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Office of Food Safety Additive Safety
Center for Food Safety and Applied Nutrition
United States Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

RE: GRAS Notification of *Lactobacillus rhamnosus* CBT LR5
II933.2-CBI.2.2

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 provide notice of a claim that the addition of the microorganism ***Lactobacillus rhamnosus* CBT LR5** to the food 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 Aegibong-ro 409 beon-gil, Wolgot-myeon, Gimpo-si, Gyeonggi-do, 415-872, Korea] has determined that the intended uses are generally recognized as safe (GRAS). The attached documents contain the specific information and data that addresses the safety of the substance for use in human food applications.

Respectfully,

Jim Lassiter, 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
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, Aegibong-ro 409 Beon-gil
Wolgot-myeon, Gimpo-si,
Republic of Korea
Tel: +82 31 987 6205

Name and Address of Manufacture:

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 rhamnosus* CBT LR5**

Intended Conditions of Use and Levels of Inclusion

The intended use of *Lactobacillus rhamnosus* CBT LR5 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 rhamnosus CBT LR5 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 rhamnosus* CBT LR5 is not intended for addition to standardized foods unless it is permitted by the applicable standard of identity.

Basis for GRAS Conclusion

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 rhamnosus* CBT LR5 is GRAS for its intended conditions of use as stated in this notification and, such use of *Lactobacillus rhamnosus* CBT LR5 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 rhamnosus* CBT LR5 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 rhamnosus* CBT LR5.

Respectfully submitted,

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

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

Common name: *Lactobacillus rhamnosus* CBT LR5 (KCTC 12202BP)

The taxonomic name for this organism has been recently changed. This organism is now known as *Lacticaseibacillus rhamnosus*. For purposes of this dossier the organism will be referred to using its previous name *Lactobacillus rhamnosus*. This is done in order to more closely associate the organism submitted in the dossier with the scientific literature that is presented as supporting evidence. (Zheng et al. 2020)

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

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Lactobacillaceae
Genus: *Lactobacillus*
Species*: rhamnosus*
Strain: CBT LR5

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).

This particular strain of *Lactobacillus rhamnosus* CBT LR5 is known under the commercial name as LAB2PRO™ as a high stability lactic acid bacterium.

Identification

The organism that is the subject of notified substance, originally isolated from cheese, is identified as Lactobacillus *rhamnosus* and has been uniquely characterized as a distinct strain known as CBT LR5 by means of genomic typing.

Carbohydrate Utilization

Fermentative characteristics of *Lactobacillus rhamnosus* CBT LR5 were analyzed using API 50 CHL kit. Results are shown in Table 1.

Table 1. Fermentative characteristics of *Lactobacillus rhamnosus* CBT LR5. 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

Strains of *L. rhamnosus* are found in diverse ecological niches including fermented dairy, plants, and human and animal intestines. The complex relationship of *L. rhamnosus* strains with their hosts, including the ability to affect mucosal physiology, is not completely understood (Ceapa et al. 2016).

Gene sequencing of 16S rRNA was used to validate *Lactobacillus* strains phenotypical characteristics (Verdenelli et al. 2009). The 16S rRNA gene sequence were aligned and compared with different *Lactobacillus* strains: *L.* rhamnosus (KCTC 12202BP), *L. rhamnosus* (ATCC 7469), *L. paracasei* (ATCC 25302), *L. acidophilus* (ATCC 4356), *L. casei* (ATCC 393), *L. salivarius* (ATCC 11741), *L. planetarium* (ATCC 14917), and *Lactococcus lactis* (ATCC 19435). Percent identity and divergence were compared between *Lactobacillus* species and strains in Table 2. As presented 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.* rhamnosus DNA was compared using RAPD with *Lactobacillus rhamnosus* ATCC 7469 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 rhamnosus* CBT LR5 (KCTC 12202BP) and *L. rhamnosus* (ATCC 7469) 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 between *Lactobacillus rhamnosus* CBT LR5 and other closely related species based on 16S rRNA gene sequences. (Cellbiotech R&D Center 2018)

Percent Identity

Divergence

	1	2	3	4	5	6	7	8
1		99.5	98.7	85.5	98.2	88.4	90.9	79.8
2	0.0		98.0	85.1	97.0	88.2	90.5	76.9
3	1.1	1.5		84.5	98.4	87.6	89.2	78.1
4	13.9	13.5	14.5		84.8	85.3	84.1	72.9
5	1.2	1.2	1.1	13.6		88.5	88.4	75.9
6	9.8	9.4	10.3	13.8	9.4		88.4	79.1
7	8.1	8.0	9.4	14.7	9.0	9.9		77.1
8	15.3	15.6	15.5	18.3	15.5	16.1	15.2	

1. *L. rhamnosus* (KCTC 12202BP)
2. *L. rhamnosus* (ATCC 7469)
3. *L. paracasei* (ATCC 25302)
4. *L. acidophilus* (ATCC 4356)
5. *L. casei* (ATCC 393)
6. *L. salivarius* (ATCC 11741)
7. *L. plantarum* (ATCC 14917)
8. *Lc. lactis* (ATCC 19435)

Figure 1. Phylogenic tree between *Lactobacillus rhamnosus* CBT LR5 (KCTC 12202BP) and other closely related Lactobacillus spp. based on 16S rRNA gene sequence. (Cellbiotech R&D Center 2018)

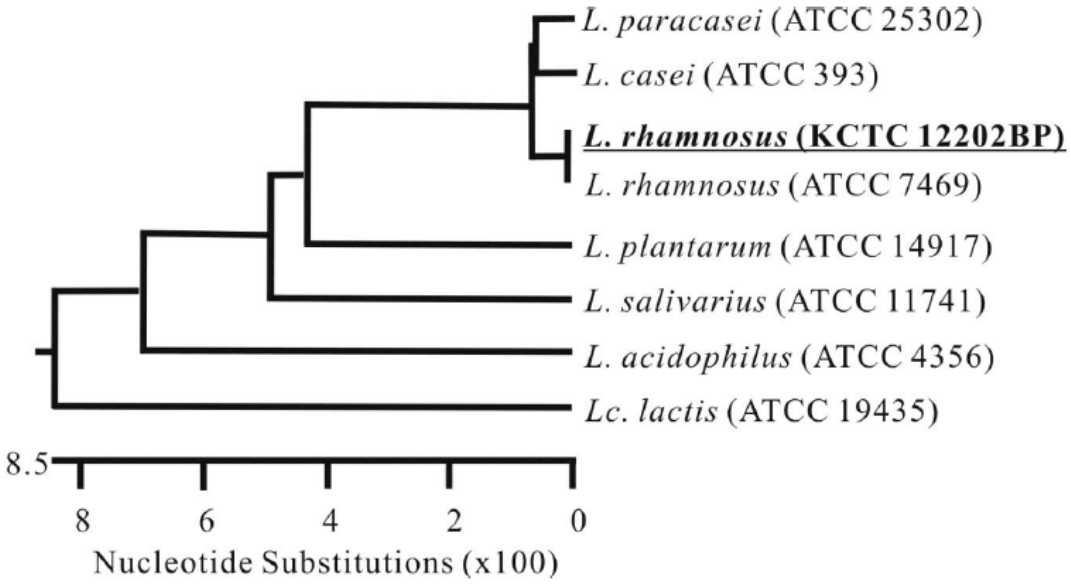
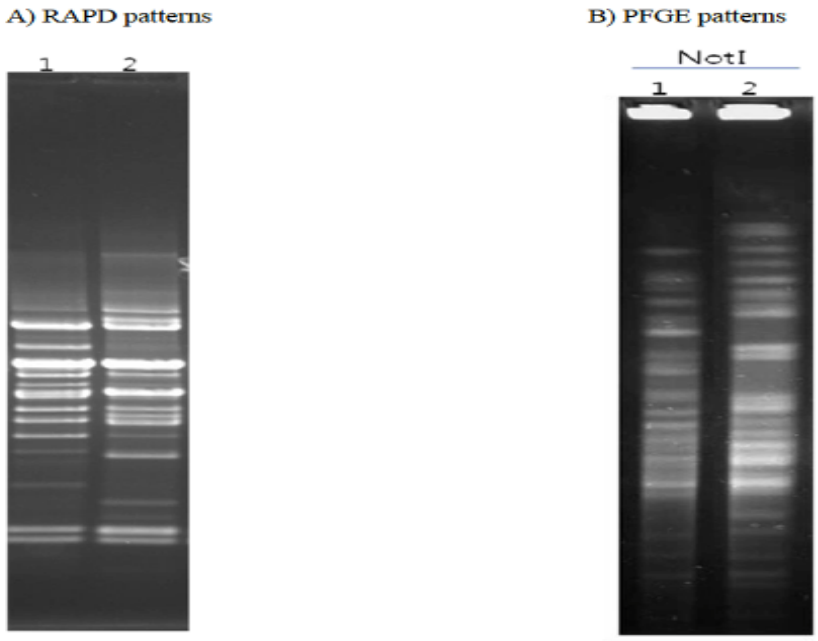


