No.	Date of Closure	Substance	Intended Use	Amount
502	2/27/14	*Lactobacillus acidophilus* La-14	Ready-to-eat breakfast cereals; bars (e.g., breakfast, energy, nutrition); milk, milk drinks (e.g., flavored milks), milk products (e.g., butter), fermented milks (e.g., Kefir, sour cream, buttermilk), yogurt, cheese (including cheese food, cheese spreads), and ice cream; soy drinks and soy products; bottled water and teas; dry beverages including sports nutrition beverages; fruit juices, fruit nectars, fruit "ades," fruit drinks, jams and jellies; nut and peanut spreads; margarines; snack foods (e.g., cookies, crackers, chips, granola); meal replacements; sauces, condiments; confections (e.g., bars, candy, coatings, drops, cookie filling); chewing gum; and in medical foods	Minimum 1 x 10^9 colony forming units per 250 gram serving of food at the time of consumption.
440	8/16/12	*Lactobacillus reuteri* strain NCIMB 30242	Beverages and beverage bases, breakfast cereals, cheeses, dairy product analogs, fats and oils, frozen dairy desserts, grain products and pastas, milk products, processed fruits and fruit juices, and sugar substitutes	At levels ranging from 3.3 X 10^8 to 1010 colony forming units per serving (CFU/serving).
410	11/16/11	*Lactobacillus reuteri* strain DSM 17938	Powdered whey-based term infant formula	Minimum level of 10^6 colony forming units per gram (cfu/g), but not higher than 10^8 cfu/g of powdered formula

GRAS No.	Date of Closure	Substance	Intended Use	Amount
357	4/19/11	*Lactobacillus acidophilus* NCFM	Ready-to-eat breakfast cereals; bars; cheeses, milk drinks, and milk products; bottled water and teas; fruit juices, fruit nectars, fruit "ades," and fruit drinks; chewing gum; and confections	10^9 colony forming units per standard serving (cfu/serving)
288	3/27/09	*Lactobacillus rhamnosus* strain HN001	Various foods, including certain beverages and beverage bases (excluding soft drinks); cheeses; milk drinks; milk products; meal replacements; energy bars; ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum, and hard candies	level to provide up to 10^9 colony forming units (cfu) per standard serving
281	8/31/09	*Lactobacillus rhamnosus* strain HN001 produced in a milk-based medium	milk-based powdered term infant formula that is intended for consumption from the time of birth, as well as in milk-based powdered follow-on formula	at a level of 10^8 colony forming units per gram (cfu/g) of the formula powder
254	11/18/08	*Lactobacillus reuteri* strain DSM 17938	Processed cheeses, yogurt, ice cream, fruit juices, fruit drinks, processed vegetables, processed vegetable drinks, beverage bases, energy bars, energy drinks, and chewing gum	Up to 10^9 colony forming units (cfu) per serving, and in a drinking straw at a level of 10^9 cfu per straw

Question 26

On page 20, the notifier states "While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of Lactobacillus acidophilus CBT LA1 in foreign countries and in multiple food products" but does not provide a summary of these food products. Please provide a brief summary of these food products.

Response

Below is a table of food products that contain Lactobacillus acidophilus CBT LA1 in foreign countries.

Product	Availability	Ingredients	Amount per Serving
DUOLAC® Care 	Singapore https://www.watsons.com.sg/duolac-care-60s/p/BP_66142	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	2.00×10^9 CFU 2.19×10^9 CFU 2.19×10^9 CFU 2.19×10^9 CFU 2.00×10^9 CFU 1.91×10^9 CFU 1.25×10^{10} Total CFU / Tablet
DUOLAC® Balance Baby 	Korea https://www.ebay.com/itm/Duolac-Baby-Probiotics-Powder-30-days-Dual-Coated-Bifidus-Triplets-Kid-Child-/232069774531	*L. acidophilus* LA1 *L. plantarum* LP3 *B. infantis* BT1 *B. longum* BG7 *B. bifidum* BF3 *B. breve* BR3	9.89×10^8 CFU 9.89×10^8 CFU 7.56×10^8 CFU 7.56×10^8 CFU 7.56×10^8 CFU 7.56×10^8 CFU 5.0×10^9 Total CFU / Stick

Product	Availability	Ingredients	Amount per Serving
DUOLAC® Gold 	Korea https://www.ebay.com/itm/Duolac-Gold-Probiotics-Adult-30-days-Dual-Coated-Lactic-Acid-Bacteria-Triplets-/231644172196	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.60×10^9 CFU 1.76×10^9 CFU 1.76×10^9 CFU 1.75×10^9 CFU 1.60×10^9 CFU 1.53×10^9 CFU 1.0×10^{10} Total CFU / Stick
DUOLAC® Normal Immune 	Denmark https://www.duolac.dk/products/duolacc-normal-immunforsvar-2/ Finland https://www.apteekkituotteet.fi/Duolac-Normal-Immune	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	5.09×10^8 CFU 6.63×10^8 CFU 7.14×10^8 CFU 6.12×10^8 CFU 5.10×10^8 CFU 3.0×10^9 Total CFU / Tablet
Lactobex® Strong 	Latvia http://www.lactobex.lt	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.12×10^9 CFU 1.23×10^9 CFU 1.23×10^9 CFU 1.23×10^9 CFU 1.12×10^9 CFU 1.07×10^9 CFU 7.0×10^9 Total CFU / Capsule

