Notice to US Food and Drug Administration of the Conclusion that the Intended Use of *Lacticaseibacillus rhamnosus* IDCC 3201 is Generally Recognized as Safe

Submitted by the Notifier:

Ildong Bioscience Co., Ltd.
17 Poseunggongdan-ro, Poseung-eup, Pyeongtaek-si
Gyonggi-do, 17957, Republic of Korea

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc
1425 Broadway, Suite 458
Seattle WA 98122

May 6, 2022

May 6, 2022

Susan Carlson, PhD
Division Director
Division of Food Ingredients
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Ildong Bioscience Co., Ltd. (the notifier), the undersigned, Maureen Dunn, ND, submits, for FD Areview, the enclosed notice that *Lacticaseibacillus rhamnosus* IDCC 3201 is GRAS under the conditions of its intended use in foods.

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

Sincerely,

Maureen Dunn, ND (agent of the notifier)
Scientific and Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

Table of Contents

Figures and Tables

Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Ildong Bioscience Co., Ltd. (the notifier), hereafter referred to as ILDONG is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that *Lacticaseibacillus rhamnosus* IDCC 3201 is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

Donghoon Oh
Manager
Ildong Bioscience Co., Ltd.
17 Poseunggongdan-ro, Poseung-eup, Pyeongtaek-si
Gyonggi-do, 17957, Republic of Korea
+82(0)70 52088308
hoonyoh@ildong.com

Agent of the Notifier

Maureen Dunn, ND
Scientific and Regulatory Consultant
AIBMR Life Sciences, Inc.
1425 Broadway, Suite 458
Seattle, WA 98122
Tel: (253) 286 -2888
maureen@aibmr.com

1.3 Name of the Substance

The name of the substance is *Lacticaseibacillus rhamnosus* IDCC 3201.

1.4 Intended Conditions of Use

L. rhamnosus IDCC 3201 is intended to be used as an ingredient added to foods where standards of identity do not preclude such use. It is not intended to be added

to infant formula, or any products that would require additional regulatory review by US DA The intended addition level to foods is up to 1 x 10^{11} CFU per serving.

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRA Sstatus of *L. rhamnosus* IDCC 3201 for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

We have concluded that *L. rhamnosus* IDCC 3201 is GRA Sfor its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of *L. rhamnosus* IDCC 3201 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRA Sconclusion will be available for review and copying during customary business hours at the office of Donghoon Oh (Ildong Bioscience Co., Ltd., 17 Poseunggongdan-ro, Poseung-eup, Pyeongtaek-si, Gyonggi-do, 17957, Republic of Korea), or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRA Snotice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

1.9 Certification of Completion

We hereby certify that, 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 *L. rhamnosus* IDCC 3201.

[redacted] May 10, 2021

Donghoon Oh
Manager
Notifier

Date

Part 2: Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

L. rhamnosus are non-motile rods, occur singly or in chains, are facultative anaerobic and are facultative heterofermentative.[1, 2] The meaning of *Lacticaseibacillus* is "*Lactobacilli* related to cheese." Members of the *Lacticaseibacillus* species are especially known for their involvement in the ripening of cheeses with concentrations in cheese products estimated at up to 10^7 CFU/g.[3] The species is known to have the ability to adapt well to various environments.[4] The most heavily studied strain of this species is currently *L. rhamnosus* GG (originally isolated from a human) about which there are a plethora of scientific publications.[4,5]

2.2 Taxonomy of *Lacticaseibacillus rhamnosus* IDCC 3201

ILDONG's *L. rhamnosus* IDCC 3201 was isolated from breast-fed infant feces and has been identified according to standard taxonomic guidelines. It has been unequivocally identified genetically based on 16S rRNA sequences with confirmed 99% sequence homology to its type strain sequence, *L. rhamnosus* JCM 1136.

In April 2020, the *Lactobacillus* genus which contained 261 species, was divided into 25 new genera, based on phylogenetic, phenotypical, and habitat differences.[6, 7] Based on this analysis, the genus and species of ILDONG's strain was reclassified from *Lactobacillus rhamnosus* to *Lacticaseibacillus rhamnosus*. It is important to note that throughout this report, the new taxonomic name is used.

The taxonomic lineage of the strain is:

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Family: Lactobacillales

Genus: *Lacticaseibacillus*

Species: *Lacticaseibacillus (Lactobacillus) rhamnosus*

Strain: *Lacticaseibacillus (Lactobacillus) rhamnosus* IDCC 3201

2.3 Manufacturing

2.3.1 Good Manufacturing Practice

ILDONG's *L. rhamnosus* IDCC 3201 is manufactured in Korea at an FDA registered facility under strict adherence to GMP standards. In addition, ILDONG maintains additional food safety management certifications:

- Hazard Analysis and Critical Control Points (HACCP)
- Bureau Veritas Certification, Food Safety System Certification (FSSC 22000)
- Bureau Veritas Certification, Food Safety Management Systems (ISO 22000)

2.3.2 Raw Materials

ILDONG confirms that the raw materials used in the production of *L. rhamnosus* IDCC 3201 are of appropriate food grade and are not genetically modified.

2.3.3 Manufacturing Narrative and Flowchart

The manufacturing flowchart is shown below, in Figure 1. The manufacturing steps are further described in the text below.

The raw ingredients are initially delivered to ILDONG and only those that are qualified during in-house inspection are weighed. The medium is prepared by dissolving the raw ingredients in a water solution and the culture medium tank is sterilized at an appropriate temperature and pressure for 30 minutes or more. Following the medium preparation, the preculture is prepared by inoculating the frozen samples of the preserved strains and incubating them at an appropriate temperature and pressure for 16 hours or more. Once the preculture reaches the exponential growth phase, the culture fluid is inoculated into the next culture medium and further incubated (same incubation temperature) for seven or more hours to prepare the middle culture.

When the middle culture reaches the exponential growth phase, the culture fluid is inoculated into the main culture medium and incubated for 14 or more hours to prepare the main culture. The main culture is centrifuged and the cell mass is recovered after the solids are separated from the liquid. The recovered cell mass is resuspended in a sterilized dispersion medium and then freeze-dried with decompression.

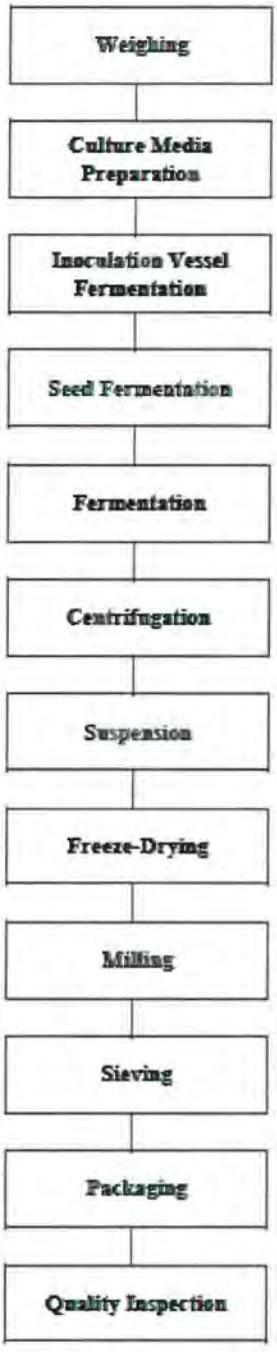

Figure 1. *Lacticaseibacillus rhamnosus* IDCC 3201 Manufacturing Flowchart

2.4 Specifications and Batch Analyses

2.4.1 Specifications

The specifications for the food-grade product *L. rhamnosus* IDCC 3201, along with the specification methods, which have been validated for their stated purpose, are listed in Table 1 below.

Table 1. *Lacticaseibacillus rhamnosus* IDCC 3201 Product Specifications

Tested Parameters	Limits/Specifications	Method
Appearance	White to light yellow powder	KFSC 8/1/1.1
Identification	*Lacticaseibacillus rhamnosus*	16S rRNA Sequencing
Cell count	$\geq 4.5 \times 10^{11}$ CFU/g	KHFSC 4/3-58
Particle size	95% Pass > 50 mesh	Ph. Eur. (Sieves method)
Water activity (Aw)	< 0.15	In-house Specifications IBS-SOP-QC-060
Microbiological Tests		
Coliforms	Negative/10g	KHFSC 8/4/4.7/4.7.1
Escherichia coli	Negative/10g	KFSC 8/4/4.8/4.8.2
Yeast & Molds	< 10 CFU/g	KFSC 8/4/4.10
Salmonella	Negative/10g	KFSC 8/4/4.11
Staphylococcus aureus	Negative/g	AOAC 2003.07
Heavy Metals*		
Lead	< 1.0 mg/kg	KFSC 8/9/9.1/9.1.2
Cadmium	< 0.3 mg/kg	KFSC 8/9/9.1/9.1.3
Mercury	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.6
Arsenic	< 0.5 mg/kg	KFSC 8/9/9.1/9.1.4

Abbreviations: CFU, colony forming units; KFSC: Korean Food Standards Codex. KHFSC, Korean Health functional Food Standards Codex; Ph. Eur., European Pharmacopoeia.
*Heavy metal specifications are set according to Korean Food Code per ILDONG.

2.4.2 Batch Analyses

Production conformity and consistency of ILDONG's *L. rhamnosus* IDCC 3201 are tested in production lots. Batch analyses of three non-consecutive lots are shown below and are reasonably consistent and met the product specifications for identity parameters, microbial analyses, and heavy metals (see Table 2).

Table 2. *Lacticaseibacillus rhamnosus* IDCC 3201 Batch Analyses

Tested Parameters	Specification	Lot No./Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0501 05/2019	Lot# IDK1001 10/2019
Appearance	White to light yellow powder	Conforms	Conforms	Conforms
Identification	*Lacticaseibacillus rhamnosus*	Conforms	Conforms	Conforms
Cell count	$\geq 4.5 \times 10^{11}$ CFU/g	4.66×10^{11} CFU/g	4.92×10^{11} CFU/g	4.8×10^{11} CFU/g

Tested Parameters	Specification	Lot No./Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0501 05/2019	Lot# IDK1001 10/2019
Particle size	95% Pass > 50 mesh	Conforms	Conforms	Conforms
W ter activity (Aw)	< 0 .15	0.0942	0.0892	0.0931
Microbiological Tests				
Coliforms	Negative/10g	Negative/10 g	Negative/10 g	Negative/10 g
Escherichia coli	Negative/10g	Negative/10 g	Negative/10 g	Negative/10 g
Yeast & molds	< 10 CFUg	Conforms	Conforms	Conforms
Salmonella	Negative/10g	Negative/10 g	Negative/10 g	Negative/10 g
Staphylococcus aureus	Negativeg	Negativeg	Negativeg	Negativeg
Heavy Metals				
Lead[a]	< 1.0 mgkg	0.01 mgkg	0.02 mgkg	0.02 mgkg
Cadmium[b]	< 0.3 mgkg	0.00 mgkg	0.00 mgkg	0.00 mgkg
Mercury[c]	< 0.1 mgkg	0.00 mgkg	0.01 mgkg	0.00 mgkg
Arsenic[d]	< 0.5 mgkg	0.00 mgkg	0.00 mgkg	0.00 mgkg

Abbreviations: CFU, colony forming units.
[a] Limit of Detection = 0.4 µg/kg
[b] Limit of Detection = 0.6 µg/kg
[c] Limit of Detection = 1.7 µgkg
[d] Limit of Detection = 0.7 µg/kg

2.5 Stability Study

A real time stability test was performed on ILDONG's *L. rhamnosus* IDCC 3201, stored at a refrigerated condition of 5 °C and no humidity as well as at Climatic Zone II at 25 °C ± 2 °C and 60% ± 5% relative humidity for a period of 24 months. The total viable cell count, expressed in CFU/g, was measured at T=0, 3, 6, 9, 12, 18, and 24 months in each study. The results essentially add to the characterization of the strain and show that there is loss of live bacteria over time for ILDONG's *L. rhamnosus* IDCC 3201 at 25 °C but not at 5 °C which is typical for this type of ingredient.

The following figure depicts the real-time stability study results for *L. rhamnosus*.

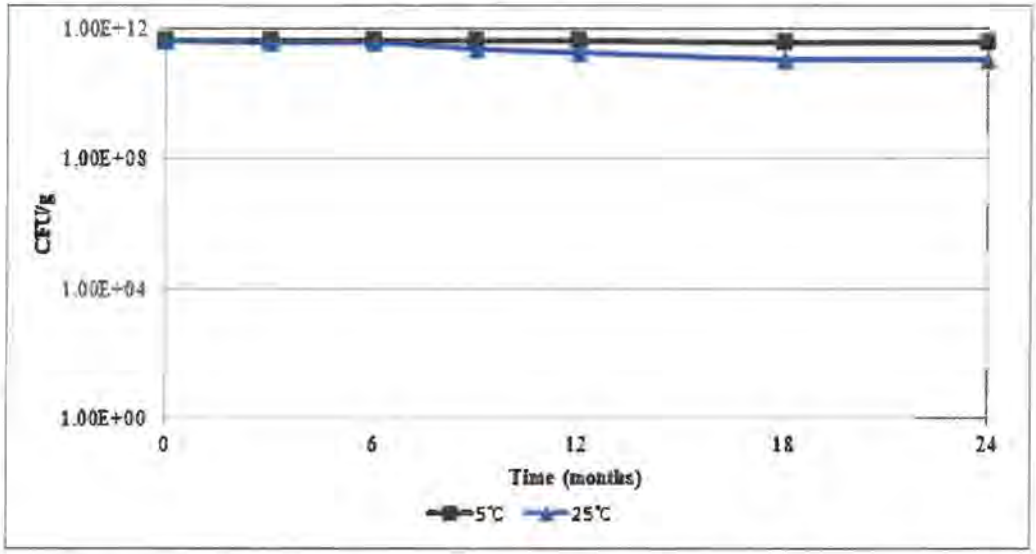

Figure 2. *Lacticaseibacillus rhamnosus* IDCC 3201 Real-Time Stability Study

2.6 Antibiotic Resistance

Resistance to therapeutic antibiotics by microbial pathogens is currently considered one of the greatest challenges in medicine and public health, as some infectious diseases may become virtually untreatable if they become non-respondent to current therapies. Antibiotic resistance may be classified into two types;

- intrinsic/natural (when resistance is inherent to a bacterial species, and is a trait generally shared by all members of that species); or
- extrinsic/acquired (when a strain of a typically susceptible species is resistant to a given antimicrobial drug).