Figure 2. RAPD and PFGE results between *Lactobacillus rhamnosus* CBT LR5 (KCTC 12202BP) – Lane 1 and Lactobacillus rhamnosus ATCC 7469 – Lane 2. (Cellbiotech R&D Center 2018)



Manufacturing

Components

All components employed in the manufacture of *Lactobacillus rhamnosus* CBT LR5 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**
Dextrose Monohydrate	[77938-63-7]	21 CFR §168.111
Soy Peptone	[73049-73-7]	21 CFR §184.1553
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
Magnesium Sulfate	[10034-99-8]	21 CFR §184.1443
Manganese Sulfate	[15244-36-7]	21 CFR §182.5461
Protease	[9001-92-7]	21 CFR §182.1
Coating Ingredient	**CAS No.**	**Reference**
Trehalose	[6138-23-4]	FEMA No. 4600 (FEMA GRAS Publication No. 24)
D-Sorbitol	[98201-93-5]	21 CFR §184.1835
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
Corn Starch	[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.

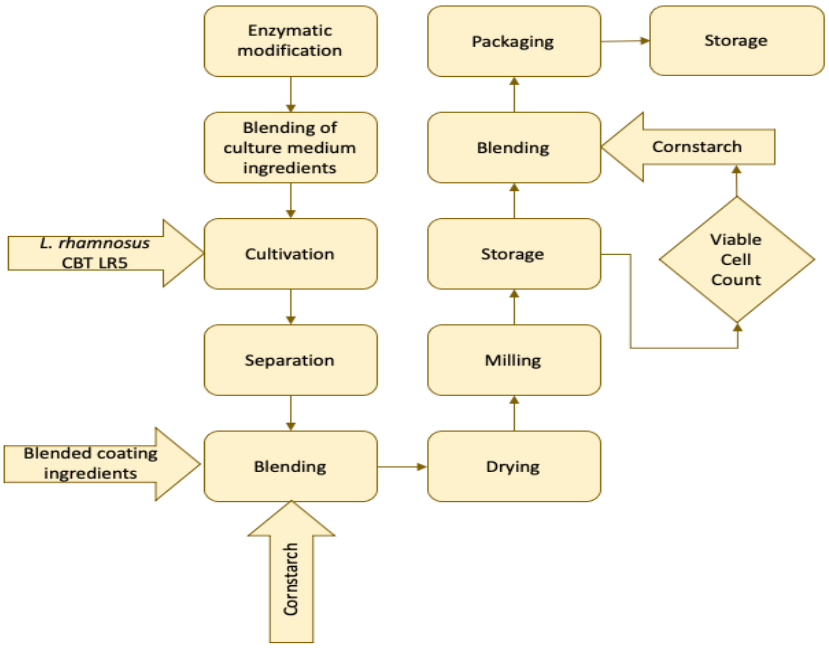
Standardization

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 rhamnosus* CBT LR5 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 rhamnosus* CBT LR5 food grade specifications and conforming test results.

Parameter	Limits	Method	Batch 15R	Batch 22R	Batch 26R
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	In-house method	Conforms	Conforms	Conforms
Coliforms	Absent in 10 g	In-house method	Conforms	Conforms	Conforms

Stability Data

In order to determine the stability of *Lactobacillus* rhamnosus CBT LR5, 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 rhamnosus* CBT LR5. 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 rhamnosus* CBT LR5 at 5 ± 3 °C.

Strain	Batch No.	Test	Time Point				
			Initial	3 Months	6 Months	9 Months	12 Months
Lactobacillus rhamnosus CBT LR5	15R	VCC (CFU/g)	1.15×10^{13}	9.90×10^{11}	8.85×10^{11}	7.90×10^{11}	7.10×10^{11}
		Survival Rate (%)	100.0	86.3%	77.1%	68.9%	61.8%
	22R	VCC (CFU/g)	1.22×10^{12}	1.02×10^{12}	9.15×10^{11}	8.11×10^{11}	7.47×10^{11}
		Survival Rate (%)	100.0	83.7%	75.2%	66.7%	61.4%
	26R	VCC (CFU/g)	1.32×10^{12}	1.17×10^{12}	1.06×10^{12}	9.67×10^{11}	8.81×10^{11}
		Survival Rate (%)	100.0	88.4%	80.1%	73.1%	66.6%
	Average Survival Rate (%)		100.0%	86.5%	77.4%	69.5%	63.2%

Technical Effects

This substance will be used to provide as a dietary source of *Lactobacillus rhamnosus* CBT LR5 as a food ingredient to dairy products.

PART 3 – DIETARY EXPOSURE

Intended Use and All Sources in the Diet

The intended use of *Lactobacillus rhamnosus* CBT LR5 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 x 10^{11} CFU per serving in dairy products would result in intakes in all users of 8.94 x 10^{10} CFU and 1.85 x 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 x 10^{11} per person per day.

Table 7. EDIs of *Lactobacillus rhamnosus* CBT LR5 from proposed uses in dairy products across all users based on 2017-2018 NHANES.

Group	% (n)	Dairy intake g/day		Dairy, serving/day		*Lactobacillus rhamnosus CBT LR5* 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 rhamnosus* CBT LR5, 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 rhamnosus* CBT LR5. 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 rhamnosus* CBT LR5**.**

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* rhamnosus CBT LR5 is included. The metabolic by-products from *Lactobacillus rhamnosus* CBT LR5 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 rhamnosus* CBT LR5 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.
- D-sorbitol is listed as a color or cooling adjunct, drying agent, flavoring agent or adjuvant, humectant, nutrient supplement, nutritive sweetener, pH control agent, solvent or vehicle, stabilizer or thickener, or texturizer.
- 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 rhamnosus* CBT LR5 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 rhamnosus* 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 Notices

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 rhamnosus* CBT LR5 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 rhamnosus* and its subspecies. GRAS notices of *Lactobacillus* organisms of species other than *rhamnosus* which FDA has no questions are presented below in Table 9.

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

GRAS No.	Date of Closure	Substance
288	27-Mar-2009	*Lactobacillus rhamnosus* strain HN001
281	31-Aug-2009	*Lactobacillus rhamnosus* strain HN001 produced in a milk-based medium

Table 9. GRAS notices of *Lactobacillus* organisms of species other than *rhamnosus* 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
254	18-Nov-2008	*Lactobacillus reuteri* strain DSM 17938
736	11-Apr-2018	*Lactobacillus casei* subsp. *paracasei* Lpc-37

Approved Use

The status of *Lactobacillus rhamnosus* 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 rhamnosus* 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. rhamnosus*) present in the 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 rhamnosus* CBT LR5 as the test strain. Observed MIC values for *Lactobacillus rhamnosus* CBT LR5 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 (2012) published by the European Food Safety Authority (EFSA), as shown in Table 10 and therefore this strain is susceptible to AMP, GEN, KAN, STM, ERM, CLM, TET, and CP. *Lactobacillus* strains exhibit high intrinsic resistance to vancomycin (Campedelli et al. 2019) and testing for such in *Lactobacillus rhamnosus* is not required (n.r.) by EFSA guidance (EFSA 2012).