Product	**Availability**	**Ingredients**	**Amount per Serving**
NBL Probiotic ATP 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/cocuk/nbl-probiotic-atp/	*L. acidophilus* LA1 *L. casei* LC5 *L. plantarum* LP3 *B. lactis* BL3	4.00×10^8 CFU 8.00×10^8 CFU 4.00×10^8 CFU 4.00×10^8 CFU 2.0×10^9 Total CFU / Stick
PRODUO Derma 	Spain http://produo.es/familia-produo-tratamiento-flora-bacteriana-intestinal/produo-derma-bacterias-intestinales/	*L. acidophilus* LA1 *L. casei* LC5 *L. plantarum* LP3 *B. lactis* BL3	1.00×10^9 CFU 2.00×10^9 CFU 1.00×10^9 CFU 1.00×10^9 CFU 5.0×10^9 Total CFU / Stick
Norgitan Care 	Belgium http://www.apomed.be/2555849-norgitan-care-5-souches-bacteries-vivantes-coloniser-votre-intestin-5425014928174-b-pharma.html	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	5.09×10^8 CFU 6.61×10^8 CFU 7.12×10^8 CFU 6.10×10^8 CFU 5.09×10^8 CFU 3.0×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
LIPROLAC 	Indonesia https://www.kalbestore.com/liprolac-vanilla-powder.html	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. longum* BG7 *B. bifidum* BF3	2.00×10^8 CFU 6.80×10^8 CFU 2.00×10^8 CFU 8.50×10^7 CFU 8.50×10^7 CFU 1.25×10^9 Total CFU / Sachet
Phital® Probiotica Plus 	Netherlands https://www.phital.nl/producten/probiotica/probiotica-plus-duolac	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	5.10×10^8 CFU 6.63×10^8 CFU 7.14×10^8 CFU 6.12×10^8 CFU 5.10×10^8 CFU 3.0×10^9 Total CFU / Stick
Nutriforte Lactoghurt 	Malaysia http://www.nutriforte.com.my/Lactoghurt+Probiotics_20_1.htm	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	3.67×10^8 CFU 4.89×10^8 CFU 3.91×10^8 CFU 4.89×10^8 CFU 3.67×10^8 CFU 2.1×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
Lacclean Gold Lab 	Vietnam https://www.alibaba.com/product-detail/LACCLEAN-GOLD-LAB-health-food_246152457.html	*L. acidophilus* LA1 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. longum* BG7 *B. bifidum* BF3	2.00×10^8 CFU 6.80×10^8 CFU 2.00×10^8 CFU 8.50×10^8 CFU 8.50×10^8 CFU 1.25×10^9 Total CFU / Sachet

Question 27

The conclusion states "the historic safe use of Bifidobacterium longum," please correct this discrepancy.

Response

The statement has been corrected to "the historic safe use of *Lactobacillus acidophilus*."

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted so far as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above responses or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly. We look forward to completing the follow up response to the Agency addressing the remaining items that are identified herein as "in progress" promptly.

Attachments

II961.1-CBI.3-A1	Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria- a minireview. *African Journal of Biotechnology* Vol. 5(9), pp. 678-683, 2 May 2006.
II961.1-CBI.3-A2	In-house analytical method for Viable Cell Count
II961.1-CBI.3-A3	Certificate of Analysis

Attachment
II961.1-CBI.3-A1

Attachment II961.1-CBI.3-A1 consisting of Seven pages have been removed in accordance with copyright laws. The removed reference citation is:

Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria- a minireview. *African Journal of Biotechnology* Vol. 5(9), pp. 678-683, 2 May 2006.

Attachment
II961.1-CBI.3-A2

Analytical Method of Viable Cell Count

Materials :

1. The diluent (Buffered peptone water)

Composition	g/L
Peptone	10
Sodium chloride	5
Disodium phosphate	3.5
Monopotassium phosphate	1.5
Tween 80	0.5
Sterilized water	979.5
pH	6.8~7.0

* Adjust pH with 0.1N NaOH

Method:

1. Dissolve precisely 1 g of the specimen in 15 mL falcon tube filled with 9 mL of the sterilized diluent (pH: 6.8 ~ 7.0)
2. Auto-vortex for 20 min. using tube adaptor at room temperature to remove the coating materials completely. If the tube adaptor is not equipped, semiauto-vortex for 20 min. in a pattern of 2-minute-vortexing-and-3-minute-resting.

   * Vortex or vortexing of the followings means semiauto-vortex or semiauto-vortexing.
3. Prepare approx. 10 glass tubes containing 9 mL of the diluent respectively. And perform the first serial dilution with a 1 in 10 (1:9) dilution method.
4. After diluting the first glass tube, vortex 3 min. and check the bacterial cells by microscope (×1,000). If the bacteria are not released completely, repeat this procedure.
5. Vortex the first glass tube for 10 sec. and continue serial dilution with a 1 in 10 (1:9) dilution method until the expected final dilution, at which 30 colonies are formed in the final culture plate. The operation between the two tubes must be done within one minute.

Dilution factor	Vortex for
10^{-1}	20 min
10^{-2}	3 min
10^{-3}	1 min
10^{-4}	30 sec
10^{-5}~	15 sec

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6. Select the last 3 tubes and vortex one tube for 10 sec. and put 1.0 mL of the diluted solution into the sterilized culture plate (Petri-dish). Pour about 20 mL of the readymade culture media (MRS or BL) carefully into the plate, cap it with the plate cover and shake the plate smoothly (clockwise 5 times and then counterclockwise 5 times). Mark the dilution ratio on the plate cover. Perform the same procedure for the other 2 tubes.
   * MRS agar for Lactobacillus, Lactococus, Enterococcus and Streptococcus species
   * BL agar for Bifidobacterium species or for total viable cell count.
   * CBT uses MRS agar and BL agar manufactured by Difco.
7. Leave the plates at room temp. until the media become hard. And then incubate the culture plate at 37℃ for 72 hrs in an aerobic incubator (for MRS agar) or for 72 hrs in an anaerobic incubator (for BL agar).
8. Select the plate at which 30~300 colonies are formed and calculate viable cells inversely using the following formula.

   Formula: Viable cells (cfu/g) = Colony number × Dilution Factor

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Attachment
II961.1-CBI.3-A3

CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 57R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 07 May. 2017

Exp. Date: 06 May. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
PHONE +82 31 987 8107 | FAX +82 31 987 6216 | www.cellbiotech.com

CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 65R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 13 Jun. 2017

Exp. Date: 12 Jun. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	$\leq$ 1.0 mg/kg	Passes test
Cadmium (Cd)	$\leq$ 0.3 mg/kg	Passes test
Mercury (Hg)	$\leq$ 0.1 mg/kg	Passes test
Arsenic (As)	$\leq$ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
| PHONE +82 31 987 8107 | FAX +82 31 987 6216 | www.cellbiotech.com

CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 80R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 30 Jul. 2017

Exp. Date: 29 Jul. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

CELL BIOTECH Co., Ltd.
Headquarters : 50, Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
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Overbey, Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Friday, June 16, 2023 8:06 PM
To: Overbey, Katie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine
Subject: Re: [EXTERNAL] FW: FDA Questions for GRN 1083
Attachments: II961.1-CBI.4.pdf

Dear Dr. Overbey,

In response to the document "GRN1083_Questions_2023-04-24" for the request for more information for GRN 001083 (*Lactobacillus acidophilus* CBT LA1) and in accordance with the below correspondence, attached you will find the second response (II961.1-CBI.4) to the remaining four (4) questions with the respective attachments included therein.