Extrinsic/acquired resistance can occur either from the gain of exogenous DNA or mutation of indigenous genes.[8, 9] While intrinsic resistance likely presents a very low risk of dissemination, extrinsic/acquired resistance, especially when the relevant genes are associated with mobile genetic elements such as plasmids and transposons, can be transferred to pathogens or other commensal bacteria.[10] It is generally recommended that resistance to antibiotics be assessed in all probiotic strains prior to marketing.[8, 11-15]

EFSA has published guidance documents with regard to antimicrobial susceptibility for bacteria that are intended to be used as feed additives and/or as production organisms.[8, 16] Phenotypic evaluation of antibiotic resistance involves testing the capacity of a microorganism to survive in a medium containing different concentrations of antibiotics. Whereas most microorganisms can survive at low

concentrations of many antibiotics, resistance is defined as the capacity to grow at antibiotic concentrations similar to those reached in the human body during therapeutic intervention.

With regard to phenotypic testing, EFSA has provided MIC values for a select list of antibiotics including ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, and chloramphenicol. The MIC cut-off values are specific to individual bacterial species and are intended to be a tool to aid in distinguishing strains with acquired resistance from susceptible strains.

A bacterial strain is defined as susceptible when its growth is inhibited at a specific antibiotic concentration that is equal to or lower than the established cut-off value for that particular strain. A bacterial strain is defined as resistant when it is able to grow at a concentration of a specific antibiotic that is higher than the established cut-off value.

In addition to phenotypic testing, ILDONG also assessed for any known antibiotic resistant genes for *L. rhamnosus* IDCC 3201 to the antibiotics detailed in the EFSA guidelines. Antibiotic resistance genes were identified based on protein homologs using the ResFinder3.2 software and compared to the Comprehensive Antibiotic Resistance Database (CARD), and the determination of resistance genes was confirmed according to the CARD criteria (search parameters for sequence identity were >80% and coverage >60%).

Phenotypic results are shown in the table below and indicate that *L. rhamnosus* IDCC 3201 is phenotypically sensitive to all antibiotics included in EFSA's guidelines for the *L. rhamnosus* species except for gentamicin and kanamycin. The genetic nature of the antibiotic resistance in these strains was evaluated and no antibiotic resistance genes were found.

Table 3. *Lacticaseibacillus rhamnosus* IDCC 3201 Assessment of Antimicrobial Susceptibility

Antimicrobial Agent	Phenotypic MIC (mg/L)			*L. rhamnosus* IDCC 3201 Genetic Resistance
	L. rhamnosus IDCC 3201 (Observed)	*L. rhamnosus* (EFSA Breakpoints)[16]	Assessment for *L. rhamnosus* IDCC 3201	
Ampicillin	1	4	Sensitive	No
Gentamicin	32[a]	16	Resistant	No
Streptomycin	32	32	Sensitive	No
Kanamycin	128[a]	64	Resistant	No
Clindamycin	<0.125	4	Sensitive	No
Chloramphenicol	<0.125	4	Sensitive	No

Antimicrobial Agent	Phenotypic MIC (mg/L)			*L. rhamnosus* IDCC 3201 Genetic Resistance
	L. rhamnosus IDCC 3201 (Observed)	*L. rhamnosus* (EFSA Breakpoints)[16]	Assessment for *L. rhamnosus* IDCC 3201	
Vancomycin	512–1024	n.r.	n.r.	No
Erythromycin	<0.125	1	Sensitive	No
Tetracycline	<0.125	8	Sensitive	No

Abbreviations: MIC, Minimum Inhibitory Concentration; n.r. = not required per EFSA guidelines for characterization of microbial strains which are the subject of applications for authorization of feed additives.[16]
[a]MIC values within one two-fold dilution of an EFSA cut-off are generally considered acceptable, see further explanation in the text.

As summarized in the table above, *Lacticaseibacillus rhamnosus* IDCC 3201 was susceptible to all of the antibiotics tested as recommended for testing by EFSA, with MIC values at or below the EFSA breakpoints, except for gentamycin and kanamycin. As stated previously, the genetic nature of the antibiotic resistance in these strains was evaluated and no antibiotic resistance genes were found.

The observed MIC for gentamicin and kanamycin was just one two-fold dilution above the EFSA cut-off value, which is still generally considered acceptable. This is due to the technical variation of the phenotypic method applied to determine antibiotic susceptibility. There is a certain amount of technical variability in all phenotypic antibiotic-resistance testing. Further, there is precedent for accepting levels that exceed the MIC cut-off by a single two-fold dilution due to normal variation around the mean; for example EFSA's "Scientific Opinion on the safety and efficacy of Oralin® (*Enterococcus faecium*) as a feed additive for calves for rearing, piglets, chickens for fattening, turkeys for fattening and dogs",[17] in which the Oralin®'s MIC value exceeded the MIC cut-off for kanamycin by a single two-fold dilution, was considered to be within normal variation and did not raise concerns for safety by EFSA.

Most *Lactobacillus* species (*Lactobacillus* is largely still discussed in the literature, as *Lacticaseibacillus* is a new taxonomic designation) have been found to be intrinsically resistant to aminoglycoside antibiotics, including gentamycin and kanamycin.[18] In most cases, the phenotypic resistance trait against aminoglycosides has been speculated to be caused by an intrinsic resistance mechanism due to a reduced uptake of aminoglycosides as a result of the lack of cytochrome-mediated transport mechanisms and general membrane properties of bacteria.[19] Gueimonde et al. (2013) stated that resistance to aminoglycosides for *Lactobacilli* bacteria are generally high.[20] Several studies have found that intrinsic resistance to aminoglycosides in the *Lactobacillus* species is higher than initially described.[9,21, 22] Current EFSA cut-off values for kanamycin are 64 mg/L but Danielsen and Wind

(2003) suggested a kanamycin breakpoint of >256 mg/L for all *Lactobacillus* species after testing 37 strains and assessing the results.[23]

While this strain presented phenotypic resistance to gentamycin and kanamycin, antibiotic resistant genes were not found. The absence of genes currently known for conveying gentamicin and kanamycin resistance in the genome of this strain implies that the antibiotic resistance is likely to be intrinsic and is not likely to be horizontally transferred. Further, per EFSA 2018 guidance, "if no known antibiotic resistance gene is identified that can be linked to the phenotype, no further studies are required."[24] In conclusion, genomic evaluation of the basis of the gentamicin and kanamycin phenotypic resistance has demonstrated that this strain does not possess any antibiotic resistance mechanisms that are known to be transferable.

2.7 Genomic Analysis for Virulence and Pathogenicity

ILDONG evaluated the potential of *L. rhamnosus* IDCC 3201 to produce toxins that have been demonstrated to be virulent to hosts, by examining genomic sequence similarities to toxigenic genes with the BLASTn algorithm, using the Virulence Factor Database (thresholds for the identification were identity >70%, coverage >70%). The results showed that *L. rhamnosus* IDCC 3201 does not contain any known genes that have been demonstrated to be virulent to hosts.

2.8 Hemolysis

ILDONG evaluated the hemolytic properties of *L. rhamnosus* IDCC 3201. A *Staphylococcus aureus* strain was used as the positive control for beta hemolysis while *Limosilactobacillus reuteri* and *Enterococcus faecium* strains were used as negative controls. The test article was streaked as a "T" in the upper right one-third of the plate, the positive control was streaked "β" in the bottom one-third of the plate, and "γ" was streaked in the upper left one-third of the plate. The plate was observed for the presence of microbial hemolysis. The β-hemolytic strain showed up as a clear zone, γ-hemolytic strains showed up as no zone, and α-hemolysis showed up as a deep green zone. ILDONG's *L. rhamnosus* IDCC 3201 showed a γ-hemolytic phenotype (no zone) on blood agar medium.

2.9 Biogenic Amine Formation

Some species and/or strains of lactic acid bacteria are able to produce biogenic amines (organic, basic, nitrogenous compounds formed mainly by the decarboxylation of amino acids), likely for use as metabolic energy and/or to increase acid resistance.[25] These amines are present in a wide range of foods (e.g., fermented food products) and although they are involved in many natural physiological processes, consuming large quantities of these amines can have undesirable consequences in some individuals. For example, if they are not properly

biotransformed in the body, they can cause release of adrenaline/noradrenaline, cause gastric acid secretion, increased cardiac output, heart rate, and blood pressure, migraines, and increased blood sugar.[25] Biogenic amine formation in fermented foods has been reviewed by EFSA (2011)[26] and Spano (2010).[25] Histamine and tyramine are considered the most concerning with regard to food safety.[26]

Per assessment using HPLC (high performance liquid chromatography) analysis, *L. rhamnosus* IDCC 3201 did not produce any of the following five biogenic amines after 24 hours of incubation: tyramine, histamine, putrescine, 2-phenethylamine, or cadaverine.

2.10 Production of D-Lactate

ILDONG tested *L. rhamnosus* IDCC 3201's ability to produce lactic acid (lactate) from the fermentation of carbohydrates. Lactate exists in two forms, a dextrorotary enantiomer (D-lactate) and a levorotary enantiomer (L-lactate). In humans, over 99% of lactate found in the blood is L-lactate. Testing D-lactate production by food microorganisms has been historically recommended likely because until relatively recently, it was believed that humans had a poor capacity of metabolizing D-lactate.[12] Some lactic acid bacteria as well as several other members of the intestinal microflora produce a mixture of L- and D-lactate.[27] More recent studies have shown that much of the human gut microbiota produces D-lactate with no evidence of D-lactic acidosis, and in fact, humans are able to metabolize this isoform.[28-34] D-lactate accumulation may only occur in cases of impaired D-lactate metabolism and/or in subjects with a disturbed gastrointestinal function following bowel resection or Short Bowel Syndrome (SBS).[30, 34-37]

L. rhamnosus IDCC 3201 predominantly produces L-lactate (99.18%) and an insignificant amount is D-lactate (0.82%). The results aid in the characterization of this strain.

2.11 Physical or Technical Effect

L. rhamnosus IDCC 3201 is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

Part 3: Intended Use and Dietary Exposure

For the purpose of this GRAS notice, ILDONG's *L. rhamnosus* IDCC 3201, manufactured in accordance with GMP, is intended to be used as an ingredient added to foods, where standards of identity do not preclude such use. For example, it may be used in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, coffee and tea, condiments and relishes, confections and frostings, dairy product analogs, fats and oils, fruit juices, frozen dairy desserts and mixes, fruit and water ices, gelatins, puddings, and fillings, grain products and pastas, hard candy and cough drops, herbs, seeds, spices, seasonings, blends, extracts, and flavorings, jams and jellies, milk, milk products, nuts and nut products, plant protein products, processed fruits, processed vegetables and vegetable juices, snack foods, soft candy, soups and soup mixes, sugar, and sweet sauces, toppings, and syrups. The addition levels for *L. rhamnosus* IDCC 3201 will be up to a maximum of 1×10^{11} CFU/serving, with an approximate 2% overage to account for loss over the shelf-life of the products. The strain is not intended to be added to infant formula, or any products that would require additional regulatory review by USDA.

Several publications were located that looked at dietary patterns of Americans by analyzing the number of servings of foods consumed in a day. A publication from the USDA's Center for Nutrition Policy and Promotion (October 2000) states that men aged 51 and older consume the largest number of servings of food per day, at 18.2 servings/day.[38] Comparatively, women aged 19–24 consumed the least, at 12.5 servings/day. This data came from detailed 14-day food diaries from 5,752 adults in the 1992–1994 time period. Millen et al. (2005) used 24-hour dietary recall and diet history questionnaire data from the Eating at America's Table study (1997–1998) to analyze the mean number of servings per day consumed of food guide pyramid food groups by adults.[39] There were 497 women and 436 men that completed the study. The results (from the study's Table 1) suggest that the mean intake for men was approximately 27.8 servings per day and for women was 19.5 servings per day.

Using a most conservative estimation of consumption, if 100% of food servings contained *L. rhamnosus* IDCC 3201 at the maximum addition level of 1×10^{11} CFU per serving, highest consumers (men) would be exposed to approximately 1.82–2.78 $\times 10^{12}$ CFU/day. Using 70 kg as a standard body weight, this is equivalent to 2.6–4.0 $\times 10^{10}$ CFU/kg bw/day. This estimation is considered extremely conservative, as realistically, most foods will not contain any of the strains due to the standards of identity of many foods, the fact that the strains will not be added to foods requiring additional USDA regulatory review, market share limitations, limited food matrix viability, and the fact that the ingredients will likely be "invisible" to many consumers, who may realize they are consuming a fermented food (or a food containing a "probiotic") but likely will not be aware that *L. rhamnosus* IDCC 3201

is the strain that they are consuming, reducing the likelihood that only food products containing this strain will be chosen and consumed. If a more realistic (but still highly conservative) estimate is used that 25% of food servings will contain the maximum intended use level, highest consumers (men) would be exposed to approximately 5.6–7.0 x 10^{11} CFU/day (using 70 kg as a standard body weight, this is equivalent to 6.5 x 10^{9}–1.0 x 10^{10} CFU/kg bw/day) of *L. rhamnosus* IDCC 3201.