Table 10. Antibiotic susceptibility for *Lactobacillus rhamnosus* CBT LR5.

Strain	**Minimum Inhibitory Concentrations (μg/mL) of Antibiotics**								
	AMP	VAN	GEN	KAN	STM	ERM	CLM	TET	CP
***L. rhamnosus* CBT LR5**	< 1	> 256	< 16	< 64	< 32	< 0.5	< 1	< 1	< 4
EFSA Cut-off Value	4	n.r.	16	64	32	1	1	8	4

Current Marketplace Availability of *Lactobacillus rhamnosus* CBT LR5

While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of *Lactobacillus rhamnosus* CBT LR5 in foreign countries and in multiple food products.

In vitro Toxicity Studies

Hemolysis Assay

The Cell Biotech R&D Center tested *Lactobacillus rhamnosus* CBT LR5 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 rhamnosus* CBT LR5 were investigated using male and female Sprague-Dawley rats. The pathogenicity of *Lactobacillus rhamnosus* CBT LR5 was examined after treating the rats with 10^{11} CFU/kg doses or 0.85% saline (control) intragastrically. 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 3), gross pathological findings (Table 12), feed and water consumption (Figure 4), organ weight (Table 13), and body temperature (Table 14) among the different treatment groups and between the treated and control rats.

Table 11. Mortality of male and female rats orally administered with 1×10^{11} CFU/kg *Lactobacillus rhamnosus* CBT LR5 ((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 LR5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$> 10^{11}$ CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Female	CBT LR5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$> 10^{11}$ CFU/kg
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Figure 3. Body weight curves for male and female rats given 10^{11} CFU/kg *Lactobacillus rhamnosus* CBT LR5 and control for 14 days. Values are mean ± SE. ((Cellbiotech R&D Center (2018))

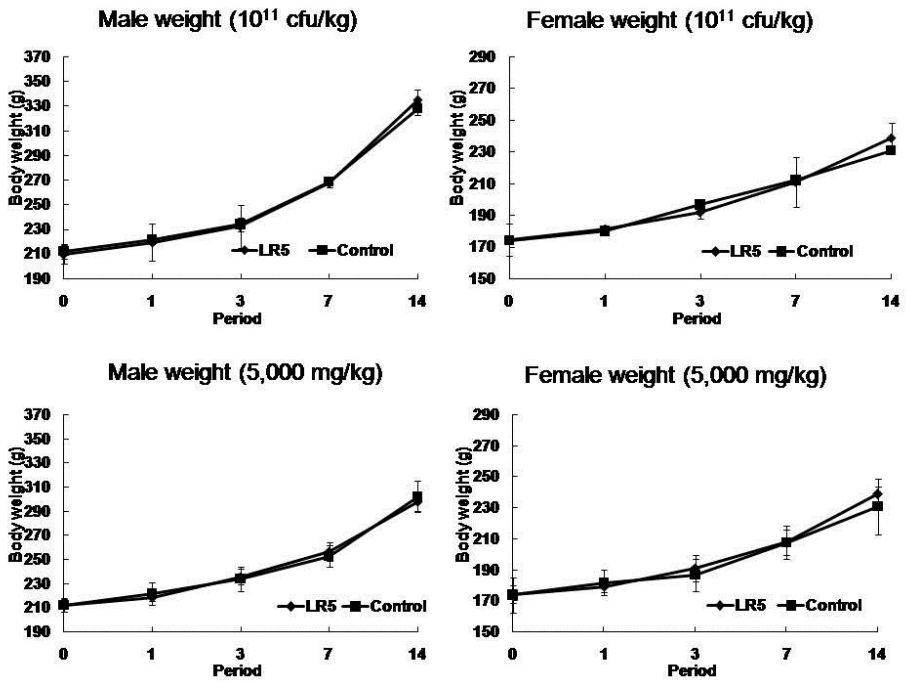


Table 12. Clinical findings of male and female rats orally administered with 10^{11} CFU/kg *Lactobacillus rhamnosus* CBT LR5 (Cellbiotech R&D Center 2018).

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

Figure 4. Food and water consumption of male and female rats given 10^{11} cfu/kg *Lactobacillus rhamnosus* CBT LR5 (Cellbiotech R&D Center 2018).

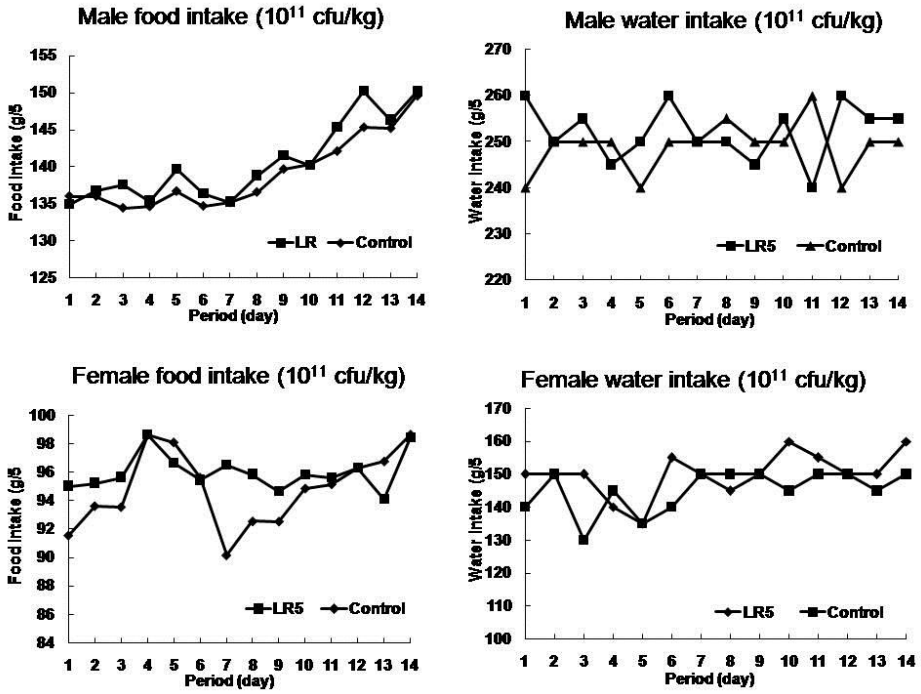


Table 13. Absolute organ weights (g) of male and female orally administered with 10^{11} CFU/kg *Lactobacillus rhamnosus* CBT LR5 (Cellbiotech R&D Center 2018).