Thank you for sending your feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Director
Quality Development Services
joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Wednesday, June 7, 2023 at 7:26 AM
To: Joel Villareal <joel@rejimus.com>
Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: RE: [EXTERNAL] FW: FDA Questions for GRN 1083

Hello Joel,
Thank you for the quick response, that time frame will work for us. Please feel free to reach out if you have any questions.

Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Tuesday, June 6, 2023 6:31 PM
To: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>
Subject: Re: [EXTERNAL] FW: FDA Questions for GRN 1083

Dear Dr. Overbey,

Thank you for your email. We have received the follow-up questions from Dr. Hice for GRNs 1078-1082 and we are working on the responses to those questions. Some of these follow up questions are pertinent to the remaining questions for GRN 1083. Therefore, we anticipate to have the responses for the remaining questions for GRN 1083 by the end of next week (Friday 6/16/23). Please let us know if this is acceptable to you.

If there are any other questions/concerns, please let us know.

Best Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Monday, June 5, 2023 at 10:24 AM
To: Joel Villareal <joel@rejimus.com>

Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: RE: [EXTERNAL] FW: FDA Questions for GRN 1083

Hello Joel,
I understand that you have been sent the follow-up questions for GRNs 1078-1082. Please let me know when we can expect additional information from you for your responses to our questions for GRN 1083.

Best,
Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Tuesday, May 16, 2023 3:28 PM
To: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>
Subject: Re: [EXTERNAL] FW: FDA Questions for GRN 1083

Dear Dr. Overbey,

In response to the document "GRN1083_Questions_2023-04-24" for the request for more information for GRN 001083 (*Lactobacillus acidophilus* CBT LA1) and in accordance with the below correspondence, attached you will find responses to the questions/comments (II961.1-CBI.3) with the respective attachments included therein.

Please note that there are still four (4) questions that will require additional time to complete the updated literature search and to address additional questions from the Agency regarding GRNs 1078-1082 as a result of the meeting with FDA on 5/10/23. The responses to the additional questions for GRN 1078-1082 would likely be identical to the questions raised for GRN 1083. Currently, we are awaiting for these questions from the Agency.

The additional information and any additional documents will be provided to the Agency for review once we have received them and we anticipate this information to be provided by Tuesday, 5/23/23. Please let us know if this suffices for this response.

Thank you for sending your initial feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Friday, May 5, 2023 at 8:52 AM
To: Joel Villareal <joel@rejimus.com>
Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>, Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: RE: [EXTERNAL] FW: FDA Questions for GRN 1083

Hello Joel,
Thank you for reaching out. Your suggested timeline makes sense, please let me know if you have questions in the meantime.

Best,
Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Thursday, May 4, 2023 9:09 PM
To: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>; Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: Re: [EXTERNAL] FW: FDA Questions for GRN 1083

Dear Dr. Overbey,

We have a meeting scheduled with Dr. Hice on 5/10/23 to discuss the responses to GRNs 001078 – 001082 about the revised use level and corresponding exposure. Owing to several similarities between the questions for GRN 1083 and GRNs 001078 - 001082, we would like to address the FDA's questions from this meeting before sending a complete response for GRN 1083. Therefore, we would like to respectfully request an extension to the responses for GRN 1083 to 5/15/23, if this is acceptable. In addition, the outcome of this meeting would also apply to the responses for GRNs 1084, 1085, 1086, 1087, and 1088.

Thank you in advance and please let us know if there are any questions.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services

joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Tuesday, May 2, 2023 at 6:56 AM
To: Joel Villareal <joel@rejimus.com>
Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: RE: [EXTERNAL] FW: FDA Questions for GRN 1083

Hello Joel,
Thank you for the update.

Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Friday, April 28, 2023 8:32 PM
To: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>; Brandon M. Griffin <brandon@rejimus.com>; Kenneth Cairns <kenneth@rejimus.com>; Kent Phan <kent@rejimus.com>; Livia Consedine <livia@rejimus.com>
Subject: [EXTERNAL] FW: FDA Questions for GRN 1083

Dear Dr. Overbey,

Thank you for your email with the questions regarding GRN 1083, on behalf of our client Cell Biotech Co. Ltd, for *Lactobacillus acidophilus* CBT LA1. We are actively working on the responses and intend to provide a response to the questions/comments within 10 business days from when the questions/comments were issued on 4/24/23. Therefore, the response is expected to be provided by 5/8/23.

In the meantime, if there are any other questions, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Monday, April 24, 2023 at 9:39 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: FDA Questions for GRN 1083

Dear Mr. Lassiter,
During our review of GRAS Notice No. 1083, we identified questions that need to be addressed. Please find the questions attached to this email.

We ask that you format your response such that each answer immediately follows the stated question and please submit responses as a PDF. Please ensure that your responses do not contain confidential business information and please do not submit a revised version of the GRAS notice.

We respectfully request a response to these questions within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options.

Thank you in advance for your attention to our comments.

Katie Overbey, Ph.D., M.S (she/her/hers)
Regulatory Review Scientist

Division of Food Ingredients
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6/16/2023

Katie Overbey, PhD
Regulatory Review Scientist
Division of Food Ingredients
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katie.overbey@fda.hhs.gov

RE: Second Response to FDA Questions/Comments Regarding GRN 001083
II961.1-CBI.4

Dear Dr. Overbey,

This is a second response following the prior response issued on 5/16/23 to address the remaining questions regarding FDA questions/comments with respect to GRN 001083.

Should you have any questions or concerns with this additional information or have additional requests based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001083

Question 20

On page 14, the notifier states *L. acidophilus* strain KCTC 11906BP is intended to be added to dairy products at concentrations needed to provide at least 10^{11} CFU per serving. According to the stability study (Table 5, page 14), the survival rate decreases ~30% during 12-months of storage. Considering the loss during storage, please provide narrative how you ensure that 1×10^{11} CFU per serving remains viable over the product shelf life.