Part 4: Self-limiting Levels of Use

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

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for *L. rhamnosus* IDCC 3201 is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information. Nevertheless, the historical use of foods fermented with *L. rhamnosus* is discussed in Section 6.

Part 6: Narrative

6.1 History of Consumption

6.1.1 Lactic Acid Bacteria

Lactic acid bacteria have been consumed around the world as part of fermented foods since the earliest records of food preservation by humans, and continue to be ubiquitous in the market today.[40-42] In recent times, consumption of lactic acid live organisms (often referred to as "probiotics") has become popular as a way to support human health and wellness.[40] It is estimated that fermented foods constitute approximately one-third of the world food production.[43]

The number of live microorganisms in fermented foods can vary quite widely, depending on how a particular product is manufactured, processed, and stored.[41] It has been estimated that fermented cheeses and milk contain at least 10^7 CFU of *Lactobacilli* per gram, but may be as high as 10^9 CFU/g and even approaching 10^{10} CFU/g in some instances.[40, 41] Yogurt products containing at least 10^8 CFU/g at the time of manufacture in the United States may use the "live and active" seal from the National Yogurt Association.[41] Note that consumption of a 100 g serving of a fermented food containing 10^{8-10} CFU/g is equivalent to consuming 10^{10-12} CFU/serving. FDA's Reference Amount Customarily Consumed for yogurt is currently 170 g per serving.

6.1.2 *Lacticaseibacillus rhamnosus*

L. rhamnosus has been found and used in fermented foods such as sausage, grains, fermented milk, unpasteurized milk, and yogurt (10^7 CFU/g).[40] *L. rhamnosus* can also be found in specialty cheeses such as Comte cheese (10^6 CFU/g), Parmigiano Reggiano, and Fiore Sardo.[40] Strains are also found in the human gastrointestinal tract and breast milk, and are sold in dietary supplements.[5, 41] *L. rhamnosus* is listed in the IDF's 2018 Inventory, with food culture usages listed as dairy, vegetables, and meat.[43]

6.2 Regulatory Opinions

6.2.1 Europe

EFS Ahas developed the Qualified Presumption of Safety (QPS) system for the assessment of microorganisms to function as a generic pre-evaluation procedure to support safety risk assessments of bacterial species intentionally added to food or feed.[44] EFS Aregularly reviews the species identity, body of knowledge, and safety concerns of various taxonomic units. Any possible safety concerns for organisms that gain QPS status are reflected by "qualifications" for status. Such qualifications

should be assessed at the strain level. There is one generic qualification that applies to all QPS bacterial taxonomic units, which is that strains should be tested to ensure the absence of acquired genes conferring resistance to clinically relevant antimicrobials.

The first QPS list was established in 2007.[45] A full evaluation of the QPS list is undertaken every three years and results are published as Scientific Opinions, while the list of QPS microorganisms is maintained and re-evaluated approximately every six months to include new notifications to EFS A and published as Panel Statements. The most recent Panel Statement was adopted in June of 2021 and includes research published through March 2021.[46] As EFS A reviews safety literature pertinent to QPS units, clinical studies discussed in Subpart 6.3.2 include those published from April 2021 to December 2021 as a gap analysis since the last publication.

Note that QPS is generally not based on a particular intended use unless stated in a particular qualification. Unless a specific provision relating to dose is included in as a qualification to the QPS status, safety is presumed at any reasonable dose, which is the case for the ILDO NG taxonomic units.[47] Microorganisms not considered suitable for QPS remain subject to full safety assessments. All of those units with QPS status are considered non-pathogenic and non-toxigenic for human consumption as long as their qualifications are met.

L rhamnosus remains on the most recent EFS AQPS list.[46] As shown in Part 2, ILDO NG has tested *L. rhamnosus* IDCC 3201 for the qualification assessment of antimicrobial resistance according to EFS Aguidelines for microorganisms used as feed additives or as production organisms, and identified no concerns in this regard.[16]

6.2.2 United States

6.2.2.1 FDA GRAS

In the US, companies can notify FDA of their conclusion of GRAS status for a particular bacterial species/strain or ingredient on an individual basis, and for specific intended uses. Four GRAS notices related to *L. rhamnosus* strains are listed in FDA's GRN inventory. Of these, two have received the no questions letter from FDA, one was ceased to be evaluated at the notifier's request and one is pending. A brief summary of these FDA notifications is shown below in Table 4.

Table 4. FDA GRAS Notifications that Include *Lacticasebacillus rhamnosus* Strains

L. rhamnosus Strain	FDA GRN	Status	Maximum Intended Use	Exposure Estimates by the Notifiers
Strain DSM 33156	GRN 1013	Pending	Pending	Pending
Strain LGG	GRN 845	Withdrawn	n/a	n/a
Strain HN001	GRN 288	NQ	10^9 CFU per serving of various foods (may add up to 10^{11} CFU/serving initially to ensure intended CFU/serving over shelf-life)	Expected maximum intake $<10^{11}$ CFU/day
Strain HN001 produced in a milk-based medium	GRN 281	NQ	Maximum level of 10^8 CFU/g in infant formula (concentration of *L. rhamnosus* strain HN001 in the prepared formula would be 1.35 x 10 CFU/100 mL)	Mean intake 10^{9-10} CFU/day

Abbreviations: CFU, colony forming units; n/a, not applicable; NQ, no questions letter from the FDA.

6.2.3 Health Canada

All natural health products (NHPs) sold in Canada are subject to the *Natural Health Products Regulations*, which came into force on January 1, 2004. To be legally sold in Canada, all natural health products must have a product license. To get a product license, proper safety and efficacy evidence must be provided. Once Health Canada has assessed a product and decided it is safe, effective, and of high quality, it issues a product license along with an eight-digit Natural Product Number (NPN), which must appear on the label. This number indicates that the product has been reviewed and approved by Health Canada.

The safety and efficacy of NHPs and their health claims must be supported by proper evidence. Evidence may include clinical trial data or references to published studies, journals, pharmacopoeias, and traditional resources. The type and amount of supporting evidence required depends on the proposed health claim of the product and its overall risks.

L. rhamnosus is considered by Health Canada to be an "acceptable non-strain specific" bacterial species for use in food at level of 1.0 x 10^9 CFU/serving without pre-market notification. Additionally, 1459 products containing *L. rhamnosus* (NPNs 00232599, 02245396, 02246224, etc.) and 160 products containing *L. rhamnosus* strain GG (NPNs 80011341, 80024350, 80027695, etc.) are approved to be marketed under the Natural Health Products Regulations of Health Canada.

6.3 Safety Information

There were ten toxicological studies found in the literature that have been published on various strains of *Lactobacillus rhamnosus* (currently taxonomic name is *Lacticaseibacillus rhamnosus*) and are summarized in subpart 6.3.1. Additionally, human studies on *Lactobacillus rhamnosus* (current taxonomy is *Lacticaseibacillus rhamnosus*) strains are discussed in subpart 6.3.2. There were no published human or toxicological studies located for *L. rhamnosus* IDCC 3201 specifically as it is considered a novel strain. The studies reviewed do not suggest any concerns related to the safety of the strain.

6.3.1 Toxicological Studies on *Lacticaseibacillus rhamnosus* strains

Table 5. Summary of *Lacticaseibacillus rhamnosus* Toxicological Studies

Author	Strain(s)	Study Type/Duration /Guidelines Utilized	Animal Number (Strain)/Group	Dose Groups/Con-centration	NOAEL/Con-clusion/Findings
Zhang et al., 2021[48]	*L. rhamnosus* MP108	90-day gavage	10 Sprague Dawley rats/sex/ group (4 groups)	Control—sterile water Dose groups—0.25, 0.50 & 1.50 g/kg bw/day	No mortalities. No treatment-related adverse clinical findings. No adverse hematological, blood biochemistry, urinalysis, ophthalmologic, or macroscopic findings. No statistically significant differences in bw, bw gain, food consumption, food utilization rate.
		BRMA (Ames) OECD Guideline 471 (OECD, 1997)	*S. typhimurium* TA97, TA98, TA100, TA102, & TA1535	Positive control -S9—Dexon & sodium azide Positive control +S9—2-aminofluorene, 1,8-dihydroxyanthraquinone & cyclophosphamide	No positive mutagenic responses ± S9 activation.

Author	Strain(s)	Study Type/Duration /Guidelines Utilized	Animal Number (Strain)/Group	Dose Groups/Con-centration	NOAEL/Con-clusion/Findings
				Negative control—sterile water & DMSO Dose groups—8, 40, 200, 1000 & 50000 µg/plate ± S9	
		Spermatocyte chromosome aberration Food Safety National Standard (China) Protocol GB15193.8-2014	5 M Kunming mice/ group (5 groups)	Positive control—40 mg/kg bw cyclophospha-mide as a single 10 mL/kg bw IP Negative control—sterile water Dose groups—1.40, 2.80 & 5.60 g/kg bw	No significant differences in the spermatocyte aberration cell & chromosome aberration rate between groups.
Bhat et al., 2019[49]	*L. rhamnosus* MTCC-5897	28-day gavage	8 M Swiss mice/ group (5 groups)	Control—100 µL sterile saline Dose groups—10^7, 10^9, 10^{11} & 10^{13} CFU/day	No significant clinical findings on body weight, clinical chemistry, or organ weight indices.
Chiu et al., 2013[50]	*L. rhamnosus* LCR177, *B. adolescentis* BA286 & *Pediococcus acidilactici* PA318	MA (72 hours) gavage OECD Guidelines No. 474	5 M ICR mice/ group	Negative control—reverse osmosis water Positive control—cyclophospha-mide Dose groups—1.25, 2.5 & 5.0 g/kg (combined total 5.0×10^{10} CFU/g)	No chromosomal damage.
		BRMT	*S. typhimurium* TA97,	Control—distilled water	Non-mutagenic.

Author	Strain(s)	Study Type/Duration /Guidelines Utilized	Animal Number (Strain)/Group	Dose Groups/Con-centration	NOAEL/Con-clusion/Findings
			TA98, TA100, TA102, & TA1535	Dose groups—100 µL solution of test substance at 3.125, 6.25, 12.5, 25, & 50 mg/mL ± S9 at 20 mL/kg	
		CAT OECD Guidelines No. 473	Chinese hamster ovary cells	Control—0.1% DMSO Dose groups—0.3125, 0.625, 1.25, 2.5, 5 mg/mL ± S9	Non-clastogenic.
Bernardeau et al., 2002[51]	*L. rhamnosus* MA27/6B & *L. acidophilus* MA27/6R	4 weeks	5 Swiss mice/sex/group	Control—milk Dose groups—0, 10^2, 10^4, 10^6 & 10^8 CFU/mL added to drinking water	Mean water consumption was 7.69 mL/mouse/day. No significant toxicological findings.
Zhou et al., 2000 (a)[52]	*L. rhamnosus* HN001, *L. acidophilus* HN017, *B. lactis* HN019 & *L. acidophilus* La-1 & *L. rhamnosus* GG (the latter 2 were used as reference strains)	8-day gavage	8 M BALB/c mice/group (6 groups)	Control—10% skim milk Dose groups—10^{11} CFU/mL/strain/day in 10% skim milk	No differences with feed intake & activity between groups. No clinical findings with weight gain & general health status.
Zhou et al., 2000 (b)[53]	*L. rhamnosus* HN001, *L. acidophilus* HN017, *B. lactis* HN019 & *L. acidophilus* LA-1 (the latter is a reference strain)	28-day gavage	78 M BALB/c mice in 5 groups	Control—10% SMP Dose groups—2.5×10^9, 5×10^{10}, or 2.5×10^{12} CFU/kg bw/day/strain (mice were inoculated with 1 of the 4 LAB strains at 3 different doses)	No toxicity up to the highest dose tested. No significant findings in clinical chemistry, macroscopic examination, feed intake, or growth.

Abbreviations: BRMA, Bacterial Reverse Mutation Assay; bw, body weight; CAT, chromosomal aberration test; CFU, colony forming units; DMSO, dimethyl sulfoxide; IP, Intraperitoneal; LAB, lactic acid bacteria; M, male; MA, Micronucleus Assay; OECD, Organization for Economic Cooperation Development; SMP, skim milk powder.

6.3.2 Human Studies

The safety of *L. rhamnosus* IDCC 3201 has not been formally investigated in healthy adult subjects. However, many recent human clinical studies have been and continue to be published on various *L. rhamnosus* strains. Twenty-four human studies relevant to *L. rhamnosus* were published since the most recent QPS review by EFSA and they are summarized in the table below. These studies range from five days to 12 months and the maximum number of participants was 2653 adults. The maximum dose in the studies was 1 x 10^{11} CFU/day, orally administered.[54] These studies do not suggest any concern for safety of this species.

Table 6. Summary of Recent *Lacticaseibacillus rhamnosus* Human Clinical Trials

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
Castro-Herrera et al., 2021[55]	To evaluate the effects of a product containing 2 species, 1 of which is *L. rhamnosus* GG (LGG). RCT Dose—1.3–1.6 x 10^9 CFU/day (combined strains)	12 months	60 seniors ages (67–97 yo)	AEs were not mentioned by authors.
Chen et al., 2021[56]	To evaluate effects of *L. rhamnosus* HN001. Two-center, double-blind, RCT Dose—6 x 10^9 CFU/day	14–16 weeks' gestation to 24–30 weeks' gestation	432 pregnant women	AEs were not mentioned by authors.
Chen et al., 2021(b)[54]	To evaluate effects of a multi-strain product, containing 4 species, 1 of which was *L. rhamnosus* GG (LGG). All subjects underwent a PCCT before and after intervention. Double-blinded RCT Dose—1 x 10^{11} CFU/day for strain GG (LGG)	4 weeks	40 healthy adult males	No AEs related to treatment were observed.