Sex	Parameters	Lab	CBT LR5	Control
		No. of Animals	5	5
Male	Body weight (g)		335.41 ± 16.13	328.39 ± 5.19
	Liver (g)		12.14 ± 0.90	11.81 ± 0.77
	Spleen (g)		0.66 ± 0.10	0.64 ± 0.05
	Kidney (g)	Right	1.16 ± 0.10	1.12 ± 0.05
		Left	1.16 ± 0.05	1.18 ± 0.12
Female	Body weight (g)		239 ± 21.38	230.73 ± 14.10
	Liver (g)		7.66 ± 1.01	7.65 ±1.15
	Spleen (g)		0.44 ± 0.03	0.41 ± 0.08
	Kidney (g)	Right	0.79 ± 0.11	0.78 ± 0.12
		Left	0.76 ± 0.04	0.77 ± 1.10

Table 14. Body temperature changes in male and female orally treated with 10^{11} CFU/kg *Lactobacillus rhamnosus* CBT LR5 ((Cellbiotech R&D Center (2018))

Day	No.	Male body temperature		Female body temperature	
		CBT LR5 (˚C)	Control (˚C)	CBT LR5 (˚C)	Control (˚C)
Pre-treatment	Ave	34.12	34.82	35.56	35.66
	SEM	0.29	0.39	0.39	0.25
Day 1	Ave	35.16	34.80	35.74	35.76
	SEM	0.58	0.35	0.50	0.65
Day 2	Ave	34.66	35.08	35.58	35.60
	SEM	0.57	0.54	0.49	0.34
Day 3	Ave	35.96	35.86	35.46	35.48
	SEM	0.49	0.34	0.34	0.37
Day 4	Ave	35.62	35.40	35.70	35.48
	SEM	0.44	0.21	0.45	0.39

Human Studies

Study 1

Yim et al. (2006) studied the therapeutic effects of specific microorganisms in sixty-four patients with atopic dermatitis. Each patient was given one sachet twice daily that contained a total of 1×10^9 CFU of four microoganisms, including 2×10^8 CFU of *L. rhamnosus*, for eight weeks. Most patients tolerated the therapy well though one patient that completed the study complained of constipation.

Study 2

Dinleyici et al. (2013) conducted a single blinded randomized study of the effect of a multispecies symbiotic mixture on the duration of diarrhea and length of hospital stay for children with acute diarrhea. The patients included forty-three girls three months to ten years old and seventy boys in the same age bracket. The microbial mixture contained a serving of 2.5×10^9 CFU of bacteria, including *L. rhamnosus*, given daily for five days. The patients reportedly suffered no adverse effects from the therapy.

Study 3

Forty-nine patients suffering from Irritable Bowel Syndrome (IBS) were enrolled in a randomized, double-blind, placebo-controlled study to determine the effect of a multispecies microbial therapy on IBS symptoms and gut microbiota. One capsule twice daily containing a total of 5×10^9 viable cell strains, including *L. rhamnosus* (KCTC 12202BP), was given to twenty-five of them for 4 weeks. The treatment was effective in symptom relief and no adverse reactions were reported (Yoon et al. 2014).

Study 4

Lee et al. (2014) conducted a randomized, double-blind, placebo-controlled clinical study on the effects of co-administration of specific microorganisms, including *L. rhamnosus*, with herbal medicine on obesity, metabolic endotoxemia and dybiosis. Fifty female patients, ages 19–65 years, were enrolled in the study and given either the microorganisms + Bofutsushosan (an Asian herbal medicine comprised of 18 components) or a placebo and the Bofutsushosan. The capsules reportedly contained 5×10^9 viable cells. All fifty patients tolerated the microbial strains with no reported negative issues.

Study 5

Yoon et al. (2015) conducted a study on the effect of administering a multispecies microorganism mixture with six organisms, including *L. rhamnosus* (KCTC 12202BP), on the changes in fecal microbiota and symptoms of irritable bowel syndrome. The study used 81 volunteers to study the effects of capsules containing 5×10^9 viable microbial cells taken over a period of four weeks.

The study concluded that while the overall composition of gut microflora did not significantly change, the concentration of most intestinal florad strains increased, and adequate irritable bowel symptom relief was higher in this group than those on placebo. None of the patients in the study arm that had been taking the multispecies microorganism mixture that included *L. rhamnosus* (KCTC 12202BP) reported adverse effects.

Study 6

Ipar et al. (2015) reported the effects of symbiotic on anthropemetry, lipid profile, and oxidative stress in obese children. Eighty-six obese children ranging in ages from four to 17 years were enrolled in the study. Each of the forty-three children in the symbiotic arm of the study were given a sachet containing five specific microorganisms, including 4.3×10^8 CFU of *L. rhamnosus*, and vitamins, daily for one month. No adverse events were reported.

Conclusion

The scientific data, information, methods, and principles described in this notification provide the basis for conclusion that *Lactobacillus rhamnosus* CBT LR5 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 Lactobacillus plantarum 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 rhamnosus* 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 rhamnosus*, 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 rhamnosus* CBT LR5.

Lactobacillus rhamnosus CBT LR5 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 rhamnosus* CBT LR5's potential for antibiotic resistance was tested in accordance with EFSA guidelines where *Lactobacillus* strains are intrinsically resistant to vancomycin.

Additional efficacy studies in humans and animals have been performed without the occurrence of observation of adverse events. An LD50 of greater than 1011 CFU/kg was established in rats which corresponds to a human equivalent amount of 9.6 × 1011 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 *Lactobacillus rhamnosus* CBT LR5 at the 90th percentile of high-level consumers of products of the intended inclusion food is 5.55 × 1011 CFU per day of *Lactobacillus rhamnosus* CBT LR5. The 90th percentile for actual consumption of 5.55 x 1011 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.

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 rhamnosus* CBT LR5

February 25, 2021

Cell Biotech Co. Ltd. intends to market ***Lactobacillus rhamnosus* CBT LR5** as an ingredient in dairy products. ***Lactobacillus rhamnosus* CBT LR5** 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 rhamnosus* CBT LR5** 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 rhamnosus* CBT LR5** 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 rhamnosus* CBT LR5**, 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 rhamnosus* CBT LR5** 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 rhamnosus* CBT LR5** 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 RHAMNOSUS* CBT LR5

February 25, 2021

Cell Biotech Co. Ltd. intends to market *LACTOBACILLUS RHAMNOSUS* CBT LR5 as an ingredient in dairy products. *LACTOBACILLUS RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5, 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 RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 is Generally Recognized as Safe by Self-determination based upon my review and participation in the appointed Expert Panel.

Signature [redacted] Date: 6 APR 21

Jeanne Moldenhauer, M. Sc.
Excellent Pharma Consulting

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Determination of Cell Biotech Co. Ltd. *LACTOBACILLUS RHAMNOSUS* CBT LR5

February 25, 2021

Cell Biotech Co. Ltd. intends to market *LACTOBACILLUS RHAMNOSUS* CBT LR5 as an ingredient in dairy products. *LACTOBACILLUS RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 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 Basis for Determination	Summary of intended exposure 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 RHAMNOSUS* CBT LR5, 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 RHAMNOSUS* CBT LR5 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 RHAMNOSUS* CBT LR5 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

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
Quality Development Services
joel@rejimus.com

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10/12/2023

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: Response to FDA Questions/Comments Regarding GRN 001084
II933.2-CBI.7

Dear Dr. Overbey,

REJIMUS, INC. received your email dated 9/28/23 regarding additional FDA questions/comments for GRN 001084. 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 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 001084

Question 1

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. rhamnosus* strain KCTC 12202BP (*L. rhamnosus* strain "CBT LR5"). Please provide a brief description of the morphology of *L. rhamnosus* strain KCTC 12202BP.

Response

Lactobacillus rhamnosus strain KCTC 12202BP is a gram-positive non-spore forming rod. The morphology of the colony is a short chain rod shape.

Question 2

2. Please provide a brief description of *L. rhamnosus* strain KCTC 12202BP, including phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites), and whether these characteristics poses a safety concern. For example, on page 17, the notifier states, "*Lactobacillus rhamnosus* CBT LR5 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 rhamnosus CBT LR5 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 rhamnosus strain KCTC 12202BP 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	NK [a]	Pediocin-like bacteriocins [b]:	Lactococcin G
Lactocin S		Pediocin PA1, Leucocin A,	Lactacin F
Lacticin 481		Sakacin P, Curvacin A,	Plantaricin E/F
Carnocin UI 49		Mesentericin Y105,	Plantaricin J/K
Cytolysin		Carnobacteriocin BM1, Carnobacteriocin B2,	Lactobin A
		Enterocin A, Piscicolin 126,	Plantaricin S[c]
		Bavaricin MN, Piscicocin V1a	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 cvclic antimicrobial peptide produced by *Enterococcus faecalis* (Martinez-Bueno et al. , 1994).