Response

After additional review and re-consideration by the Sponsor relative to the available safety information included in this notification as well as the prior notices cited, and current products in the marketplace as well as published clinical studies, on of L. acidophilus KCTC 11906BP, the intended maximum use levels have been updated to up to 1 x 10^9 CFU/serving of the ingredient.

With respect to the updated intended maximum use level of up to 1 x 10^9 CFU/serving, there should not be a concern over the viability of the ingredient over a 12-months shelf-life owing to the original stability study performed at 10^{11} CFU/serving level. Even at an approximate 30% decrease in the survival rate over the identified storage period (12 months), the ingredient is capable of meeting the updated intended maximum level of use. Furthering this, the Sponsor intends to market the ingredient as a bulk ingredient only. The producer of the milk product is responsible for determinations regarding inclusion of this microorganism, but the limits of inclusion, as established in this notification, remain at not higher than 1 x 10^9 CFU/serving.

Question 21

Please provide food subcategories included in the estimation of consumption of "dairy products" in Table 7. In addition, please specify a serving size for each food subcategory and provide the reference that was used as the basis for determining the serving size.

Response

Below is a table of food subcategories used in the estimation of consumption with the respective food code from NHANES as well as the respective serving size. As mentioned previously, the producer of the milk product is responsible for determinations regarding inclusion of this microorganism, but the limits of inclusion as established in this notification remain at not higher than 1 x 10^9 CFU/serving. The food serving size for the food subcategories of milk is based on the reference amounts customarily consumed (RACC).

Food Code	***Food Subcategories***	***Serving Size***	***Food Serving***
11100000	*Milk, NFS*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11111000	*Milk, whole*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11111100	*Milk, low sodium, whole*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11111150	*Milk, calcium fortified, whole*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11111160	*Milk, calcium fortified, low fat (1%)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11111170	*Milk, calcium fortified, fat free (skim)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11112110	*Milk, reduced fat (2%)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11112210	*Milk, low fat (1%)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11113000	*Milk, fat free (skim)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11114300	*Milk, lactose free, low fat (1%)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11114320	*Milk, lactose free, fat free (skim)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11114330	*Milk, lactose free, reduced fat (2%)*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*
11114350	*Milk, lactose free, whole*	*Up to 1 x 10^9 CFU/serving*	*8 fl oz or 240mL*

Based on clinical studies provided in the GRAS notification, intended levels of previous GRAS notifications, updated literature search, and current products in the marketplace outside the United States, the serving size of 1 x 10^9 CFU/serving is reasonable to be safe for consumption.

Question 22

Please clarify what population is represented by "all users" in your dietary exposure estimate (Table 7). If the dietary exposure estimate is not for the U.S. population aged 2 years and older, please provide mean and 90th percentile eaters-only dietary exposure estimates for U.S. population aged 2 years and older.

Response

Based on the intended food uses and the intended maximum use level of up to 1 x 10^9 CFU/serving, the estimated dietary exposure, based suggested three daily servings, is shown below:

Population Group	***Age Group***	***Eaters only (CFU/day)***	
		Mean	***90th Percentile***
Total Population (eaters-only)	*2 years old and older*	*2.68×10^9*	*5.55×10^9*

Question 24

Please provide an updated literature search that discusses the safety of *L. acidophilus*, including the safety of Lactobacilli, this strain, or closely related strains, as applicable. Please do not limit your discussion solely to studies in human populations and include a discussion on pathogenicity and toxigenicity. Further, any reports of bacteremia or foodborne illness involving Lactobacilli should also be discussed. Please include the date (month and year) the literature search was performed and the dates or years the search spanned (e.g., 1990-present). Also, discuss whether findings from any publications contradict your GRAS conclusion.

Response

A PubMed and Google Scholar search was performed for "Lactobacillus acidophilus", and "CBT LA1" to determine if there are any adverse events in a human populations or animal studies. Published studies are summarized below.

Reference	***Study Title***	***Subjects***	***Dose***	***Duration***	***Summary of Safety***
Lim et al. (2020)	*The effect of Lactobacillus acidophilus YT1 (Menolacto) on improving menopausal symptoms: a randomized, double-blinded, placebo-controlled clinical trial*	*Female subjects*	*Daily dose of 1×10^8 CFU/day.*	*12 weeks*	*12 adverse events were from experimental group and 10 adverse effects were from the placebo group. The authors concluded no "causal relationship" to the product.*

Reference	***Study Title***	***Subjects***	***Dose***	***Duration***	***Summary of Safety***
Preston et al. (2018)	*Lactobacillus acidophilus CL1285, Lactobacillus casei LBC80R, and Lactobacillus rhamnosus CLR2 improve quality of life and IBS symptoms: a double-blind, randomized, placebo-controlled study*	*113 human subjects*	*2 capsules containing Minimum 50 x 10^9 CFU of the blend daily*	*12 weeks*	*4 adverse events were from experimental group and 3 adverse effects were from the placebo group. The authors concluded no relationship to the product.*
Ahn et al. (2019)	*Randomized, double-blind, placebo-controlled study of a multispecies probiotic mixture in nonalcoholic fatty liver disease*	*68 obese adults*	*10^9 CFU/1.4g mixture of 6 organisms mixture which contains Lactobacillus acidophilus CBT LA1*	*12 weeks*	*1 patient died in the placebo group but there was no relationship to the product.*
Mitrovic et al. (2023)	*The impact of symbiotic treatment on the levels of gut-derived uremic toxins, inflammation, and gut microbiome of chronic kidney disease patients – a randomized trial*	*34 adults*	*2 pills containing 16 billion CFU mixture of 3 organisms mixture which contains Lactobacillus acidophilus CBT LA1 (4 x 10^9 CFU)*	*Once daily for 12 weeks*	*Other than 2 subjects with increased flatulence, "no safety issues were noted" from the authors.*

Reference	***Study Title***	***Subjects***	***Dose***	***Duration***	***Summary of Safety***
Shahriari et al. (2021)	*The effect of probiotic supplementation on the risk of gestational diabetes mellitus among high-risk pregnant women: A parallel double-blind, randomized, placebo-controlled clinical trial*	*542 pregnant women*	*1 capsule containing a mixture of 3 organisms containing at least 7.5 x 10^9 CFU of Lactobacillus acidophilus LA1*	*Daily for 24 weeks*	*Other than 3 subjects with increased flatulence, there were no adverse effects.*
Karyana et al. (2022)	*The efficacy of probiotics supplementation of the lipid profiles of obese adolescents: a randomized trial*	*58 obese adolescents*	*1.25 x 10^9 CFU of 5 strains containing Lactobacillus acidophilus*	*8 weeks*	*No adverse effects were observed.*

Kunz et al. (2004) discusses two cases of Lactobacillus bacteremia. The authors specifies that these two cases of bacteremia occur in patients who had a gastrointestinal tract condition. Boyle et al. (2006) presented a review publication on what may cause bacteremia as well as several cases of bacteremia or bacterial sepsis related to Lactobaclli. However, the author mentions "all cases of bacteremia or fungemia gave occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons." Therefore, these publications conclude that food-borne illness, such as bacteremia, are typically caused by medical or external causes.