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
Chen et al., 2021 (c)[57]	To evaluate 2 species, 1 of which was *L. rhamnosus*. Double-blind, RCT Dose—6 x 10^9 CFU/sachet (combined strains)	4 weeks	40 adults with *Heliobacter pylori*	In the intervention group there were reports of flatulence, abdominal pain, constipation, diarrhea, and others (unspecified). In the placebo group there were reports of dizziness, headache, flatulence, abdominal pain, nausea, constipation, diarrhea, and other (unspecified).
Cukrowska et al., 2021[58]	To evaluate effects of a daily oral product containing 3 species, 2 of which were *L. rhamnosus* LOCK 0900 & *L. rhamnosus* LOCK 0908. Multicenter RCT Dose—10^9 CFU/day (25% of LOCK 0908 & 25% LOCK 0900)	3 months with subsequent 9-month follow-up	151 children under 2 yo with AD & a cow's milk protein allergy	Authors reported sporadic reports of AEs, most commonly changes in stool consistency in 3 from the treatment group and 4 from the placebo group.
Damholt et al., 2021	To evaluate *L. rhamnosus* GG DSM 33156. Double-blind, placebo-controlled RCT Dose—10^9 CFU/day	16 weeks	619 children (2–6 yo)	4 events in intervention led to withdrawal from the trial including pain after dental extractions, asthma, emotional lability, & respiratory allergy, & 2 of the AEs (radius fracture & gastroenteritis) in the intervention group were evaluated as severe. None of the AEs were determined to be caused by the intervention. 4 were possibly related to the treatment including 3 in the intervention group (change in bowel habits & 2 loose stools) & 1 in the placebo group (loose stools).
Folwarski et al., 2021[59]	To evaluate the effects of *L. rhamnosus* GG. Prospective single-center randomized trial	30 days	40 adults undergoing pylorus-preserving Longmire-Traverso PPPD	No statistically significant difference between groups with IC, wound infections, pneumonia, abdominal abscess, UTI. 2 patients died due to septic shock (placebo group) & multiorgan dysfunction in

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	Dose—5 million CFU every 12 hours from day of surgery for 30 days			course of anastomosis leakage, 4 had a pancreatic fistula (it was not discussed which group later 2 AEs were associated with).
Freedman et al., 2021[60]	To study 2 species, 1 of which was *L. rhamnosus*. *Priori* planned multicenter, randomized, double-blinded, placebo-controlled ancillary PROGUT trial Dose—4 x 10^9 CFU including both strains (95:5 ratio). 5 extra sachets were provided & repeat dosing was administered if vomiting occurred within 15 minutes of administration	Participants received product for 5 days & submitted stool specimens on days 0, 5 & 28.	133 of 886 PROGUT children (3–48 mo) provided all 3 specimens. presenting in the ED, experienced ≥ 3 episodes of watery stools in a 24-hour period, had diarrhea or vomiting <72 hour & diagnosed with AGE	No AEs discussed by authors.
Groele et al., 2021[61]	To evaluate effects of a 2 species, 1 of which was *L. rhamnosus* GG. Double-blind, RCT Dose—10^9 CFU/day (combined strains)	6 months (participants were followed up with every 3 months up to 12 months from start of intervention)	96 children (8–17 yo) with newly diagnosed T1D	No AEs related to the study products were reported.
Guillemard et al., 2021[62]	To evaluate a multi-strain fermented milk product containing 3 species, 1 of which was *L. rhamnosus* CNCM I-3690. Double-blind, RCT with 2 parallel arms Dose—3.40 x 10^7–2.2 x 10^8 CFU/g for CNCM I-3690	28 days including a screening phase, 14-days of *Hp* eradication treatment, 28-days of product consumption & 14-days of follow-up with dietary restriction	136 adults under 14-day *Hp* treatment	42 participants in each group reported AEs, including headaches, nasopharyngitis, vulvovaginal mycotic infection, dysgeusia & rash. AEs from which the causality was not established, were considered by authors as unlikely related to the study products.
Johnstone et al., 2021[63]	To evaluate the effect of *L. rhamnosus* GG. RCT	Up to 60 days or until discharged from ICU or	2653 adults in ICU predicted to require mechanical	There were 15 patients (1.1%) in treatment group who experienced AEs, 2 of which were serious AEs and

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	Dose—2 x 10^{10} CFU/day	until *Lactobacillus* species were isolated from a sterile site or cultured as the sole or predominant organism from a nonsterile site	ventilation for at least 72 hours	the patients died. 1 patient (0.1%) in the placebo group experienced an AE. It is important to note that this trial involved critically ill patients.
Kosenoe et al., 2021[64]	To study 2 species, 1 of which was *L. rhamnosus* R0011. Double-blind, placebo-controlled RCT Dose—not provided	6 months	554 healthy elderly subjects (ranging from 60–90 yo)	AEs were not discussed by authors.
Lundtorp-Olsen et al., 2021[65]	To study the effects of 3 bacterial species, 1 of which was *L. rhamnosus* PB01 DSM14870. Double-blind RCT Dose—2 x 10^9 CFU/day (combined strains)	12 weeks	110 oral & systemic healthy adults	Authors reported that there were a "limited number" of participants who reported discomfort in regard to the treatment, but "at a minor level and all completed the trial."
Margiotta et al., 2021[66]	To evaluate 2 bacterial species, 1 of which was *L. rhamnosus* LR04-DSM. Monocentric survey Dose—1 x 10^9 CFU/day	15 days	457 patients admitted to the PED from 1 month–18 yo with a diagnosis of functional abdominal pain, gastroenteritis & gas colic	Authors reported there were no AEs.
Moludi et al., 2021[67]	To evaluate effects of *L. rhamnosus* GG (LGG). Double-blind RCT Dose—1.6 x 10^9 CFU/day	12 weeks	44 patients with CAD	2 AEs in treatment group & 1 in placebo, including stomach upset & gastrointestinal problems.
Morales et al., 2021[68]	To study effects of *L. rhamnosus* SP1.	12 months	47 systemically healthy	No AEs discussed by authors.

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	Triple-blind parallel-arm RCT Dose—2 x 10^7 CFU/day		participants with stage III periodontitis	
Onning et al., 2021[69]	To evaluate effects of a daily oral product combination with 2 species, 1 of which was *L. rhamnosus* 271. RCT. Dose—10^9 CFU/cap/day	8 weeks	32 healthy infants aged 4–83 days completed the study	No observed differences between groups with regard to growth parameters, AEs & intestinal performance. 2 participants had serious AEs (1 in placebo & 1 in treatment group) & authors stated that the events were unlikely related to the intervention.
Piatek et al., 2021[70]	To evaluate a multi-strain product with 8 species, 1 of which *is L. rhamnosus* GG and FOS. Open-label 2 parallel treatment group study Dose—10^9 CFU (equal amounts per strain)	4 weeks	87 infants aged 3–6 weeks with infantile colic	No AEs were reported for the two treatment groups.
Ryan et al., 2021[71]	To evaluate an 8-strain product, 1 of which was *L. rhamnosus* HN001 Single-arm, open-label study Dose—5 billion CFU/day for *L. rhamnosus* HN001	10 days	10 healthy adults	No serious AEs. 22 nonserious AEs, 2/3 of which were gastrointestinal in nature. As there was no control group, it is impossible to determine if the AEs were related to the intervention.
Shin et al., 2021[72]	To evaluate 2 strains, 1 of which was *L. rhamnosus* GR-1. Participants were also given *Saccharomyces boulardii.* Type of study was not discussed (note they did include a control). Dose—4 x 10^8 CFU/day (combined strains)	2 months	25 pairs of breastfeeding mothers & their infants	Authors did not discuss AEs.
Quero et al., 2021[73]	To evaluate the effects of Gasteel Plus® which contains 3 species, 1 of	30 days	27 male participants, 13	There were no AEs discussed by the authors.

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	which was *L. rhamnosus* CNCM I-4036 & vitamins including zinc & selenium. Triple-blinded, RCT Pilot study. Dose—10^9 CFU/day		professional soccer plays & 14 sedentary students	
Trachootham et al., 2021[74]	To evaluate *L. rhamnosus* GG (LGG) fermented milk followed by 5 glasses of beer with a 1-week wash-out. Blinded crossover RCT Dose—10^8 CFU of *L. rhamnosus* GG in 150 mL of fermented milk/day	28 days	20 adult healthy Thai M (20–50 yo)	Authors reported that there were no serious AEs.
Zhang et al., 2021[75]	To evaluate 2 strains, 1 was *L. rhamnosus* GR-1 in a probiotic drink, along with 7 days of vaginally administered metronidazole. Prospective, parallel-group, RCT Dose—$\geq 1 \times 10^9$ CFU/day	30 days	126 Chinese adult women with BV	There were 30 day and 90-day follow-up visits. No serious AEs were reported. Authors stated that there was no significant difference in external genital itching and burning between the groups.
Zheng et al., 2021[76]	To evaluate 4 strains, 1 of which was *L. rhamnosus* LGG-19. RCT Dose—10^9 CFU/cap for *L. rhamnosus* LGG-19 (up to 3 times/day)	Duration is unclear.	100 adults with gastric cancer	Authors did not discuss AEs.

Abbreviations: AD, atopic dermatitis; AE, adverse event; AGE, acute gastroenteritis; BV, bacterial vaginosis; CAD, cardiovascular disease; CFU, colony forming unit; ED, emergency department; FOS, fructooligosaccharides; *Hp, Heliobacter pylori*; IC, infectious complications; ICU, intensive care unit; M, male; mo, months old; PCCT, phosphatidylcholine challenge test; PED, pediatric emergency department; PPPD, pancreatoduodenectomy; PROGUT, Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment; RCT, randomized control trial; T1D, type 1 diabetes; UTI, urinary tract infection; yo, years old.

6.3.3 Opportunistic Infections

Infections caused by lactic acid bacteria have been described in the literature (e.g., sepsis and endocarditis) but for the most part occur at very low rates.[45] Infections associated with *Lactobacillus* genus (*Lactobacillus* is largely still discussed in the literature, as *Lacticaseibacillus* is a new taxonomic designation) almost always occur in immunocompromised patients, those who have suffered surgical or accidental insult, or have a serious underlying illness.[45] For example, infective endocarditis is caused by bacterial colonization of heart valves or endocardial tissue and generally occurs in individuals with valve defects (congenital or acquired), valve replacements, history of rheumatic endocarditis, etc.[45, 77] Bacteria, usually from the host's own commensal microflora, generally enter the bloodstream and adhere to the heart valves.[77] The vast majority of all infections occur from commensal bacteria, and the ingestion of lactic acid bacteria does not seem to be of additional concern with regard to infection possibilities.[45, 77]

Lactobacillus genus infections have been estimated as one case per 10 million people, which has been considered "unequivocally negligible" and such infections are rarely fatal.[1 4 42, 78] There have been some cases reported of neonates with related conditions such as sepsis, however they had complications with medical procedures, pre-existing conditions or compromised immune systems.[79-82] *L. rhamnosus* species appears to predominate with regard to *Lactobacillus* infections. Yet, EFSA determined that the at-risk population is not placed at added risk by the use of this species in food/feed, and thus, the taxonomic unit continue to have QPS status.[44, 45, 79, 83, 84]

6.4 Allergenicity

The U.S. Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 lists nine major allergens that could result in a requirement for allergy labeling on food products, including: milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, sesame, and soybeans. *L. rhamnosus* IDCC 3201 is grown in culture medium that contains milk and soy components. Thus, food products that contain this strain may be required to declare the allergens in their labeling per FALCPA. Otherwise, *L. rhamnosus* IDCC 3201 does not contain gluten, celery, mustard, sulfur dioxide and sulfites, lupin, or mollusks.

It is worth noting that the literature suggests there is an inverse relationship between lactic acid bacteria consumption and allergies and atopic eczema.[85]

6.5 Past Sales and Reported Adverse Events

ILDONG has been selling *L. rhamnosus* IDCC 3201 on all continents, mainly for human consumption, since 2016. According to the company, nearly 132,562

kilograms of the *L. rhamnosus* IDCC 3201 represented in this report were sold over the six years from January 1, 2016, to November 31, 2021. Commercial products in which *L. rhamnosus* IDCC 3201 is currently sold are shown below.

- BIO MCHEON Premium
- BIO MTA Family
- BIO MTA Family mini tablet
- gQlab 10B Alive Probiotics Gold (gQlab S 10 billion live probiotics)
- gQlab BeBe Plus (gQlab BeBe)
- gQlab Bifido plus (gQlab Bifido Postbiotics, gQlab Bifido Multibiotics)
- gQlab Daily
- gQlab Kids Plus (gQlab Kids)
- gQlab My Kids Probiotics
- gQlab My Kids Probiotics Chewable (gQlab Kids Plus Chewable, gQlab Kids Chewable Probiotics, gQlab Kids Chewable)
- gQlab Postbiotics
- gQlab Power Active (gQlab Active Probiotics)
- gQlab S Alive Probiotics
- gQlab S Synbiotics
- GUT HEALTH N PROBIOTICS
- HIGHLACTO Kids Chewable
- HIGHLACTO Premium (HIGHLACTO)
- HIGHLACTO Pro & Pre
- IBL Alive Probiotics Chewable Tablet
- IBL Diet Probiotics
- IMMUNE N PROBIOTICS
- Lactogold plus
- LACTONIA Diet Probiotics
- LACTONIA Vitamin C Probiotics
- MyNi GoodMorning Probiotics 100B (MyNi GoodMorning Probiotics)
- MICROBIOME PROBIOTICS

- PROBIO TRIPLE

Related to this six-year sales period, complaints and non-serious adverse events were registered. There were 134 complaints over this period. 118 complaints are for products currently being sold, which are shown in the list above. These products all contain *L. rhamnosus* IDCC 3201, along with other strains and excipients. The majority of complaints (75%) were minor and gastrointestinal in nature, including abdominal pain, constipation, diarrhea, loose stools, incomplete evacuation, flatulence, gastrointestinal discomfort, stomachache, sour stomach, bloating, indigestion, reflux, nausea, and four cases of vomiting. Twenty-four percent were minor skin reactions including itch, facial flushing, rash, and redness around mouth. There were two complaints of dizziness and headaches. Finally, there were four complaints of allergy and one complaint of a cold sore.