Attachment II933.2-CBI.7-A1

Question 3

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. rhamnosus* strain KCTC 12202BP 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 rhamnosus strain KCTC 12202BP is non-pathogenic and non-toxigenic.

Question 4

4. Please state whether *L. rhamnosus* strain KCTC 12202BP is genetically engineered.

Response

Lactobacillus rhamnosus strain KCTC 12202BP is not genetically engineered.

Question 5

5. On page 9, the notifier describes how pulsed field gel electrophoresis was performed on *L. rhamnosus* strain KCTC 12202BP and *L. rhamnosus* strain ATCC 7469; however, the notifier does not provide a discussion of these results. 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 rhamnosus strain KCTC 12202BP are different from the reference Lactobacillus rhamnosus strain ATCC 7469. Therefore, it can be indicated that Lactobacillus rhamnosus strain KCTC 12202BP is a new strain of Lactobacillus rhamnosus species.

Question 6

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 specify the method of analysis for each.

Response

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

Question 7

7. Please briefly specify how the purity of *L. rhamnosus* strain KCTC 12202BP 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

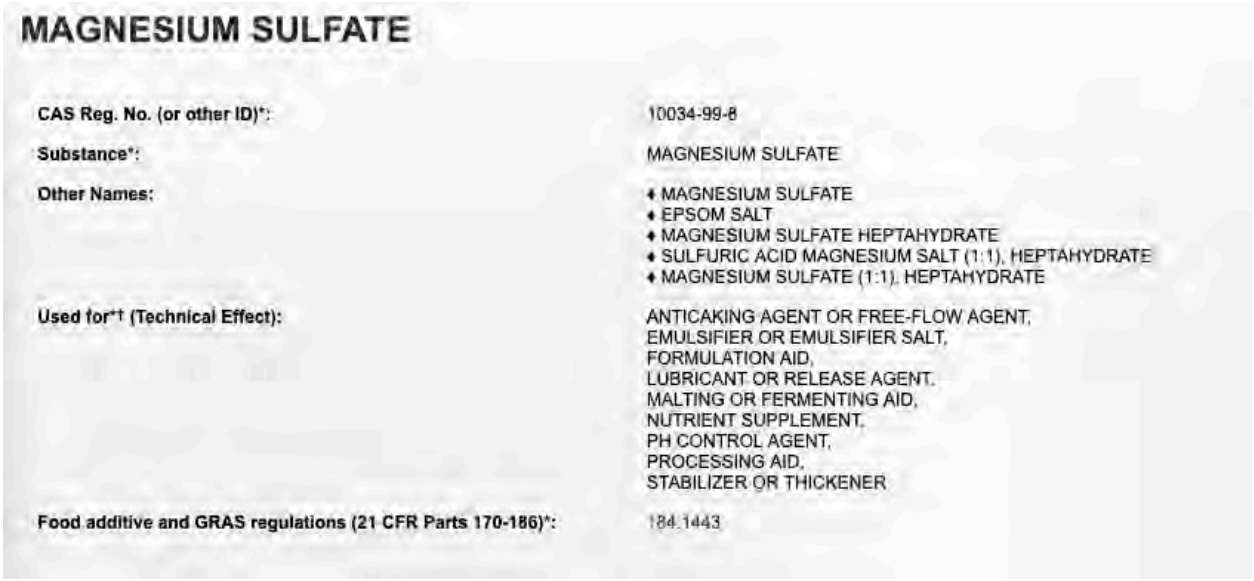
8. In Table 3 (page 11), the notifier provides a list of raw materials used during the manufacturing process. The CAS numbers provided for soy peptone, soy protein isolate, yeast extract powder, and corn starch do not appear to correspond to the correct substances. For the administrative record, please provide the correct CAS numbers for these four substances. In addition, we note that the correct name of the substance designated by CAS No. 10034-99-8 is magnesium sulfate heptahydrate and the correct name of the substance designated by CAS No. 15244-36-7 is manganese sulfate hydrate. Please confirm.

Response

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

Ingredient	CAS No.
Soy peptone	[91079-46-8]
Soy Protein Isolate	[977076-84-8]
Yeast Extract Powder	[8013-01-2]
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

Question 9

9. In Table 3 (page 11), the notifier provides a list of raw materials used during the manufacturing process. The references provided for manganese sulfate (21 CFR 182.5461) and corn starch (21 CFR 182.70 and 21 CFR 182.90), do not correspond to a regulation in the CFR. Please provide a clarified or appropriate reference for these substances. Further, the references provided for trehalose (FEMA No. 4600) and protease (21 CFR 182.1), 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
Corn starch	SCOGS Report No. 115
Trehalose	GRN 000045
Protease	21 CFR§184.1027

Question 10

10. In Table 3 (page 11), the notifier lists the components of the fermentation media, along with 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 soy peptone and 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 11

11. 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 12

12. 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 13

13. 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 182.1 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 14

14. In Table 4 (page 13), the notifier lists specifications for some microorganisms, including coliforms, but does not provide specifications for other common, notable foodborne pathogens, such as *Salmonella* serovars. Please address and provide clarification on the following:

    a. Please explain why there is not a specification for each microorganism. If a specification is needed for a microorganism, please provide it. For each specification, please state the analytical method used and an affirmation that the methods are validated for their intended purpose.

    b. 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 alone is sufficient.

    c. Please briefly describe how microorganism contamination is controlled during the manufacturing process.

Response

a. Microbiological testing such as E. coli, S. aureus, Salmonella, L. monocytogenes are performed and meets specifications as shown in the attached Certificate of Analysis for three non-consecutive batches. Below is a table of the analytical method used for each microbial test. The methods shown below are validated for their intended purpose and are attached.

Parameter	***Limits***	***Method***
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)*

b. Testing of presumptive positive coliform results are further conducted to confirm the genus and species of any presumptive coliforms identified during the initial testing.

c. 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(s) II933.2-CBI.7-A2, II933.2-CBI.7-A3, II933.2-CBI.7-A4, II933.2-CBI.7-A5, II933.2-CBI.7-A6, II933.2-CBI.7-A7, II933.2-CBI.7-A8

Question 15

15. The notifier does not provide specifications for heavy metals (Table 4, page 13).Please include limits for lead, arsenic, cadmium, and mercury in the specifications for *L. rhamnosus* strain KCTC 12202BP and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet these specification limits. Please note that the limits for heavy metals should be as low as possible and be reflective of the results of the batch analyses. In addition, 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.

Response

Heavy metals are being performed as identified in the attached Certificate of Analysis. These include results for Lead, Arsenic, Cadmium, and Mercury in three non-consecutive batches. The Certificate of Analysis also provides the test results in actual measured levels and all test results met specifications regarding the level of these heavy metals. 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.

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 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).

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) II933.2-CBI.7-A2

Question 16

16. For the administrative record, please provide a revised copy of Table 4 for all specifications, including microorganisms and heavy metals. Please include any recen revisions made to the specifications in response to these questions, 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*
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*

Question 17

17. Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications have been validated for that particular purpose. This includes the in-house method listed in Table 4 for the measurement of viable cell count and coliforms.

Response

All analytical methods used in the testing of the batches, inclusive of the in-house methods in the revised Table 4, are validated for their respective purposes.

Question 18

18. On page 14, the notifier states *L. rhamnosus* strain KCTC 12202BP 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 ~40% 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. rhamnosus KCTC 12202BP, 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 40% 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 19

19. 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 20

20. 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

"All users" is clarified as the eaters-only population. 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, is shown below. As such, the dietary exposure estimates would not affect the GRAS conclusion.