Owing to the results of the updated literature search performed on May – June 2023 from studies from 2004 to current and additional publication on the pathogenicity and toxigenicity as well as no significant adverse effects of Lactobacillus acidophilus, none of the published studies is contradictory with the GRAS conclusion.

Attachment(s) II961.1-CBI.4-A1, II961.1-CBI.4-A2, II961.1-CBI.4-A3, II961.1-CBI.4-A4, II961.1-CBI.4-A5, II961.1-CBI.4-A6, II961.1-CBI.4-A7, II961.1-CBI.4-A8.

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted so far as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above responses or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

Attachments

II961.1-CBI.4-A1	Lim EY, Lee S-Y, Shin HS, Lee J, Nam Y-D, Lee DO, Lee JY, Yeon SH, Son RH, Park CL, Heo YH, Kim YT (2020). The effect of Lactobacillus acidophilus YT1 (Menolacto) on improving menopausal symptoms: A randomized, double-blinded, placebo-controlled clinical trial. *J. Clin Med.* 2020, 9, 2173
II961.1-CBI.4-A2	Preston K, Krumian R, Hattner J, de Montigny D, Stewart M, Gaddam S (2018). Lactobacillus acidophilus CL1285, Lactobacillus casei LBC80R, and Lactobacillus rhamnosus CLR2 improve quality-of-life and IBS symptoms: a double-blind, randomised, placebo-controlled study. *Beneficial Microbes,* 2018 online.
II961.1-CBI.4-A3	Ahn SB, Jun DW, Kang B-K, Lim JH, Lim S, Chung M-J (2019). Randomized, double-blind, placebo-controlled study of a multispecies probiotic mixture in nonalcoholic fatty liver disease. *Scientific Reports* (2019) 9:5688.
II961.1-CBI.4-A4	Mitovic M, Stankovic-Popovic V, Tolinacki M, Golic N, Bajic SS, Veljovic K, Nastasijevic B, Soldatovic I, Svorcan P, Dimkovic N (2023). The impact of symbiotic treatment on the levels of gut-derived uremic toxins, inflammation, and gut microbiome of chronic kidney disease patients – a randomized trial. *Journal of Renal Nutrition*, Vol 33, No 2 (March), 2023: pp 278-288.
II961.1-CBI.4-A5	Shahriari A, Karimi E, Shahriari M, Aslani N, Khooshideh M, Arab A (2021). The effect of probiotic supplementation on the risk of gestational diabetes mellitus among high-risk pregnant women: a parallel double-blind, randomized, placebo-controlled clinical trial. *Biomedicine & Pharmacotherapy* 141 (2021) 111915.
II961.1-CBI.4-A6	Karyana PG, Apsari NLS, Artana WD, Suarta K, Yantie PVK, Nesaa NNM, Putra GNS, Soetjiningsih (2022). The efficacy of probiotics supplementation of the lipid profiles of obese adolescents: a

	randomized trial. *Bali MedJ* 2022, Volume 11, Number
II961.1-CBI.4-A7	Kunz AN, Noel JM, Fairchok MP (2004). Two cases of Lactobacillus bacteremia during probiotic treatment of short gut syndrome. *J Pediatr Gastroenterol Nutr*, Vol. 38, No. 4, April 2004
II961.1-CBI.4-A8	Boyle RJ, Robins-Browne RM, Tang MLK (2006). Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006; 83:1256-64.

All attachments in the prior list have been removed in accordance with copyright laws.

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9/6/2023

Katie Overbey, PhD
Regulatory Review Scientist
Division of Food Ingredients
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Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
katie.overbey@fda.hhs.gov

RE: Response to FDA Questions Regarding GRN 001083 Received on 8/23/23
II961.1-CBI.5

Dear Dr. Overbey,

REJIMUS, INC. received your email dated 8/23/23 regarding additional FDA questions to GRN 001083. This is the response to address the questions presented.

Should you have any questions or concerns with this additional information based on the information provided, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS REGARDING GRN 001083 RECEIVED ON 8/23/23

Question 1

1. In the May 16, 2023 amendment (response to question 18), the notifier provides a requested specification limit for lead as well as the limits for arsenic, cadmium, and mercury along with the results (reported as "Passes test") from the analyses of three non-consecutive batches. We note that we typically do not see limits for lead as high as ≤1 mg/kg (the limit proposed by the notifier) for fermentation derived ingredients manufactured in accordance with good manufacturing practices. In addition, we would like to bring to your attention a relevant FDA's "Closer to Zero" initiative that focuses on reducing dietary exposure to heavy metals.

    a. Please specify the limit of detection (LOD) and/or limit of quantitation (LOQ) for the analytical method(s) used to test for heavy metals and provide the results for heavy metals as the actual measured levels or state that the levels are below the specified LOQ or LOD. If ≤1 mg/kg is the LOQ or LOD of the analytical method used to test for lead, we recommend that the notifier use a more sensitive method to measure the actual levels of lead in the ingredient and propose a specification limit that reflects the results of the batch analyses and is as low as possible. If a new method is employed, please provide a statement that it is validated for its purpose.

    b. Furthermore, we note that the results of the batch analyses provided for cadmium were similar to those for arsenic and mercury. For consistency with the specifications proposed for arsenic and mercury and in keeping with FDA's Closer to Zero initiative for heavy metals, please consider lowering the specification for cadmium to at least ≤ 0.1 mg/kg.

Response

a. A limit of detection (LOD) and Limit of quantitation (LOQ) for the analytical method used to test for heavy metals is provided in the attached Certificate of Analysis. The same Certificate of Analysis provides the test results in actual measured levels and all test results met specifications regarding the level of these heavy metals.