It is important to note that while these complaints correlated with consumption of the products, there is no way to absolutely determine whether they were caused by consumption of the products or by other factors. As discussed above, ILDONG's *L. rhamnosus* IDCC 3201 is produced with milk. Thus, it is possible that the allergic reactions were due to exposure to this known allergen, exposure to one of the excipients, or could be completely unrelated to consumption of the products. As also mentioned above, ILDONG may be required to declare milk on the labeling for this strain.

As described above, *L. rhamnosus* has a long history of safe consumption by humans and animals.[40-43] Today, it is available in supplements from numerous companies. According to a search of the National Institutes of Health's Dietary Supplement Label Database, which contains information taken from the labels of dietary supplement products available in the U.S. marketplace, the search term "*Lactobacillus rhamnosus*" returned 6015 products and "*Lacticaseibacillus rhamnosus*" returned 2562 products that contain this species as an ingredient.

FDA

A search of MedWatch and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine had no mention of *L. rhamnosus*. All information was accessed from the databases on January 4, 2022.

FAERS

FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System (FAERS AE) revealed 36 cases of relevant adverse events which included one death. These events are summarized in the table below.

Table 7. Adverse Events reported for *Lacticaseibacillus rhamnosus* on FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System

# of AE cases reported/year (# of cases)	Ages (# of cases)	Reaction Classification (# of cases)	Deaths
36 cases in total 2021 (1) 2020 (3) 2019 (4) 2018 (9) 2016 (2) 2015 (7) 2014 (5) 2013 (3) 2012 (1) 2011 (1)	0–1 month (1) 2 months–2 yo (1) 3–11 yo (6) 12–17 yo (3) 18–64 (14) 65–85 (5) >85 yo (2) Not specified (4)	General disorders & administration site conditions (17) Infections & infestations (10) Gastrointestinal disorders (9) Im mune system disorders (7) Skin & subcutaneous tissue disorders (7) Injury, poisoning & procedural complications (6) Nervous system disorders (5) Congenital, familial & genetic disorders (5) Cardiac disorders (3) Vascular disorder (3) Investigations (2) Respiratory, thoracic & mediastinal disorders (2) Ear & labyrinth disorders (2) Neoplasms benign, malignant & unspecified (1) Reproductive system & breast disorders (1) Pregnancy, puerperium & perinatal conditions (1) Hepatobilliary disorders (1)	1 death—male, fetus (2016) Suspect product name listed (note that it does not mean they are the cause)—Augmentin

Abbreviations: AE, adverse events; yo, years old.

Adverse event reports are only associations and reported products may not be causally related to the adverse events. The FAERS website include the following caveats regarding their AERs as seen below.

"...while FAERS contains reports on a particular drug or biologic, this does not mean that the drug or biologic caused the adverse event. Importantly, the FAERS data by themselves are not an indicator of the safety profile of the drug or biologic. Some additional limitations to note include:

***Duplicate and incomplete reports are in the system**: There are many instances of duplicative reports and some reports do not contain all the necessary information.*

***Existence of a report does not establish causation**: For any given report, there is no certainty that a suspected drug caused the reaction. While consumers and healthcare professionals are encouraged to report adverse events, the reaction may have been related to the underlying disease being treated, or caused by some other drug being taken concurrently, or occurred for other reasons. The information in these reports reflects only the reporter's observations and opinions.*

***Information in reports has not been verified**: Submission of a report does not mean that the information included in it has been medically confirmed nor it is an admission from the reporter that the drug caused or contributed the event.*

***Rates of occurrence cannot be established with reports**: The information in these reports cannot be used to estimate the incidence (occurrence rates) of the reactions reported."*

6.6 Basis for the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with a conclusion that ILDONG's *L. rhamnosus* IDCC 3201 is reasonably certain to be safe under the conditions of its intended use.

6.6.1 Data and Information that Establish Safety

The scientific data, information, and methods forming the basis of this conclusion are:

- The establishment of identity via 16S rRN A sequence as well as complete genome sequencing, with confirmed 99% sequence homology to its type strain sequence, *L. rhamnosus* JCM 1136;
- The analyses and resulting data showing *L. rhamnosus* IDCC 3201 lacks resistance to clinically relevant antibiotics per European Food Safety Authority (EFS A)minimal inhibitory concentration cut-offs and guidelines, with the exception of gentamicin and kanamycin, where further investigation by ILDO NG showed that resistance did not correlate with any related genetic sequences, and is not expected to be transferrable;
- The lack of potential of *L. rhamnosus* IDCC 3201 to produce toxins or virulence factors that have been demonstrated to be virulent to hosts (via comparison of genomic sequences to known virulence sequences using the Virulence Factor Database);

- The methods of manufacture, specifications, as well as batch analyses, showing that all specifications are met for each batch, demonstrating safe production methods and robust quality control standards for *L. rhamnosus* IDCC 3201;
- Two previous GRAS notices to FDA for *L. rhamnosus* strain HN 001, one of which has intended uses similar to ILDO NG's *L. rhamnosus* IDCC 3201 (GRN 288), received no questions letters from FDA at addition levels in the 10^9 CFU/serving range. One of two GRNs was for use in infant formulas, suggesting a high degree of recognized safety of this species;
- L. rhamnosus' EFS AQPS status for food and feed use, at any reasonable dose/intended use, suggesting no further regulatory review prior to introduction of new strains into the European food supply, other than the qualification that it must be verified to not harbor acquired antimicrobial resistance genes;

6.6.2 Data and Information that are Corroborative of Safety

- The documented long history of safe human consumption of *L. rhamnosus* as a common bacterial species in dairy products such as yogurt, Comte cheese, fermented milk, Parmigiano Reggiano, Fiore Sardo, and unpasteurized milk;
- The lack of serious adverse events reported in clinical trials using other *L. rhamnosus* strains at daily dosages up to 1×10^{11} CFU/day;
- Published toxicology studies on other *L. rhamnosus* species, showing no indication of safety issues in rodents; and
- Agreement in the literature that it is highly unlikely that a microorganism maintained in pure culture, with a history of safe use, would become unsafe as a result of mutation (genetic drift), production changes, or delivery format changes.[86-88]

6.6.3 General Recognition

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of *L. rhamnosus* IDCC 3201 for its intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions provide ample evidence of general recognition among qualified experts that there is reasonable certainty that consumption of *L. rhamnosus* IDCC 3201 for its intended use is not harmful. The

general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

6.6.4 Data and Information that are Inconsistent with the GRAS Conclusion

- We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.6.5 Information that is Exempt from Disclosure under FOIA

- There are no data or information in this report that are considered trade secret or commercial or financial information that is privileged or confidential.

Part 7: Supporting Data and Information

Literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted through January 4, 2021.

7.1 Data and Information that are *not* Generally Available

All of the information described in this report is generally available.

7.2 References that *are* Generally Available

1. Pot B, Felis G, et al. The genus Lactobacillus. In: Lactic acid bacteria: biodiversity and taxonomy. W Holzapfel and B Wood: John Wiley & Sons: 2014. 249-353.
2. Mu Q, Tavella VJ, et al. Role of Lactobacillus reuteri in Human Health and Diseases. *Front Microbiol*. 2018;9:757
3. Bottari B, Levante A, et al. How the Fewest Become the Greatest. L. casei's Impact on Long Ripened Cheeses. *Front Microbiol*. 2018;9:2866
4. Lebeer S, Vanderleyden J, et al. Adaptation factors of the probiotic Lactobacillus rhamnosus GG. *Benef Microbes*. 2010;1(4):335-42
5. Lahtinen S, Ouwehand A, et al. Lactic acid bacteria. Microbiological and functional aspects. Fourth edition. Boca Raton: CRC Press; 2012
6. EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis K, et al. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: suitability of taxonomic units notified to EFSA until March 2020. *EFSA Journal*. 2020;18(7)
7. Zheng J, Wittouck S, et al. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *Int J Syst Evol Microbiol*. 2020;70(4):2782-2858
8. EFSA. Scientific Opinion. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*. 2012;10(6):2740
9. Ammor MS, Florez AB, et al. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiol*. 2007;24(6):559-70
10. Mathur S and Singh R. Antibiotic resistance in food lactic acid bacteria--a review. *Int J Food Microbiol*. 2005;105(3):281-95
11. WHO. The world is running out of antibiotics, WHO report confirms; 2017.
12. FAO/WHO. Guidelines for the evaluation of probiotics in food. 2002. 1-11.
13. EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, et al. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA. *EFSA Journal*. 2017;15(3):4664
14. Bernardeau M, Vernoux JP, et al. Safety assessment of dairy microorganisms: the Lactobacillus genus. *Int J Food Microbiol*. 2008;126(3):278-85

15. Reid G. The importance of guidelines in the development and application of probiotics. *Curr Pharm Des.* 2005;11(1):11-6
16. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal.* 2018;16(3):5206
17. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Scientific Opinion on the safety and efficacy of Oralin® (Enterococcus faecium) as a feed additive for calves for rearing, piglets, chickens for fattening, turkeys for fattening and dogs. *EFSA Journal.* 2014;12(6):3727
18. Campedelli I, Mathur H, et al. Genus-Wide Assessment of Antibiotic Resistance in Lactobacillus spp. *Appl Environ Microbiol.* 2019;85(1):e01738-18
19. Krause KM, Serio AW, et al. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med.* 2016;6(6)
20. Gueimonde M, Sanchez B, et al. Antibiotic resistance in probiotic bacteria. *Front Microbiol.* 2013;4:202
21. Shao Y, Zhang W, et al. Comparative studies on antibiotic resistance in Lactobacillus casei and Lactobacillus plantarum. *Food Contr.* 2015;50:250-258
22. Zhou JS, Pillidge CJ, et al. Antibiotic susceptibility profiles of new probiotic Lactobacillus and Bifidobacterium strains. *Int J Food Microbiol.* 2005;98(2):211-7
23. Danielsen M and Wind A. Susceptibility of Lactobacillus spp. to antimicrobial agents. *Int J Food Microbiol.* 2003;82(1):1-11
24. EFSA. Outcome of the public consultation on the draft guidance on characterisation of microorganisms used as feed additives or as production organisms. *EFSA Supporting publication.* 2018;EN-1389
25. Spano G, Russo P, et al. Biogenic amines in fermented foods. *Eur J Clin Nutr.* 2010;64 Suppl 3:S95-100
26. EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal.* 2011;9(109):2393
27. Stiles ME and Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol.* 1997;36(1):1-29
28. Ewaschuk JB, Naylor JM, et al. D-lactate in human and ruminant metabolism. *J Nutr.* 2005;135(7):1619-25
29. Petersen C. D-lactic acidosis. *Nutr Clin Pract.* 2005;20(6):634-45
30. Connolly E, Abrahamsson T, et al. Safety of D(-)-lactic acid producing bacteria in the human infant. *J Pediatr Gastroenterol Nutr.* 2005;41(4):489-92
31. Haschke-Becher E, Brunser O, et al. Urinary D-lactate excretion in infants receiving Lactobacillus johnsonii with formula. *Ann Nutr Metab.* 2008;53(3-4):240-4
32. Haschke-Becher E, Baumgartner M, et al. Assay of D-lactate in urine of infants and children with reference values taking into account data below detection limit. *Clin Chim Acta.* 2000;298(1-2):99-109

33. Hove H and Mortensen PB. Colonic lactate metabolism and D-lactic acidosis. *Dig Dis Sci*. 1995;40(2):320-30
34. Uribarri J, Oh MS, et al. D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. *Medicine (Baltimore)*. 1998;77(2):73-82
35. Munakata S, Arakawa C, et al. A case of D-lactic acid encephalopathy associated with use of probiotics. *Brain Dev*. 2010;32(8):691-4
36. Ku W, Lau D, et al. Probiotics Provoked D-lactic Acidosis in Short Bowel Syndrome: Case Report and Literature Review. *HK J Paediatr*. 2006;11:246-254
37. de Vrese M, Koppenhoefer B, et al. D-lactic acid metabolism after an oral load of DL-lactate. *Clin Nutr*. 1990;9(1):23-8
38. Basiotis P, Lino M, et al. Consumption of Food Group Servings: People's Perceptions vs. Reality. *Nutrition Insights*. 2000. 20: 1-2
39. Millen AE, Midthune D, et al. The National Cancer Institute diet history questionnaire: validation of pyramid food servings. *Am J Epidemiol*. 2006;163(3):279-88
40. Bernardeau M, Guguen M, et al. Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiol Rev*. 2006;30(4):487-513
41. Rezac S, Kok CR, et al. Fermented Foods as a Dietary Source of Live Organisms. *Front Microbiol*. 2018;9:1785
42. International Dairy Federation. Safety demonstration of microbial food cultures (MFC) in fermented food products. Bulletin of the International Dairy Federation 455/2012. 2012. 62.
43. International Dairy Federation. Inventory of microbial food cultures with safety demonstration in fermented food products. Update of the Bulletin of the IDF No. 455/2012: Safety demonstration of Microbial Food Cultures (MFC) in fermented food cultures. Bulletin of the International Dairy Federation 495/2018. 2018. 71.
44. EFSA Panel on Biological Hazards (BIOHAZ). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: Suitability of taxonomic units notified to EFSA until March 2019. *EFSA Journal*. 2019;17(7):5753
45. EFSA. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Opinion of the Scientific Committee. *The EFSA Journal*. 2007;587:1-16
46. EFSA Panel on Biological Hazards (BIOHAZ). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 14: suitability of taxonomic units notified to EFSA until March 2021. *EFSA Journal*. 2021;19(7):6689
47. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Scientific Opinion on the removal of a maximum dose from the authorisation of microbial products assessed using the Qualified Presumption of Safety approach. *EFSA Journal*. 2012;10(5):2680
48. Zhang B, Lynch B, et al. Lactobacillus rhamnosus MP108: Toxicological evaluation. *J Food Sci*. 2021;86(1):228-241