Population Group	***Age Group***	***Eaters only (CFU/day)***	
		Mean	***90th Percentile***
Total Population (eaters-only)	*2 years old and older*	*8.94 x 10^8*	*1.85 x 10^9*

Question 21

21. 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. rhamnosus* strain KCTC 12202BP. For the administrative record, please confirm that the term "cumulative" was incorrectly used in the statements from the original notice 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, L. rhamnosus strain KCTC 12202BP is considered a novel ingredient in food. As dairy products are the only proposed food, the dietary exposure of the ingredient is only based on the dairy products only. Therefore, the term "cumulative" was inappropriately used.

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

Question 22

22. Please provide an updated literature search and review that discusses the safety of *L. rhamnosus*, including the safety of Lactobacilli, this strain, or closely related strains, as applicable. To do this, please include the following:

    a. Please do not limit your review 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.

    b. Please include the date (month and year) the literature search was performed and the dates or years the search spanned (e.g., 1990-present), the resource database(s) used (e.g., PubMed), and the principal search terms used.

    c. Please also discuss whether findings from any publications contradict your GRAS conclusion.

Response

A literature search was conducted during the period September 28 through October 3, 2023, using the research sites Google Scholar, PubMed, ResearchGate, and ScienceDirect. The findings of the search are discussed below. The principal search terms used are "[Lactobacillus] and [pathogenicity]", "[Lactobacillus] and [toxigenicity]", "[Lactobacillus rhamnosus] and [pathogenicity]", "[Lactobacillus rhamnosus] and [toxigenicity]", "[Lactobacillus] and [bacteremia]", "[Lactobacillus rhamnosus] and [bacteremia]." Time period of the search: 2004 – 2023. The overall findings of this additional literature review do NOT contradict our GRAS conclusion.

The overall safety of species of microorganisms in the genus Lactobacillus is recognized in concert with the demonstrated benefits of these microorganisms. As the general recognition of safety is shown in both formal regulatory and GRAS self-determinations for a range of individual species of this genus. As regards the toxigenicity of these microorganisms, there are reports in the literature detailing infrequent instances of bacteremia presented most frequently as single cases. With regard to the pathogenicity of the genus, in 2019 Rossi et. al. reviewed whether or not there was evidence that Lactobacillus species could be considered pathogenic organisms. Their conclusion was that some of these microorganisms are identified as being pathogenic likely owing to their ability to affect platelet aggregation capacity and biofilm formation. These microorganisms have been identified as pathogens outside of the gastrointestinal tract. A review of case study reports for the years 2019 through 2021 was performed by Rossi et. al. (2022) finding that although there appears to have been an increase in the incidence of Lactobacillus infection, the findings were sufficient only to recommend the continuing monitoring of such events. The lack of demonstration of toxic effect and pathogenicity outside of translocation points to the continuing consideration of the safety of these microorganisms. Moreover, the absence of clear linkage between oral probiotic consumption and adverse events remains.

A review of the literature conducted by Kullar et. al. (2023) presented findings that are clearly current and significant regarding infections attributed to Lactobacillus spp. and L. rhamnosus particularly. Their findings indicate the magnitude of the challenge to be on the order of 0.1% - 0.2% of all isolates found in hospitalized patients and 0.5% of immunocompromised patients. The authors additionally found that from

1980 to 2023 the literature presented a total of 23 cases where the blood isolate from the patient was identical to the probiotic strain consumed by the patient. They found that the incidence of bacteremia associated with Lactobacillus spp. is infrequent although more common in individuals consuming probiotic microorganisms orally.

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 Lactobacilli. 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.

Among the commonalities in nearly all of the individual case studies of bacteremia infections involving Lactobacillus spp. is that nearly all the individuals were severely compromised in one way or another. Even in the sternest evaluations of probiotic microorganisms and potential disease initiation, the recognition is that the occurrence is owed to an opportunity for the microorganism to translocate owing to some insult or injury.

To be certain, there are a number case studies in the literature that describe Lactobacillus infection. Representative examples are listed below. These individual cases are, as noted previously, infrequent and invariably tied to some other condition likely allowing for the translocation of the microorganism into the bloodstream. Even when case studies indicate an otherwise healthy individual, the initiation of the infection is not understood. A single-center descriptive analysis over a 4-year period by Albarillo et. al. (2020) noted that Lactobacillus is considered generally safe, but in instances where individuals have a number of comorbidities, it is shown that Lactobacillus species has demonstrated the ability to translocate given circumstances that include some compromise of the gastrointestinal tract. This position is little changed from that found in 2007 by Anukam et. al. The expressed concerns then are little more than "negligible".

The evidence regarding the safety of the genus Lactobacillus and with specificity, the species Lactobacillus rhamnosus CBT LR5 continues to be considered safe for consumption by the general population under the conditions presented in GRN 1084. The studies shown in the requested literature review do not raise questions concerning the consumption of this microorganism in the additive and substitutive fashion described in the GRAS determination filed.

Reference	**Study Title**
Rossi et. al. (2019)	*Members of the Lactobacillus Genus Complex (LGC) as Opportunistic Pathogens: A Review*
Rossi et. al. (2022)	Lactobacilli Infection Case Reports in the Last Three Years and Safety Implications
Kullar et. al. (2023)	Lactobacillus Bacteremia and Probiotics: A Review
Chiang et. al. (2021)	*Lactobacillus rhamnosus* sepsis associated with probiotic therapy in an extremely preterm infant: Pathogenesis and a review for clinicians

Reference	Study Title
Sendil et. al (2020)	*Lactobacillus rhamnosus* Bacteremia in an Immunocompromised Renal Transplant Patient
Sherid et. al. (2016)	Liver abscess and bacteremia caused by lactobacillus: role of probiotics? Case report and review of the literature
Tribe et. al. (2015)	*Lactobacillus rhamnosus* Infection of a Metal on Metal Hip Arthroplasty
Vyas et. al. (2020)	*Lactobacillus* masticator abscess after probiotics consumption
Omar et. al. (2019)	Breaking Bad: a case of Lactobacillus bacteremia and liver abscess
Pasala et. al. (2020)	Lactobacillus endocarditis in a healthy patient with probiotic use
Albarillo et. al. (2020)	*Lactobacillus rhamnosus* Infection: A Single-center 4-year Descriptive Analysis
Anukam et. al. (2007)	Probiotic Toxicity, Any Evidence?
Kunz et al. (2004)	Two cases of Lactobacillus bacteremia during probiotic treatment of short gut syndrome
Boyle et al. (2006)	Probiotic use in clinical practice: what are the risks?

Attachment(s): II933.2-CBI-A9, II933.2-CBI-A10, II933.2-CBI.7-A11, II933.2-CBI.7-A12, II933.2-CBI.7-A13, II933.2-CBI.7-A14, II933.2-CBI.7-A15, II933.2-CBI.7-A16, II933.2-CBI.7-A17, II933.2-CBI.7-A18, II933.2-CBI.7-A19, II933.2-CBI.7-A20, II933.2-CBI.7-A21, II933.2-CBI.7-A22

Question 23

23. In Tables 8 and 9, the notifier lists several GRAS notices where the subject of the notice was a strain of *L. rhamnosus* 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 rhamnosus* CBT LR5" 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, findings, and/or comparable exposure levels from the selected previous notices that support the basis of the notifier's argument for the safety of the intended use of their ingredient, beyond a statement of the use levels from each notice. Given each GRAS notice is considered to stand on its own, please briefly summarize the information incorporated by reference from the listed previous GRAS notices within the context of the notifier's safety conclusion for this GRAS notice.