   The established LOD and LOQ for Lead for this analytical method used is 0.017 ppb (0.000017 mg/kg) and 0.050 ppb (0.00005 mg/kg), respectively. Owing to the very low LOQ and LOD, the analytical method used is sensitive enough to detect or quantify a small amount of Lead in the product. In addressing the specification of Lead at ≤ 1mg/kg, the specification in the attached COAs was based on production from 2017. However, based on more current batch analysis results and in recognition of FDA's "Closer to Zero" initiative, future production batches of this ingredient will have an updated Lead specification of ≤ 10 ppb (≤ 0.01 mg/kg).

b. For consistency with the specifications proposed for arsenic and mercury and in keeping with FDA's Closer to Zero initiative for heavy metals, the specification for cadmium has been updated to ≤ 0.1 mg/kg.

Attachment(s): II961.1-CBI.5-A1

Question 2

2. In the May 16, 2023 amendment, the notifier provides several specifications for microbial analyses, including yeast and mold, *Escherichia coli, Staphylococcus aureus, Salmonella* serovars, and *Listeria monocytogenes*. The notifier affirms that all the analytical methods employed are validated for their intended purpose; however citations are not provided for the analytical methods used for the listed microbial specifications. Please provide complete citations for the above listed analyses.

Response

Below are the citations for the above listed microbial analyses:

Parameter	***Method***
Yeast and Mold	*Analytical Method of Yeast and Mold (In-house test method)*
E. coli	*Korean FDA Food Code, VIII. Food Analytical Method, 4.8 E. coli*
S. aureus	*Analytical Method of S. aureus (In-house test method)*
Salmonella	*Analytical Method of Salmonella (In-house test method)*
L. monocytogenes	*Analytical Method of L. monocytogenes (In-house test method)*

Attachment(s): II961.1-CBI.5-A2, II961.1-CBI.5-A3, II961.1-CBI.5-A4, II961.1-CBI.5-A5, II961.1-CBI.5-A6,

Question 3

3. For the administrative record, please provide a revised copy of Table 4 for all specifications, including microorganisms (i.e., coliforms, yeast and mold, *E. coli*, *S. aureus*, *Salmonella* serovars, and *L. monocytogenes*) and heavy metals. Please include the most recent revisions made to the specifications (e.g., revision of the lead and cadmium specifications), the method of analysis for each specification, and the sample size for microbial specifications and ensure that the stated sample sizes align with the referenced analytical methods.

Response

Below is the revised Table 4 that includes all specifications for the ingredient:

Parameter	***Limits***	***Method***
Appearance	*Light brown powder*	*Visual*
Viable Cell Count	*≥ 1.0 x 10^{11} CFU/g*	*Analytical Method of Viable Cell Count (In-house test method)*
Coliforms	*Absent in 10 g*	*Korean FDA Food Code VIII. Food Analytical Method, 4.7 Coliforms*
Yeast and Mold	*≤ 10 CFU/g*	*Analytical Method of Yeast and Mold (In-house test method)*
E. coli	*Absent in 1 g*	*Korean FDA Food Code, VIII. Food Analytical Method, 4.8 E. coli*
S. aureus	*Absent in 25 g*	*Analytical Method of S. aureus (In-house test method)*
Salmonella	*Absent in 25 g*	*Analytical Method of Salmonella (In-house test method)*
L. monocytogenes	*Absent in 25 g*	*Analytical Method of L. monocytogenes (In-house test method)*
Lead	*≤ 0.01 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Parameter	***Limits***	***Method***
Cadmium	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Mercury	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*
Arsenic	*≤ 0.1 mg/kg*	*Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal*

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions on the above responses, please let us know at your earliest convenience and we will do everything we can to address those promptly.

Attachments

II961.1-CBI.5-A1	Certificate of Analysis reports
II961.1-CBI.5-A2	Analytical Method for Yeast and Mold
II961.1-CBI.5-A3	Analytical Method for *Escherichia coli*
II961.1-CBI.5-A4	Analytical Method for *Staphylococcus aureus*
II961.1-CBI.5-A5	Analytical Method for *Salmonella*
II961.1-CBI.4-A6	Analytical Method for *Listeria monocytogenes*

Attachment
II961.1-CBI.5-A1

CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 57R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 07 May. 2017

Exp. Date: 06 May. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	≤ 1.0 mg/kg	0.0032 mg/kg
Cadmium (Cd)**	≤ 0.3 mg/kg	0.0024 mg/kg
Mercury (Hg)***	≤ 0.1 mg/kg	0.0008 mg/kg
Arsenic (As)******	≤ 0.1 mg/kg	0.0082 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

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CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 65R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 13 Jun. 2017

Exp. Date: 12 Jun. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	≤ 1.0 mg/kg	0.0016 mg/kg
Cadmium (Cd)**	≤ 0.3 mg/kg	0.0013 mg/kg
Mercury (Hg)***	≤ 0.1 mg/kg	0.0029 mg/kg
Arsenic (As)****	≤ 0.1 mg/kg	0.0057 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

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Certificate of Analysis

Product Name : ***Lactobacillus acidophilus***

Place of Production: KOREA

Batch(Lot) No. : LA1 80R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 30 Jul. 2017

Exp. Date: 29 Jul. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light brown powder	Light brown powder
Initial viable cell	$\geq 1.0 \times 10^{11}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	$\leq$ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)*	$\leq$ 1.0 mg/kg	0.0090 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0008 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0015 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0100 mg/kg

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

* LOD: 0.017 ppb, LOQ: 0.050 ppb ** LOD: 0.026 ppb, LOQ: 0.080 ppb

*** LOD: 1.400 ppb, LOQ: 5.400 ppb **** LOD: 0.049 ppb, LOQ: 0.148 ppb

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Attachment
II961.1-CBI.5-A2

Analytical Method of Yeast and Mould

1. SCOPE

This work instruction defines the procedures for counting yeast and mould colony-forming units.

2. MEDIA AND REAGENTS

Dichloran rose Bengal chloramphenicol agar (DRBC)

3. METHODS

1. Prepare a 1 in 5 dilution of sample by emulsifying 10 grams in 40 mL of 0.1% peptone water.
2. Pipette 0.2mL of the (1 in 5) diluted sample onto 3 plates of DRBC.
3. Incubate upright at 25°C for 5 days.
4. Examine each plate and count yeast and mould colonies.