49. Bhat MI, Singh VK, et al. Adherence capability and safety assessment of an indigenous probiotic strain Lactobacillus rhamnosus MTCC-5897. *Microb Pathog*. 2019;130:120-130
50. Chiu YJ, Nam MK, et al. Genotoxicity assessment of multispecies probiotics using reverse mutation, mammalian chromosomal aberration, and rodent micronucleus tests. *Scientific World Journal*. 2013;2013:254239
51. Bernardeau M, Vernoux JP, et al. Safety and efficacy of probiotic lactobacilli in promoting growth in post-weaning Swiss mice. *Int J Food Microbiol*. 2002;77(1-2):19-27
52. Zhou JS, Shu Q, et al. Acute oral toxicity and bacterial translocation studies on potentially probiotic strains of lactic acid bacteria. *Food Chem Toxicol*. 2000;38(2-3):153-61
53. Zhou JS, Shu Q, et al. Safety assessment of potential probiotic lactic acid bacterial strains Lactobacillus rhamnosus HN001, Lb. acidophilus HN017, and Bifidobacterium lactis HN019 in BALB/c mice. *Int J Food Microbiol*. 2000;56(1):87-96
54. Chen S, Jiang PP, et al. Effects of probiotic supplementation on serum trimethylamine-N-oxide level and gut microbiota composition in young males: a double-blinded randomized controlled trial. *Eur J Nutr*. 2020
55. Castro-Herrera VM, Fisk HL, et al. Combination of the Probiotics Lacticaseibacillus rhamnosus GG and Bifidobacterium animalis subsp. lactis, BB-12 Has Limited Effect on Biomarkers of Immunity and Inflammation in Older People Resident in Care Homes: Results From the Probiotics to Reduce Infections iN CarE home reSidentS Randomized, Controlled Trial. *Front Immunol*. 2021;12:643321
56. Chen Y, Lu J, et al. Effect of Lactobacillus rhamnosus Probiotic in Early Pregnancy on Plasma Conjugated Bile Acids in a Randomised Controlled Trial. *Nutrients*. 2021;13(1)
57. Chen MJ, Chen CC, et al. The efficacy of Lactobacillus acidophilus and rhamnosus in the reduction of bacterial load of Helicobacter pylori and modification of gut microbiota-a double-blind, placebo-controlled, randomized trial. *Helicobacter*. 2021;26(6):e12857
58. Cukrowska B, Ceregra A, et al. The Effectiveness of Probiotic Lactobacillus rhamnosus and Lactobacillus casei Strains in Children with Atopic Dermatitis and Cow's Milk Protein Allergy: A Multicenter, Randomized, Double Blind, Placebo Controlled Study. *Nutrients*. 2021;13(4)
59. Folwarski M, Dobosz M, et al. Effects of Lactobacillus rhamnosus GG on early postoperative outcome after pylorus-preserving pancreatoduodenectomy: a randomized trial. *Eur Rev Med Pharmacol Sci*. 2021;25(1):397-405
60. Freedman SB, Horne R, et al. Probiotic stool secretory immunoglobulin A modulation in children with gastroenteritis: a randomized clinical trial. *Am J Clin Nutr*. 2021;113(4):905-914
61. Groele L, Szajewska H, et al. Lack of effect of Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12 on beta-cell function in children with newly diagnosed type 1 diabetes: a randomised controlled trial. *BMJ Open Diabetes Res Care*. 2021;9(1)

62. Guillemard E, Poirel M, et al. A Randomised, Controlled Trial: Effect of a Multi-Strain Fermented Milk on the Gut Microbiota Recovery after Helicobacter pylori Therapy. *Nutrients*. 2021;13(9)
63. Johnstone J, Meade M, et al. Effect of Probiotics on Incident Ventilator-Associated Pneumonia in Critically Ill Patients: A Randomized Clinical Trial. *JAMA*. 2021;326(11):1024-1033
64. Koesnoe S, Masjkuri N, et al. A randomized controlled trial to evaluate the effect of influenza vaccination and probiotic supplementation on immune response and incidence of influenza-like illness in an elderly population in Indonesia. *PLoS One*. 2021;16(12):e0250234
65. Lundtorp-Olsen C, Enevold C, et al. Probiotics Do Not Alter the Long-Term Stability of the Supragingival Microbiota in Healthy Subjects: A Randomized Controlled Trial. *Pathogens*. 2021;10(4)
66. Margiotta G, Ferretti S, et al. Effect of Lactobacillus reuteri LRE02-Lactobacillus rhamnosus LR04 combination and gastrointestinal functional disorders in an Emergency Department pediatric population. *Eur Rev Med Pharmacol Sci*. 2021;25(7):3097-3104
67. Moludi J, Kafil HS, et al. Effect of probiotic supplementation along with calorie restriction on metabolic endotoxemia, and inflammation markers in coronary artery disease patients: a double blind placebo controlled randomized clinical trial. *Nutr J*. 2021;20(1):47
68. Morales A, Contador R, et al. Clinical effects of probiotic or azithromycin as an adjunct to scaling and root planning in the treatment of stage III periodontitis: a pilot randomized controlled clinical trial. *BMC Oral Health*. 2021;21(1):12
69. Onning G, Palm R, et al. New Lactiplantibacillus plantarum and Lacticaseibacillus rhamnosus strains: well tolerated and improve infant microbiota. *Pediatr Res*. 2021
70. Piatek J, Bernatek M, et al. Effects of a nine-strain bacterial synbiotic compared to simethicone in colicky babies - an open-label randomised study. *Benef Microbes*. 2021;12(3):249-257
71. Ryan JJ and Patno NM. Short-Term Tolerability, Safety, and Gut Microbial Composition Responses to a Multi-Strain Probiotic Supplement: An Open-Label Study in Healthy Adults. *Integr Med (Encinitas)*. 2021;20(1):24-34
72. Shin DY, Park J, et al. Comprehensive Analysis of the Effect of Probiotic Intake by the Mother on Human Breast Milk and Infant Fecal Microbiota. *J Korean Med Sci*. 2021;36(8):e58
73. Quero CD, Manonelles P, et al. Differential Health Effects on Inflammatory, Immunological and Stress Parameters in Professional Soccer Players and Sedentary Individuals after Consuming a Synbiotic. A Triple-Blinded, Randomized, Placebo-Controlled Pilot Study. *Nutrients*. 2021;13(4)
74. Trachootham D, Whanmek K, et al. Intake of Lactobacillus rhamnosus GG (LGG) fermented milk before drinking alcohol reduces acetaldehyde levels and duration of flushing in drinkers with wild-type and heterozygous mutant ALDH2: a randomized, blinded crossover controlled trial. *Food Funct*. 2021;12(20):10147-10159

75. Zhang Y, Lyu J, et al. Probiotic Lacticaseibacillus rhamnosus GR-1 and Limosilactobacillus reuteri RC-14 as an Adjunctive Treatment for Bacterial Vaginosis Do Not Increase the Cure Rate in a Chinese Cohort: A Prospective, Parallel-Group, Randomized, Controlled Study. *Front Cell Infect Microbiol*. 2021;11:669901
76. Zheng C, Chen T, et al. Adjuvant treatment and molecular mechanism of probiotic compounds in patients with gastric cancer after gastrectomy. *Food Funct*. 2021;12(14):6294-6308
77. Adams MR. Safety of industrial lactic acid bacteria. *J Biotechnol*. 1999;68(2-3):171-8
78. Sanders ME, Akkermans LM, et al. Safety assessment of probiotics for human use. *Gut Microbes*. 2010;1(3):164-85
79. Rossi F, Amadoro C, et al. Members of the Lactobacillus Genus Complex (LGC) as Opportunistic Pathogens: A Review. *Microorganisms*. 2019;7(5)
80. Land MH, Rouster-Stevens K, et al. Lactobacillus sepsis associated with probiotic therapy. *Pediatrics*. 2005;115(1):178-81
81. Tran L, Begue R, et al. Lactobacillus rhamnosus sepsis in an infant without probiotic use: A case report and literature review. *Journal of Neonatal-Perinatal Medicine*. 2011;4(2):163-167
82. Divyaswathi C and Gourishankar A. Lactobacillus causing urinary tract infection in a neonate. *J Med Cases*. 2013;4(10):682-685
83. EFSA Panel on Biological Hazards (BIOHAZ). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 9:suitability of taxonomic units notified to EFSA until September 2018. *EFSA Journal*. 2018;17(1):5555
84. Cannon JP, Lee TA, et al. Pathogenic relevance of Lactobacillus: a retrospective review of over 200 cases. *Eur J Clin Microbiol Infect Dis*. 2005;24(1):31-40
85. Zukiewicz-Sobczak W, Wroblewska P, et al. Probiotic lactic acid bacteria and their potential in the prevention and treatment of allergic diseases. *Cent Eur J Immunol*. 2014;39(1):104-8
86. Pariza MW, Gillies KO, et al. Determining the safety of microbial cultures for consumption by humans and animals. *Regul Toxicol Pharmacol*. 2015;73(1):164-71
87. Stevens H and Nabors L. Microbial food cultures: a regulatory update. *Food Tech*. 2009;63(3):36-41
88. Sanders ME, Klaenhammer TR, et al. Effects of genetic, processing, or product formulation changes on efficacy and safety of probiotics. *Ann N Y Acad Sci*. 2014;1309:1-18

Kaiping Deng
Staff Fellow/Regulatory Review Scientist
Regulatory Review Branch
Division of Food Ingredients
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 708-924-0622
kaiping.deng@fda.hhs.gov

April 4, 2023

Re: Responses to FDA's GRN 1093 Questions

Dear Dr. Deng,

Please find responses to FDA's questions concerning *L. rhamnosus* 3201 (GRN 1093) below. FDA's questions are in BLACK, while the notifier responses are in BLUE:

1. Please provide a statement that all processing aids used in the manufacture of *L. rhamnosus* IDCC 3201 are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses or are subjects of effective food contact notifications.
   - Response: Subpart 2.3.2 (Raw Materials, page 10 of 47) of GRN 1093 is amended to include the following statement: All processing aids used in the manufacture of *L. rhamnosus* IDCC 3201 are used in accordance with applicable US regulations, were concluded to be GRAS for their respective uses, or are subjects of effective food contact notifications.

2. In Table 2 (page 13), you provided the results from the analyses of three non-consecutive batches of *L. rhamnosus* IDCC 3201 including the results for heavy metals. We note that the batch analyses show that the results for lead, cadmium, mercury, and arsenic are all consistently and significantly lower than the corresponding specification limits.
   a. We recommend that you lower the specification limits for the heavy metals to reflect the results of the batch analyses and to be as low as possible.

      Response: The notifier has lowered their specification limits for lead and arsenic to better reflect the results of the batch analyses and can be seen in the table below.

Heavy Metals		
	New Specifications	Previous Specifications
Lead	< 0.5 mg/kg	< 1.0 mg/kg
Cadmium	< 0.3 mg/kg	< 0.3 mg/kg

Heavy Metals		
Mercury	< 0.1 mg/kg	< 0.1 mg/kg
Arsenic	< 0.3 mg/kg	< 0.5 mg/kg

b. In addition, please confirm that the analytical results for heavy metals expressed as "0.00 mg/kg" represent the levels below the corresponding limits of the detection (LOD) listed in the footnotes to Table 2.

Response: The notifier confirms that the analytical results for heavy metals expressed as "0.00 mg/kg" represent levels below the corresponding limits of detection (LOD) listed in the footnotes to Table 2. Table 2 has been amended to reflect this update and is shown in the table below:

Tested Parameters	Specification	Lot No./Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0501 05/2019	Lot# IDK1001 10/2019
Heavy Metals				
Lead[a]	< 1.0 mg/kg	0.01 mg/kg	0.02 mg/kg	0.02 mg/kg
Cadmium[b]	< 0.3 mg/kg	< LOD	< LOD	< LOD
Mercury[c]	< 0.1 mg/kg	< LOD	0.01 mg/kg	< LOD
Arsenic[d]	< 0.5 mg/kg	< LOD	< LOD	< LOD

Abbreviations: CFU, colony forming units; LOD, limit of detection.
[a] Limit of Detection = 0.4 µg/kg
[b] Limit of Detection = 0.6 µg/kg
[c] Limit of Detection = 1.7 µg/kg
[d] Limit of Detection = 0.7 µg/kg

3. In Part 3, you provided a list of broad food categories in which *L. rhamnosus* IDCC 3201 is intended to be used as an ingredient. Please specify a serving size for each food category (or food subcategory if needed) and provide the reference that was used as the basis for determining the serving size. In addition, please confirm that the maximum use level of the ingredient is up to 1.02 x 10^{11} CFU/serving regardless of the food category and that the intended food uses exclude alcoholic beverages.

- Response, part a: As stated in the GRN notice 1093 Part 3 on page 19 of 47, *L. rhamnosus* IDCC 3201 is intended to be used as an ingredient added to foods where standards of identity do not preclude such use. It is not intended to be added to infant formula or any products that would require additional regulatory review by USDA. While not stated in the original notice, we now amend Part 3 of the notice to state that *L. rhamnosus* IDCC 3201 is also NOT intended to be used in alcoholic beverages.
- Response, part b: The food categories listed in Part 3 of the GRN are merely examples of the types of food categories to which the ingredient could be added but are not intended to be all inclusive. We confirm that the maximum use level of the ingredient is 1.02 x 10^{11} CFU per serving (which includes

overage) regardless of the food category. Please let us know if any additional detail is required, as we will be happy to quickly provide it.