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 rhamnosus receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).*

GRAS No.	Date of Closure	Substance	Intended Use	Amount
288	27-Mar-2009	*Lactobacillus rhamnosus* strain HN001	As an ingredient in 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	up to 10^9 (cfu) per standard serving
281	31-Aug-2009	*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 levels not to exceed 10^8 cfu/g of the formula powder

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

GRAS No.	Date of Closure	Substance	Intended Use	Amount
847	30-Sep-2019	*Lactobacillus plantarum* ECGC 13110402	Conventional foods (excluding infant formula and foods	At levels up to 1 x 10^{10} CFU per serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
			under the jurisdiction of the United States Department of Agriculture (USDA))	
840	27-Aug-2019	*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 CFU/serving
810	05-Apr-2019	*Lactobacillus paracasei* ssp. *paracasei* strain F-19	Non-exempt powdered infant formulas for term infants	Levels of 10^9 CFU/800 mL of reconstituted formula
758	20-Aug-2018	*Lactobacillus helveticus* R0052, *Bifidobacterium longum* ssp. *infantis* R0033, and *Bifidobacterium bifidum* R0071, for use individually, or in combination at a 80:10:10 ratio of *L. helveticus* R0052, *B. longum* ssp. *infantis* R0033, and *B. bifidum* R0071, repectively	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 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
736	11-Apr-2018	*Lactobacillus casei* subsp. *paracasei* Lpc-37	An ingredient in yogurt and other dairy products, soy products, beverages, chewing gum, and confectionary snacks	At least 10^{10} CFU/serving throughout the shelf life of the product

GRAS No.	Date of Closure	Substance	Intended Use	Amount
722	16-Feb-2018	*Lactobacillus plantarum* Lp-115	An ingredient in conventional foods, including yogurt and other dairy products, soy products, beverages, chewing gum, and confectionary snacks	At 1 × 10^{10} CFU / serving
685	31-Oct-2017	*Lactobacillus plantarum* strain 299v	An ingredient in conventional foods	Up to 1 x 10^{10} CFU/serving
531	14-Aug-2014	*Lactobacillus fermentum* CECT5716	Term infant formulas	Maximum level of 10^{7} cfu/g of powdered non-exempt milk-based infant formula
502	27-Feb-2014	*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,	Minimum 1 x 10^{9} CFU per 250 gram serving of food

GRAS No.	Date of Closure	Substance	Intended Use	Amount
			chips, granola); meal replacements; sauces, condiments; confections (e.g., bars, candy, coatings, drops, cookie filling); chewing gum; and in medical foods	
440	16-Aug-2012	*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 10^{10} CFU/serving
410	16-Nov-2011	*Lactobacillus reuteri* strain DSM 17938	Powdered whey-based term infant formula	Minimum level of 10^6 cfu/g, but not higher than 10^8 cfu/g of powdered formula
357	19-Apr-2011	*Lactobacillus acidophilus* NCFM	An ingredient to 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	At a level to provided 10^9 CFU per standard serving
254	18-Nov-2008	*Lactobacillus reuteri* strain DSM 17938	An ingredient in processed cheeses, yogurt, ice cream, fruit juices, fruit drinks, processed vegetables, processed vegetable drinks, beverage bases,	Levels up to 10^9 cfu per serving, and in a drinking straw at a level of 10^9 cfu per straw

GRAS No.	Date of Closure	Substance	Intended Use	Amount
			energy bars, energy drinks, and chewing gum	
171	07-Dec-2005	*Mixture of Lactobacillus acidophilus (NP35, NP51) L. lactis (NP7),* and *Pediococcus acidilactici (NP3)*	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 CFU of lactobacilli per gram of product

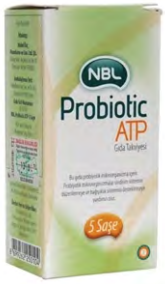
Question 24

24. 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 rhamnosus* CBT LR5 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

Product	Availability	Ingredients	Amount per Serving
DUOLAC® Care 	Singapore https://www.watsons.com.sg/duolac-care-60s/p/BP_66142	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	2.00 x 10^9 CFU 2.19 x 10^9 CFU 2.19 x 10^9 CFU 2.19 x 10^9 CFU 2.00 x 10^9 CFU 1.91 x 10^9 CFU 1.25 x 10^{10} Total CFU / Tablet
DUOLAC® Derma	Singapore https://duolac.sg/product/duolac-derma/	*L. rhamnosus* LR5 *L. casei* LC5 *L. plantarum* LP3 *B. lactis* BL3	2.00 x 10^9 CFU 4.00 x 10^9 CFU 2.00 x 10^9 CFU 2.00 x 10^9 CFU 1.0 x 10^{10} Total CFU / Stick

Product	Availability	Ingredients	Amount per Serving
			
DUOLAC® Derma Plus 	Denmark https://www.webapoteket.dk/kosttilskud/maelkesyrebakterier/duolac-derma-plus-p-220803	*L. rhamnosus* LR5 *L. casei* LC5 *L. plantarum* LP3 *B. lactis* BL3	2.00 x 10^9 CFU 4.00 x 10^9 CFU 2.00 x 10^9 CFU 2.00 x 10^9 CFU 1.0 x 10^{10} Total CFU / Stick
DUOLAC® Daglig Vitalitet 	Denmark https://duolac.dk/produkter/duolac-daglig-vitalitet/	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.12 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.12 x 10^9 CFU 1.07 x 10^9 CFU 7.0 x 10^9 Total CFU / Capsule
Lactobex® Strong 	Latvia https://www.herba.lt/en/lactobex-strong-kapsules-n6	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.12 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.12 x 10^9 CFU 1.07 x 10^9 CFU 7.0 x 10^9 Total CFU / Capsule
Lactobex®	Latvia https://e-menessaptieka.lv/en/p/lactobex-lactic-acid-and-bifidobacteria-complex-	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. longum* BG7 *B. lactis* BL3	2.0 x 10^8 CFU 2.0 x 10^8 CFU 2.0 x 10^8 CFU 2.0 x 10^8 CFU 2.0 x 10^8 CFU

Product	Availability	Ingredients	Amount per Serving
	sachets-10-pcs-1132536?tab=use		1.0 x 10^9 Total CFU / Stick
NBL Probiotic ATP 	Turkey https://nbl.md/en/nbl-probiotic-en/nbl-probiotic-atp/	*L. rhamnosus* LR5 *L. casei* LC5 *L. plantarum* LP3 *B. lactis* BL3	8.2 x 10^8 CFU 1.64 x 10^8 CFU 8.2 x 10^8 CFU 8.2 x 10^8 CFU 2.0 x 10^9 Total CFU / Stick
NBL Probiotic Gold 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/yetiskin/nbl-probiotic-gold/	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. longum* BG7 *B. bifidum* BF3	4.3 x 10^8 CFU 8.2 x 10^8 CFU 4.3 x 10^8 CFU 4.3 x 10^8 CFU 4.3 x 10^8 CFU 2.5 x 10^9 Total CFU / Stick
NBL Gynobiotic 	Turkey https://nblturkiye.com/en/urunler/nbl-gynobiotic/	*L. rhamnosus* LR5 *L. acidophilus* LA1	2.50 x 10^9 CFU 2.50 x 10^9 CFU 5.0 x 10^9 Total CFU / Capsule
PRODUO Flora	Spain http://produo.es/familia-produo-tratamiento-flora-bacteriana-intestinal/produo-flora-tratamiento-microflora-intestinal/	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	5.10 x 10^8 CFU 6.60 x 10^8 CFU 7.10 x 10^8 CFU 6.10 x 10^8 CFU 5.10 x 10^8 CFU 3.0 x 10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
			