4. RESULTS

0.2mL of a 1 in 5 dilution sample is spread onto 3 DRBC plates, so the "limit of detection", one colony out of the 3 plates, is equivalent to 8 colony-forming units per gram.

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Attachment
II961.1-CBI.5-A3

Analytical Method of Coliform and E.coli

1. Test Method Summary

This test method defines the procedures for isolation and identification of Coliforms and E.coli in 1 gram of sample using most probable number technique and for E.coli in 1 gram of sample.

2. Media and Reagents

2.1 Single-strenth BGLB broth

2.2 Double-strenth BGLB broth

2.3 Eosin methylene blue agar (EMB)

2.4 EC broth (ECB)

2.5 Tryptone water

2.6 Kovac's reagent

3. Test Method

3.1 Prepare a 1 in 10 dilution of sample by emulsifying 10 grams in 90 ml of 0.1% peptone water. Also prepare a 1 in 100 dilution by transferring 1 ml of the initial suspension into 9 ml of 0.1% peptone water.

3.2 Take three tubes of double-strength BGLB broth. Using a sterile pipette, transfer to each of these tubes 10 ml of the 1 in 10 diluted sample.

3.3 Then take three tubes of single-strength BGLB broth. Using a fresh sterile pipette transfer to each of these tubes 1 ml of the 1 in 10 diluted samples.

3.4 Then take three tubes of single-strength BGLB broth. Using a fresh sterile pipette transfer to each of these tubes 1 ml of the 1 in 100 diluted samples.

3.5 Incubate all tubes at 37℃ for 2 days if neither gas formation nor opacity preventing the observation of gas formation is observed at this stage for 3 days.

3.6 Steak any presumptive positives (i.e. positive in BGLB) onto EMB agar and incubate at 37℃ for one day.

3.7 Examine for coliforms. Typical coliform colonies on EMB are dark purple. They may also have a green metallic sheen or be mucoid and pink on the surface but are dark purple when viewed from the back of the plate. Record any dark colonies as coliform positive.

3.8 Subculture from EMB into EC broth and tryptone water and incubate in a water bath at 44.0℃ to 44.5℃ for up to 48 hours.

3.9 Tap the tubes gently before reading reading to counter ant gas supersaturation. E.coli produce gas in ECB at 44.5℃.

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3.10 Test the tryptone water cultures for indole production by adding about 0.2 ml Kovac's reagent. E.coli is indole positive at 44.5℃.

4. Result

4.1 If no gas formation is observed in the BGLB tube, the result is reported as not detected in samples for E.coli.

Reference: KFDA Food Code, Ⅷ. Food Analytical Method, 4.7 Coliforms, 4.8 E.coli

Attachment
II961.1-CBI.5-A4

Analytical Method of *S. aureus*

1. Add 25g or 25mL of Test Solution to 225mL of Tryptic Soy Broth(BD REF 211825) with 10% NaCl concentration and cultivate at 35~37℃ for 18~24 hours. Then, Inoculate the cultured solution to Baird-Parker agar (BD REF 276840) and cultivate at 35~37℃ for 18~24 hours. Conduct confirmatory test on the agar if glossy black colonies surrounded by transparent rim or black colonies surrounded by opaque circles as a result of cultivation on Baird-Parker agar.

2. Suspected Staphylococcus-positive when black colonies proliferate on Baird-Parker agar then move those onto normal agar (BD REF 213000) and cultivate at 35~37℃ for 18~24 hours. Do Gram staining to check Gram positive coccus which has Staphylococcus pattern, then if found, conduct coagulase test to determine coagulation within 24 hours.

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Attachment
II961.1-CBI.5-A5

Analytical Method of Salmonella

1. SCOPE

This work instruction defines the procedures for isolation and identification of Salmonella.

2. MEDIA AND REAGENTS

- Buffered peptone water (BPW)
- Muller-Kaufman tetrathionate/novobiocin broth (MKTTn broth)
- Rapport Vassiliadis medium with soya (RVS broth)
- XLD(xylose lysine desoxycholate) medium
- API20E

3. METHODS

1. Inoculate 25g of sample into 225mL of BPW and incubate at 37^{0}C for 16-20 hours. This is known as the pre-enrichment stage.
2. Transfer 1ml of pre-enrichment into 10ml MKTTn broth, and another 0.1ml of pre-enrichment into 10ml of RVS broth.
3. Incubate MKTTn broth at 37℃ and incubate RVS broth at 42℃, both for 24 hours.
4. Streak MKTTn an RVS selective enrichment broths onto one plate XLD agar.
5. Invert the dishes and place in the incubator set at 37℃ for 1-2 days for XLD agar.
6. Examine the plate for the presence of typical colonies of Salmonella and atypical colonies that may be Salmonella. Typical colonies of Salmonella grown on XLD agar have a black center and a slightly transparent zone of reddish color due to the color change of the indicator. Confirm any pink colonies.

   Note: Salmonella H_2S negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive Salmonella grown on XLD agar are yellow with or without blackening.
7. If the API20E result shows that Salmonella is very unlikely, the result should be reported as Salmonella-negative, quoting the API 20E result code, regardless of whether a unique identification is achieved.

4. RESULTS

Report result as presence or absence for Salmonella in 25g sample.

Attachment
II961.1-CBI.5-A6

Analytical Method of *L.monocytogenes*

1. SCOPE

This work instruction defines the procedures for isolation and identification of *Listeria*.

2. MEDIA AND REAGENTS

- Buffered listeria enrichment broth (BLEB)
- Oxford Agar
- Tryptone soya yeast extract agar (TSYEA)
- Tryptone soya yeast extract broth (TSYEB)
- API Listeria
- Motility medium
- Hydrogen peroxide solution 3% (v/v)

3. METHODS

1. Inoculate 25g of sample into 225mL of BLEB and incubate at 30^0C for 46-50 hours.

2. Using a technique ensuring isolated colonies, streak the enrichment broth onto Oxford agar, and incubate at 37^0C for 48±2 hours.

3. Examine each plate for typical Listeria colonies, which are small dark colonies with possible greenish sheen and are about 2mm in diameter with black halos and sunken centres.

4. Streak each suspect colonies onto tryptone soya yeast extract agar (TSYEA), and incubate at 37^0C for 24 hours or until growth is satisfactory.