4. In Part 3, you provided the maximum number of servings consumed per day by men (~27.8 servings/day) and women (~19.5 servings/day) calculated based on the data published by Millen et al. (2006). We note the following:
    a. The maximum numbers of servings/day provided in the GRAS notice are higher than expected based on the data in Millen et al. (2006). Please note that the number of ounces/day reported for "Red meat, poultry, fish" accounts for all "Lean meat".
        - Response: We acknowledge your point and agree that our exposure estimations were higher than would be expected based on the Millen et al. (2006) data. Also, we note, from a practical perspective, "lean meats" are not a suitable format of food for addition of *L. rhamnosus* IDCC 3201, nor are other whole food products such as vegetables and fruits, making our exposures estimates all the more conservative.
    b. To estimate dietary exposure based on the number of servings, we typically use 20 servings/day (the average number of servings for men and women from both the 24-hour dietary recall and the diet history questionnaire).
        - Response: Noted, and thank you; this is helpful information.
    c. For an ingredient that is intended for use in all food categories except infant formula and products under the jurisdiction of USDA, we typically presume that half the servings (10 out of 20 servings) of food will contain the ingredient.
        - Response: Again, noted and thank you for this information.

    Please verify your calculations and provide an updated dietary exposure estimate based on our recommendations above.
        - Response: Utilizing FDA's recommendations shared above (assuming individuals will consume approximately 20 servings/day of foods containing the ingredient) and using the intended addition level of 1.02×10^{11} CFU *L. rhamnosus* IDCC 3201 per serving, the new estimated dietary exposure to the ingredient is 1.02×10^{13} CFU/day. Using 70 kg as an average body weight, the new exposure is equivalent to 1.46×10^{11} CFU/kg bw/day.
        - Utilizing FDA's recommendations shared above (assuming individuals will consume approximately 10 servings/day of foods containing the ingredient) and using the intended addition level of 1.02×10^{11} CFU *L. rhamnosus* IDCC 3201 per serving, the new estimated dietary exposure to the ingredient is 1.02×10^{12} CFU/day. Using 70 kg as an average body weight, the new exposure is equivalent to 1.46×10^{10} CFU/kg bw/day.
5. In Section 2.2, you identified *L. rhamnosus* IDCC 3201 taxonomically according to standard taxonomic guidelines. You stated that the *Lactobacillus* genus which

contained 261 species, was divided into 25 new genera in April 2020, based on phylogenetic, phenotypical, and habitat differences (Ref.6 and 7). Based on this analysis, the genus and species of ILDONG's strain was reclassified from *Lactobacillus rhamnosus* to *Lacticaseibacillus rhamnosus.*

a. Please clarify where in the two references the ILDONG's strains were reclassified.

Response: We apologize for the miscommunication and want to clarify that the *Lactobacillus rhamnosus* species was reclassified (not ILDONG's strain specifically) to *Lacticaseibacillus rhamnosus*. In reference #6 (EFSA 2020), the reference discusses the taxonomic change on page 21. Please see a screenshot of the text from this page in the publication below. Further, *List of Prokaryotic Names with Standing in Nomenclature* (LPSN) and the *National Library of Medicine taxonomy browser* list, on their websites, *Lacticaseibacillus rhamnosus* (JCM 1136) as a type strain of the reclassified *Lactobacillus rhamnosus* . *Lacticaseibacillus rhamnosus* (JCM 1136) is the type strain against which ILDONG identified *L. rhamnosus* IDCC 3201 using 16S rRNA DNA. Please see links provided above.

BIOHAZ statement on QPS: suitability of taxonomic units notified until March 2020

'Classical' denomination	'Updated' denomination
Lactobacillus crispatus	*Lactobacillus crispatus*
Lactobacillus curvatus	*Latilactobacillus curvatus*
Lactobacillus delbrueckii	*Lactobacillus delbrueckii*
Lactobacillus dextrinicus	*Lapidilactobacillus dextrinicus*
Lactobacillus diolivorans	*Lentilactobacillus dioliovorans*
Lactobacillus farciminis	*Companilactobacillus farciminis*
Lactobacillus fermentum	*Limosilactobacillus fermentum*
Lactobacillus gallinarum	*Lactobacillus gallinarum*
Lactobacillus gasseri	*Lactobacillus gasseri*
Lactobacillus helveticus	*Lactobacillus helveticus*
Lactobacillus hilgardii	*Lentilactobacillus hilgardii*
Lactobacillus johnsonii	*Lactobacillus johnsonii*
Lactobacillus kefiranofaciens	*Lactobacillus kefiranofaciens*
Lactobacillus kefiri	*Lentilactobacillus kefiri*
Lactobacillus mucosae	*Limosilactobacillus mucosae*
Lactobacillus panis	*Limosilactobacillus panis*
Lactobacillus paracasei	*Lacticaseibacillus paracasei*
Lactobacillus paraplantarum	*Lactiplantibacillus paraplantarum*
Lactobacillus pentosus	*Lactiplantibacillus pentosus*
Lactobacillus plantarum	*Lactiplantibacillus plantarum*
Lactobacillus pontis	*Limosilactobacillus pontis*
Lactobacillus reuteri	*Limosilactobacillus reuteri*
Lactobacillus rhamnosus	*Lacticaseibacillus rhamnosus*
Lactobacillus sakei	*Latilactobacillus sakei*
Lactobacillus salivarius	*Ligilactobacillus salivarius*
Lactobacillus sanfranciscensis	*Fructilactobacillus sanfranciscensis*

In reference #7 (Zheng et al. 2020), the taxonomic change is discussed on page 2814. Please see a screenshot of the text from this page in the publication below.

DESCRIPTION OF *LACTICASEIBACILLUS RHAMNOSUS* COMB. NOV.

Lacticaseibacillus rhamnosus (rham.no'sus. N.L. masc. adj. *rhamnosus* pertaining to rhamnose).

Basonym *Lactobacillus rhamnosus* (Hansen 1968, Collins *et al.* 1989, 108[VP] (*Lactobacillus casei* subsp. *rhamnosus* Hansen 1968 [172],

Original characteristics of *L. rhamnosus* strains are described in by [172]. The genome size of the type strain is 2.95 Mbp. The mol% G+C content of DNA is 46.7.

The species has a nomadic lifestyle and was isolated from a broad range of habitats including dairy products, fermented meat, fish, vegetables and cereals, sewage, humans (oral, vaginal and intestinal), invertebrate hosts and clinical sources [17, 169].

The type strain is ATCC 7469[T]=CCUG 21452[T]=CIP A157[T]=DSM 20021[T]=NBRC 3425[T]=JCM 1136[T]=LMG 6400[T]=NCAIM B.01147=NCCB 46033[T]=NCIMB 6375[T]=NCTC 12953[T] = NRRL B-442[T]=VKM B-574[T].

Genome sequence accession number: AZCQ00000000.

16S rRNA gene accession number: D16552.

b. Has the strain been deposited? If yes, please provide the depository of the strain.
    - Response: Yes, the strain has been deposited with the American Type Culture Collection with deposit number BAA-2836.

6. In Section 2.3, you stated that the preculture is prepared by inoculating the frozen samples of the preserved strain.
    a. Please provide a statement that the frozen sample is pure culture that has been verified by selective plating, biochemical or serological testing.
        - Response: The frozen sample is pure culture that has been verified by Next-generation sequencing analysis (Metagenome).

    b. Do you continuously monitor fermentation process for contaminants? If so, please provide a statement for that.
        - Response: Yes, the fermentation process is monitored per every lot and every inoculation process, for contaminants including culture condition, culture temperature, pH, type of bacteria and presence of contaminants by culture medium sampling.

7. You listed the methods and batch analysis results in the Tables 1 and 2. Most of the testing methods are based on KFSC (Korean Food Standards Codex) or KHFSC (Korean Health functional Food Standards Codex).
    - Please provide a statement that the KFSC and KHFSC methods are validated against a standardized method such as ISO, AOAC or FDA BAM methods for its intended use.

- Response: In Subpart 2.4.1 (page 12 of 47) of the submitted GRAS notice (GRN 1093), we included a statement that the methods cited in Table 1 had been validated for their stated purposes.

  In response to the current query, the notifier confirms that the KFSC and KHFSC methods are recognized by the Ministry of Food and Drug Safety, Republic of Korea, and are comparable to the corresponding internationally recognized AOAC, ISO, and USP methods as shown in the amended specifications table below:

Table 1. *Lacticaseibacillus rhamnosus* IDCC 3201 Product Specifications

Tested Parameters	New Limits/Specifications	Method	Corresponding Internationally Recognized Methods
Appearance	White to light yellow powder	KFSC 8/1/1.1	
Identification	*Lacticaseibacillus rhamnosus*	16S rRNA Sequencing	
Cell count	≥ 4.5 x 10^{11} CFU/g	KHFSC 4/3-58	USP<64>
Particle size	95% Pass > 50 mesh	Ph. Eur. (Sieves method)	
Water activity (Aw)	< 0.15	In-house Specifications IBS-SOP-QC-060	
Microbiological Tests			
Coliforms	Negative/10g	KHFSC 8/4/4.7/4.7.1	ISO 4831
Escherichia coli	Negative/10g	KFSC 8/4/4.8/4.8.2	AOAC 991.14
Yeast & Molds	< 10 CFU/g	KFSC 8/4/4.10	AOAC 2002.11
Salmonella	Negative/10g	KFSC 8/4/4.11	AOAC 989.14
Staphylococcus aureus	Negative/g	AOAC 2003.07	
Heavy Metals*			
Lead	< 0.5 mg/kg	KFSC 8/9/9.1/9.1.2	AOAC 2013.06
Cadmium	< 0.3 mg/kg	KFSC 8/9/9.1/9.1.3	AOAC 2013.06
Mercury	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.6	AOAC 2013.06
Arsenic	< 0.3 mg/kg	KFSC 8/9/9.1/9.1.4	AOAC 2013.06

Abbreviations: AOAC, Association of Official Analytical Collaboration; CFU, colony forming units; ISO, International Organization for Standardization; KFSC: Korean Food Standards Codex. KHFSC, Korean Health functional Food Standards Codex; Ph. Eur., European Pharmacopoeia; USP, United States Pharmacopeia.
*Heavy metal specifications are set according to Korean Food Code per ILDONG.

8. In Section 2.9, you evaluated biogenic amine formation using HPLC (high performance liquid chromatography) analysis.
   - Please briefly describe the HPLC method. Is it an internal protocol? Has the method been validated for its intended use?
     - The method is an internal protocol based on EFSA guidelines, using the reference method specified in the European Commission Regulation (EC) No 2073/2005. In this method, the biogenic amine

and samples were derivatized by dansyl chloride, and then analyzed using HPLC (C18 column, UVD). Further, the method has been validated for its intended use per Mao et al. (2009).[1]

9. Adverse event complaints reported that include allergy to products containing *L. rhamnosus* IDCC 3201 are discussed on pg. 37 of the notice. You concluded that *L. rhamnosus* IDCC 3201 is produced using milk and that "it is possible that the allergic reactions were due to exposure to this known allergen, exposure to one of the excipients, or could be completely unrelated to consumption of the products." Thus, this conclusion suggests that you cannot rule out that *L. rhamnosus* IDCC 3201 itself carries allergenic potential. Considering this is a noted novel strain that lacks published toxicological studies, please discuss why risk from allergenicity from your intended use is minimal. If an allergenicity assessment (e.g., *in silico* sequence alignment) has been performed on IDCC 3201, or other strains of *L. rhamnosus*, please discuss any relevant findings to support your GRAS conclusion.
    - Response: There are a number of reasons that we believe risk of allergenicity from the intended use of *L. rhamnosus* IDCC 3201 is minimal, including the following:
    - Adverse event reporting supplied by ILDONG over a period of six years showed approximately 3% of the 134 complaints were potential allergic reactions. The highest amount of *L. rhamnosus* IDCC 3201 in a product serving for which a complaint received was 150 mg. Thus, the notifier has sold approximately 884 million 150 mg servings over 6 years and received 134 complaints. If each complaint is associated with one serving, the total complaints account for 0.000015% of sales or one complaint per 6.6 million users, thus, complaints of any nature can be considered a rare occurrence. If considering only the 3% of complains that may reasonably be considered potential allergic reactions, the rate of occurrence is reduced to 0.00000045% of sales (or one in 220 million users). Further, while these complaints were reported in association with consumption of products containing *L. rhamnosus* IDCC 3201, causation cannot be proven.[2] Self-reporting of adverse events is notoriously confounded by outside factors. Similarly, according to the FDA CAERS webpage, it is stated that "*The adverse event reports about a product and the total number of adverse event reports for that product in CAERS only reflect information AS REPORTED and do not represent any conclusion by FDA about whether the product actually caused the adverse events. For any given report, there is no certainty that a suspected product caused a reaction. Healthcare practitioners, firms, agencies, consumers, and others are encouraged to report suspected reactions; however, the event may have been related to a concurrent underlying condition or activity or to co-consumption of another product, or it may have simply occurred by chance at that time.*"
    - Further, as discussed in Subpart 6.5 on page 37 of 47, ILDONG's *L. rhamnosus* IDCC 3201 is produced with milk and is made with excipients.

These ingredients could possibly contribute to these reactions, assuming the ingredient had a causal roll in the reported adverse event. Per FALCPA and as stated below, milk is required to be declared in the labeling.