PRODUO Daily Care 	Spain https://produo.es/producto/produo-daily-care/	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.10 x 10^6 CFU 1.23 x 10^6 CFU 1.23 x 10^6 CFU 1.23 x 10^6 CFU 1.12 x 10^6 CFU 1.07 x 10^6 CFU 7.0 x 10^6 Total CFU / Capsule
PRODUO Digestive Vitality 50+ 	Spain https://produo.es/producto/produo-digestive-vitality-50/	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7 *B. bifidum* BF3	1.4 x 10^6 CFU 7.0 x 10^8 CFU 7.0 x 10^8 CFU 2.1 x 10^6 CFU 1.1 x 10^6 CFU 1.1 x 10^6 CFU 7.1 x 10^6 Total CFU / Capsule
Norgitan Care 	Netherlands https://www.pharmacodel.com/fr/home/37800-orgitan-care-15-comprimes.html	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	3.8 x 10^8 CFU 5.0 x 10^8 CFU 5.4 x 10^8 CFU 4.6 x 10^8 CFU 3.8 x 10^8 CFU 2.26x 10^9 Total CFU / Tablet
Floradicol7	Belgium https://www.pharmamarket.be/be_nl/floradicol-7-30-capsules-126578.html	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. plantarum* LP3 *L. acidophilus* LA1 *B. lactis* BL3	1.12 x 10^9 CFU 1.23 x 10^9 CFU 2.30 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU

Product	Availability	Ingredients	Amount per Serving
		*B. longum* BG7 *B. bifidum* BF3	1.12 x 10^9 CFU 1.07 x 10^9 CFU 7.0 x 10^9 Total CFU / Capsule
Nutriforte Lactoghurt 	Malaysia https://shopee.com.my/Nutriforte-Lactoghurt-Probiotics-with-FOS-(60s-60s-x-2-30s-30s)-i.11530378.84547619	*L. rhamnosus* LR5 *S. thermophilus* ST3 *L. acidophilus* LA1 *B. lactis* BL3 *B. longum* BG7	4.1 x 10^8 CFU 5.4 x 10^8 CFU 4.3 x 10^8 CFU 5.4 x 10^8 CFU 2.0 x 10^8 CFU 2.1 x 10^9 Total CFU / Tablet
Lacclean Gold Lab 	Vietnam https://droppii.store/lacclean-gold-lab	*L. rhamnosus* LR5 *E. faecium* EF4 *L. acidophilus* LA1 *B. longum* BG7 *B. bifidum* BF3	6.25 x 10^7 CFU 1.0 x 10^9 CFU 1.25 x 10^8 CFU 3.125 x 10^7 CFU 3.125 x 10^7 CFU 1.25 x 10^9 Total CFU / Sachet

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

II933.2-CBI.7-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.
II933.2-CBI.7-A2	Certificate of Analysis
II933.2-CBI.7-A3	Analytical Method for Viable Cell Count
II933.2-CBI.7-A4	Analytical Method for Coliforms and *E.coli*
II933.2-CBI.7-A5	Analytical Method for *Yeast and Mold*
II933.2-CBI.7-A6	Analytical Method for *S. aureus*
II933.2-CBI.7-A7	Analytical Method for *Salmonella*
II933.2-CBI.7-A8	Analytical Method for *L. monocytogenes*
II933.2-CBI.7-A9	Rossi F, Amadoro C, Colavita G (2019). Members of the *Lactobacillus* genus complex (LGC) as opportunistic pathogens: a review. *Microorganisms* 2019, 7, 126; doi:10.3390/microorganisms7050126
II933.2-CBI.7-A10	Rossi F, Amadoro C, Gasperi M, Colavita G (2022) Lactobacilli infection case reports in the last three years and safety implications. *Nutrients* 2022, 14, 1178. https//doi.org/10.3390/nu14061178
II933.2-CBI.7-A11	Kullar R, Goldstein EJC, Johnson S, McFarland LV (2023). Lactobacillus Bacteremia and Probiotics: A Review. *Microorganisms* 2023, 11, 896, https://doi.org/10.3390/microorganisms11040896
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II933.2-CBI.7-A15	Tribe H, Stammers J, Ranawat V, Petkar H, Skinner J (2015). *Lactobacillus rhamnosus* Infection of a Metal on Metal Hip Arthroplasty. *Austin J Orthopade & Rheumatol.* 2015;2(1):1010
II933.2-CBI.7-A16	Vyas V, Mian S, Paolino K, Siddique Z (2021) *Lactobacillus* masticator abscess after probiotics consumption. *Proc Bayl Univ Med Cent* 2021; 34(1) 93-94
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II933.2-CBI.7-A19	Albarillo FS, Shah U, Joyce C, Slade D (2020) *Lactobacillus rhamnosus* Infection: A Single-center 4-year Descriptive Analysis. *J Global Infect Dis* 2020; 12:119-23
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One-hundred and nineteen pages have been removed in accordance with copyright laws. The removed reference citations can be found at in the attachments list after the conclusion section of the notifier response.

The following 7 attachments remain:

- II933.2-CBI.7-A2 Certificate of Analysis
- II933.2-CBI.7-A3 Analytical Method for Viable Cell Count
- II933.2-CBI.7-A4 Analytical Method for Coliforms and E.coli
- II933.2-CBI.7-A5 Analytical Method for Yeast and Mold
- II933.2-CBI.7-A6 Analytical Method for S. aureus
- II933.2-CBI.7-A7 Analytical Method for Salmonella
- II933.2-CBI.7-A8 Analytical Method for L. monocytogenes

Attachment
II933.2-CBI.7-A2

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Certificate of Analysis

Product Name : ***Lactobacillus rhamnosus***

Place of Production: KOREA

Batch(Lot) No. : LR5 26R

Issued Date: 03 Apr. 2017

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 30 Mar. 2017

Exp. Date: 29 Mar. 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	0.0039 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0041 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0008 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0084 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

Director, Head of Quality Management Division

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CELL BIOTECH

Certificate of Analysis

Product Name : ***Lactobacillus rhamnosus***

Place of Production: KOREA

Batch(Lot) No. : LR5 15R

Issued Date: 27 Feb. 2017

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 23 Feb. 2017

Exp. Date: 22 Feb. 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	0.0022 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0018 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0024 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0103 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 rhamnosus***

Place of Production: KOREA

Batch(Lot) No. : LR5 22R

Issued Date: 27 Mar. 2017

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 23 Mar. 2017

Exp. Date: 22 Mar. 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	0.0017 mg/kg
Cadmium (Cd)**	$\leq$ 0.3 mg/kg	0.0005 mg/kg
Mercury (Hg)***	$\leq$ 0.1 mg/kg	0.0013 mg/kg
Arsenic (As)****	$\leq$ 0.1 mg/kg	0.0064 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

Director, Head of Quality Management Division

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Attachment
II933.2-CBI.7-A3

CELL BIOTECH

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
II933.2-CBI.7-A4

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

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Attachment
II933.2-CBI.7-A5

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
II933.2-CBI.7-A6

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
II933.2-CBI.7-A7

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
II933.2-CBI.7-A8

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^{0}C for 46-50 hours.

2. Using a technique ensuring isolated colonies, streak the enrichment broth onto Oxford agar, and incubate at 37^{0}C 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^{0}C 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: Friday, October 20, 2023 9:27 PM
To: Overbey, Katie
Cc: Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine; Jonathan Fink
Subject: [EXTERNAL] FW: GRN 1084 - Additional Question

Dear Dr. Overbey,

Thank you for your email. We would like to respectfully respond to the question below:

Request

1. Please state whether *L. rhamnosus* CBT LR5 has been deposited in a recognized culture collection.

Response:
L. rhamnosus CBT LR5 is deposited in a recognized culture collection (Korean Collection for Type Cultures) as KCTC 12202BP.

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
joel@rejimus.com

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From: Overbey, Katie <Katie.Overbey@fda.hhs.gov>
Date: Friday, October 20, 2023 at 11:36 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 1084 - Additional Question

Hello Mr. Lassiter,
We had an additional clarifying question during our review of GRN 1084. You can reply directly to this email with your response.

1. Please state whether *L. rhamnosus* CBT LR5 has been deposited in a recognized culture collection.

Thank you,
Katie

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

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Center for Food Safety and Applied Nutrition
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