5. Perform a Gram stain on each suspect culture.
   a) *Listeria* spp. are Gram-positive slim rods.
   b) If the Gram result is convincingly atypical, report the culture as *Listeria*-negative, otherwise continue.

6. Perform a catalase test on each of the suspect culture:
   a) *Listeria* spp. are catalase positive.
   b) If the culture is catalase-negative, report as *Listeria*-negative, otherwise continue.

7. Perform a motility test on each suspect culture; using the stabbing technique and or using a hanging drop technique to determine typical tumbling motility.
   a) *Listeria* are motile, with a typical umbrella like growth pattern in motility medium and an unmistakable tumbling motion in fresh hanging drops preparations.
   b) If the culture is non-motile, report as *Listeria*-negative, otherwise continue.

8. Report presumptive *Listeria* identification immediately, if the Gram, catalase and motility results are atypical.

9. Confirm the genus *Listeria* and identify the species using API *Listeria* kit.

4. RESULTS

Report result as presence or absence for *Listeria* in 25g sample.

Motility test

Take a typical colony obtained on the TSYEA and suspend in a tube containing TSYEB.

Incubate at 25^{0}C for 8 - 24h until a cloudy medium is observed.

Deposit a drop of the above culture using a loop onto a clean glass microscope slide. Place a cover slip on top and examine it with the microscope. *Listeria* spp. appears slim, short rods with tumbling motility.

Cultures grown above 25^{0}C may fail to exhibit this motion. Always compare to known culture. Cocci, large rods, or rods with rapid swimming motility are not *Listeria* spp.

As an alternative test for motility, using an inoculating needle, stab the motility agar with a culture from a typical colony on TSYEA. Incubate for 48h at 25^{0}C.

Examine for growth around the stab. *Listeria* spp. are motile, giving a typical umbrella-like growth pattern. If growth is not sufficient, incubate for up to an additional 5 days and observe the stab again.

Overbey, Katie

From: Joel Villareal <joel@rejimus.com>
Sent: Monday, October 2, 2023 2:45 PM
To: Overbey, Katie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Livia Consedine; Kent Phan
Subject: [EXTERNAL] FW: Additional Question - GRN 1083

Dear Dr. Overbey,

Thank you for your email. We would like to respectfully respond to the question below and confirm the estimated dietary exposure of the ingredient.

Request:

1. In the amendment dated June 16, 2023 (response to Question 22), the notifier multiplied the values of 8.94×10^8 CFU/person (p)/d and 1.85×10^9 CFU/p/d by three (i.e., by the number of suggested daily servings) to obtain the eaters-only estimate of dietary exposure at the mean and 90th percentile, respectively. We note that based on the information provided in Table 7 of GRN 001083, the values of 8.94×10^8 CFU/p/d and 1.85×10^9 CFU/p/d already account for the number of servings consumed per person day that were estimated based on food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). Therefore, we consider that multiplying these values by three suggested daily servings was inappropriate. Please confirm that the estimated eaters-only dietary exposure to the ingredient would be 8.94×10^8 CFU /p/d at the mean and 1.85×10^9 CFU/p/d at the 90th percentile for the U.S. population aged 2 years and older and that these updated dietary exposure estimates would not affect your GRAS conclusion.

Response:
We confirm for GRN 001083 that the estimated eaters-only dietary exposure of the ingredient for the U.S. population aged 2 years and older is 8.94×10^8 CFU/p/d at the mean and 1.85×10^9 CFU/p/d at the 90th percentile. As such, the updated dietary exposure estimates would not affect the GRAS conclusion for GRN 001083.

If there are any questions regarding this response, please let us know and we will be sure to address that promptly.

Sincerely,

Joel Villareal | Regulatory Director
Quality Development Services
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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Monday, October 2, 2023 at 7:54 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: Additional Question - GRN 1083

Hello Mr. Lassiter,
Per our phone call, the FDA had an additional clarifying question for GRN 1083, please see below:

2. In the amendment dated June 16, 2023 (response to Question 22), the notifier multiplied the values of 8.94×10^8 CFU/person (p)/d and 1.85×10^9 CFU/p/d by three (i.e., by the number of suggested daily servings) to obtain the eaters-only estimate of dietary exposure at the mean and 90th percentile, respectively. We note that based on the information provided in Table 7 of GRN 001083, the values of 8.94×10^8 CFU/p/d and 1.85×10^9 CFU/p/d already account for the number of servings consumed per person day that were estimated based on food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). Therefore, we consider that multiplying these values by three suggested daily servings was inappropriate. Please confirm that the estimated eaters-only dietary exposure to the ingredient would be 8.94×10^8 CFU /p/d at the mean and 1.85×10^9 CFU/p/d at the 90th percentile for the U.S. population aged 2 years and older and that these updated dietary exposure estimates would not affect your GRAS conclusion.

We ask that you format your response such that each answer immediately follows the stated question and that you submit responses as a PDF or as a direct response to this email. Please ensure that your responses do not contain confidential business information and please do not submit a revised version of the GRAS notice.

We respectfully request a response to these questions within 10 business days. If you are unable to complete the response within that time frame, please contact me to discuss further options.

Thank you in advance for your attention to our comments.

Best,
Katie

Katie Overbey, Ph.D., M.S (she/her/hers)
Regulatory Review Scientist

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From: Joel Villareal
To: Highbarger, Lane A
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Livia Consedine; Kent Phan
Subject: [EXTERNAL] FW: Wash step in GRNs 1078-1088
Date: Friday, October 6, 2023 7:53:13 PM
Attachments: image001.png

Dear Dr. Highbarger,

Thank you for your email. Below is the response to the following question.

Request:
Is there a wash step in the purification process in GRNs 1078-1088 after the microorganisms are separated by filtration?

Response:
There is no wash step in the purification process after the microorganisms are separated.

The Sponsor has brought to our attention a translation issue concerning the separation process. As a clarification, microorganisms are separated not by filtration, but by using a centrifugation method. During this process, the microorganisms are spun down and concentrated. Following this step, all fermentation medium is removed and the microorganisms are transferred into the blending process. Please note that this process applies to all notified microorganisms from Cell Biotech Co. Ltd.

If there are any questions regarding this response, please let us know and we will be sure to address that promptly.

Sincerely,

Joel Villareal | Regulatory Director
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