- We did not find scientific reports of allergy to *L. rhamnosus* in the public domain. As the species has a long history of use in fermented foods, the lack of this finding suggests a very low likelihood that the species is allergenic.[3, 4] *L. rhamnosus* also has QPS status (see Subpart 6.2.1, pages 23 & 24 of 47) without any known history of causing allergenicity. The IDCC 3201 strain is not genetically modified, and thus no genes coding for potentially allergenic proteins have been inserted.
- Genetic drift is highly unlikely to lead to the presence of a novel allergenic component or other toxicologically relevant characteristics. [5-7]
- Finally, as an aside to this question, it is important to note that as of very recently, ILDONG soy components are no longer used in the manufacturing process (when the notice was submitted, soy was still being used and thus it is listed in the notice). Therefore, in addition to our response above, we also amend the first paragraph of Subpart 6.4 on page 35 of 47 to read as follows: "The U.S. Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 lists nine major allergens, the presence of which would result in a requirement for allergy labeling on food products, as follows: milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, sesame, and soybeans. *L. rhamnosus* IDCC 3201 is grown in culture medium that contains milk-derived components. Thus, food products that contain this strain are required to declare milk in their labeling. *L. rhamnosus* IDCC 3201 does not contain other major allergens listed in FALCPA and does not contain gluten, celery, mustard, sulfur dioxide and sulfites, lupin, or mollusks.

10. It is unclear if safety data discussed in the GRAS notice were collected as part of a comprehensive literature search and that no additional safety concerns were found. If a comprehensive literature search was performed, please provide the details of these search(es), including date (month and year), search engine(s) used, and search terms. If this was not done, please provide a comprehensive updated literature search, and discuss whether any publications were found that may be considered contradictory to a GRAS conclusion.
    - Response: A comprehensive literature search was performed related to the safety of the ingredient. Literature searches for the safety assessment described in Part 6 were conducted through January 4, 2022. The search engines utilized included PubMed and Medline. The search terms included "*Lactobacillus rhamnosus*", "*L. rhamnosus*", and "*Lacticaseibacillus rhamnosus*".

11. In Section 6.1.1, you wrote that "In recent times, consumption of lactic acid live organisms (often referred to as 'probiotics') has become popular as a way to support human health and wellness.40".
    - Reference #40 was a review article published back in 2006. The authors reviewed the *Lactobacillus* genus, its long history of use, its biotope and biodiversity (focusing specifically on its "probiotic" use), the associated biological hazard and the European regulatory framework (focusing on the guidelines for safety assessment). In general, submissions should not include discussion of purported benefits or language implying dietary supplement uses (e.g., "probiotic", dose, capsule, sachet, efficacy as an endpoint, health benefit, etc.). We recommend that GRAS notices focus on the substance's identity, intended use and safety.
        - Response: Thank you for this important reminder. The above query #11 appears to read as an FYI without a requested action. However, we would be happy to amend the GRN to remove the quoted sentence if so requested.

12. For supporting your safety conclusion, you listed four GRAS notices related to *L. rhamnosus* strains. Please note that GRN 845 was withdrawn and GRN 1013 is pending. For each of the other two successful GRAS notices, please provide a brief paragraph summarizing the information pertaining to safety.
    - A GRAS notice to FDA (GRN 281) for *L. rhamnosus* strain HN001 received a no questions letter from FDA for use as an ingredient in milk-based powdered term infant formula for term infants at a level of 10^8 CFU/g of formula powder. In FDA's no questions letter dated on August 31, 2009, they summarized the GRN safety narrative for the ingredient, including the following: The notifier "discusses data from published and unpublished studies that include in vitro testing methods, genetic sequencing, animal models, and studies in human subjects (adults and infants) using the bacterium *L. rhamnosus* strain HN001. The notifier states that the body of evidence confirms the safety of *L. rhamnosus* strain HN001."
    - A GRAS notice to FDA (GRN 288) for *L. rhamnosus* strain HN001 received a no questions letter from FDA for use as an ingredient in various foods at a level of up to 10^9 CFU/serving. Conventional foods include certain beverages and beverage juices, nectars, ades, and drinks; confections; chewing gum, and hard candies. The maximum intake is expected to be less than 10^{11} CFU/day. In FDA's no questions letter dated on November 1, 2009, they summarized the GRN safety narrative for the ingredient, including the following: The notifier "discusses data from published and unpublished studies that include in vitro testing methods, genetic sequencing, animal models, and studies in human subjects (adults and infants) using the bacterium *L. rhamnosus* strain HN001."

- It should be noted that GRN 1013 is no longer pending and received a no questions letter, and is briefly summarized. A GRAS notice to FDA (GRN 1013) for *L. rhamnosus* DSM 33156 received a no questions letter from FDA for use as an ingredient in conventional foods at a level of up to 10^{11} CFU/serving and in cow milk-, soy milk-, and partially hydroyzed protein-based, non-exempt infant formula for term infantas at a level of 10^{8} CFU/g. In FDA's no questions letter dated on December 15, 2021, they summarized the GRN safety narrative for the ingredient, including the following: The notifier "discusses data and information that support the safety of *L. rhamnosus* DSM 33156, including a history of safe use of *L. rhamnosus* DSM 33156 in dairy products and infant formulas in European markets. The notifier incorporates into the notice summaries of surveillance studies from GRN 000231 showing that no increases in *Lactobacillus* bacteremia were evident with increased *L. rhamnosus* DSM 33156 consumption. The notifier also discusses newly published reports of adverse events associated with consuming *L. rhamnosus* DSM 33156 and incorporates into the notice previous adverse case reports from GRN 000231. The notifier concludes that adverse events were rare and occurred only in subjects with an underlying disease or health condition. The notifier also states that *L. rhamnosus* DSM 33156 is recognized by the European Food Safety Authority with a Qualified Presumption of Safety."

References

1. Mao H-m, Chen B-g, et al. Simultaneous determination of twelve biogenic amines in serum by high performance liquid chromatography. *Microchemical Journal*. 2009;91(2):176-180
2. FDA. Questions and Answers on FDA's Adverse Event Reporting System (FAERS). [2018; Retrieved March 29, 2023] https://www.fda.gov/drugs/surveillance/questions-and-answers-fdas-adverse-event-reporting-system-faers.
3. Bernardeau M, Guguen M, et al. Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiol Rev*. 2006;30(4):487-513
4. Rezac S, Kok CR, et al. Fermented Foods as a Dietary Source of Live Organisms. *Front Microbiol*. 2018;9:1785
5. Pariza MW, Gillies KO, et al. Determining the safety of microbial cultures for consumption by humans and animals. *Regul Toxicol Pharmacol*. 2015;73(1):164-71
6. Stevens H and Nabors L. Microbial food cultures: a regulatory update. *Food Tech*. 2009;63(3):36-41
7. Sanders ME, Klaenhammer TR, et al. Effects of genetic, processing, or product formulation changes on efficacy and safety of probiotics. *Ann N Y Acad Sci*. 2014;1309:1-18

Kaiping Deng
Staff Fellow/Regulatory Review Scientist
Regulatory Review Branch
Division of Food Ingredients
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 708-924-0622
kaiping.deng@fda.hhs.gov

April 20, 2023

Re: Responses to FDA's GRN 1093 Second Round of Questions

Dear Dr. Deng,

Please find responses to the second round of FDA's questions concerning *L. rhamnosus* 3201 (GRN 1093) below. FDA's questions are in BLACK, while the notifier responses are in BLUE:

1. In the amendment dated April 4, 2023 (response to Question 2), you provide the revised specification limits for lead, cadmium, and arsenic (< 0.5 mg/kg, < 0.3 mg/kg, and <0.3 mg/kg, respectively). We note that the provided results of the analyses of three batches for lead, cadmium, and arsenic are significantly lower (at least 30 to 50 times) than the revised specification limits. In line with FDA's "Closer to Zero" initiative, we recommend that you consider further lowering the specification limits for lead, calcium, and arsenic to better reflect the results of the batch analyses and to be as low as possible.
    - Response: The notifier has further amended their specification limits for cadmium, mercury, and arsenic, as shown in the table below, to better reflect the results of the batch analyses. The initial specifications of the heavy metals were in set in accordance with the Korean Health Functional Food Codex standards. The limits have been revised based on the upper end of the results range observed on analysis of historical batch analysis data of the commercial product.

Heavy Metal Specification Limits			
	Acceptance Criteria		
	Revised 4/17/2023	Revised 4/4/2023	Original
Lead	< 0.3 mg/kg	< 0.5 mg/kg	< 1.0 mg/kg
Cadmium	< 0.2 mg/kg	< 0.3 mg/kg	< 0.3 mg/kg
Mercury	< 0.1 mg/kg	< 0.1 mg/kg	< 0.1 mg/kg
Arsenic	< 0.2 mg/kg	< 0.3 mg/kg	< 0.5 mg/kg

2. In the amendment dated April 4, 2023 (response to Question 4), you provide the updated dietary exposure estimate of 1.02×10^{13} CFU/day based on the maximum use level and the consumption of 20 servings of food/day. We note that the provided dietary exposure estimate value is incorrect and assuming a maximum use level of 1.02×10^{11} CFU/serving of food and consumption of 20 servings/day, this would result in a dietary exposure of 2.04×10^{12} CFU/day. Please provide the correct dietary exposure estimate.
    - Response: We confirm that we made an error in the mathematical calculation of the previous amendment resulting in an incorrect maximum exposure estimate. We are grateful that FDA recognized the error and pointed it out, and we further confirm the FDA's result is correct. We further amend Part 3 of the notice to state the following.
        i. Utilizing FDA's recommendations of consumption of an average number of 20 serving/day of food for men and women and assuming the ingredient will be present at the maximum addition level of 1.02×10^{11} CFU *L. rhamnosus* IDCC 3201 per serving, the maximum estimated dietary exposure from the intended use of *L. rhamnosus* IDCC 3201 is 2.04×10^{12} CFU/day. Using 70 kg as an average body weight, this exposure is equivalent to 2.91×10^{10} CFU/kg bw/day. This is estimate is highly conservative as it assumes the ingredient will be present at the maximum addition level in all foods.
        ii. In addition to the above amendment, to be thorough, we further note that the calculations of our previous April 4, 2023 amendment were correct as given for the more realistic (yet still highly conservative) exposure estimate for an ingredient intended for use in all food categories except infant formula and products under the jurisdiction of USDA for which FDA presumes that one half of all food (10 out of 20 servings) will contain the ingredient.
3. In the amendment dated April 4, 2023 (response to Question 10), you state that literature search was conducted through January 4, 2022. It should be noted that a relevant article was published since then: https://pubmed.ncbi.nlm.nih.gov/35458216/. Zafar et al., 2022, demonstrate that *Lacticaseibacllus rhamnosus* fed to male rats at a concentration of 2×10^{8} CFU/d can "improve blood lipid profiles as effectively as statins", suggesting exposure at this level has clinical implications on blood cholesterol levels.

    Considering a significant percentage of the U.S. adult population use cholesterol-lowering medications, the vast majority of which are statins, you would need to address issues on any adverse effects that may result from consumption of *L. rhamnosus* at its intended use level on blood cholesterol levels, such as physiologically-low cholesterol or hypocholesterolemia, including in individuals taking statins.

- Response: Zafar et al. (2022) conducted a 28-day study in rats of eight bacterial strains, including *L. rhamnosus* FM9 and *L. rhamnosus* Y59 to evaluate their effects on serum lipids.[1] Fifty-five male Wistar rats were divided into 11 groups (5 rats/group) and were administered a negative control standard diet, a positive control high-fat hypercholesterolemic diet (HFCD), the HFCD plus 10 mg/kg bw/day atorvastatin (comparator group), or the the HFCD plus each of the eight test articles at a concentration of 2 x 10^8 CFU/mL in distilled water.
- There are many limitations to the in vitro study by Zafar et al. 2022; however, it should first be noted that beneficial effects of bacterial ingredients should be evaluated at the strain, not the species, level. For example, Reis et al (2017), in their review of the hypercholesteremic effect of bacteria and yeasts, notes that the effects produced are not always reproduced in humans, conflicting across studies, the results are modest (relative to statins), and importantly, are strain specific.[2] As such, it is fundamentally uncertain how, or whether, the *L. rhamnosus* FM9 and *L. rhamnosus* Y59 results of Zafar et al. are translatable to *L. rhamnosus* IDCC 3201.
- Limitations of the Zafar study include:
    - Translatability of the observed results in a diet induced hypercholesterolemic rat model to humans (clinical trials are necessary), as noted by the authors.
    - As documented in hypercholesterolemia treatment standards of care, exogenous cholesterol biosynthesis, is an important consideration in human hypercholesterolemia, perhaps more so than dietary cholesterol intake. Zafar et al. studied only a dietary induction in rats; thus, it is unknown whether their results would be similar with respect to exogenous hypercholesterolemia.
    - Further, the authors hypothesized that the mechanism of action for hypocholesterolemic effects of *L. rhamnosus* FM9 and *L. rhamnosus* Y59 is, at least in part, due to the "bacteria's ability to assimilate cholesterol molecules in the small intestine," which suggests that at least part of the effect is limited to dietary cholesterol consumption and, thus, would be attenuated for exogenous cholesterol biosynthesis. Additionally, bacterial colonization of the gastrointestinal track in humans is predominantly colonic, suggesting that any potential effects in humans via this mechanism would be attenuated.
    - The small group 'n' and testing only in male rats.
    - Statistical analyses were carried out only for intragroup comparisons. Thus, it is unknown whether the groups of rats were comparable at baseline or whether, or which, intergroup comparisons may have been statistically significant following treatment compared to the negative control or comparator groups. At least visually, total, HDL, and LDL cholesterol were higher in the negative control and comparator (i.e.,

statin) groups than in the positive control group and *L. rhamnosus* FM9 and *L. rhamnosus* Y59 groups while the comparator group LDL-C was higher than the negative control at baseline. Further the intragroup comparison for LDL was not statistically significant for *L. rhamnosus* Y59. As such, the between group conclusions (including the statement, "improve blood lipid profiles as effectively as statins" quoted by FDA above) drawn by the authors are not scientifically valid.

- Finally, FDA asked us to address "any adverse effects that may result from consumption of *L. rhamnosus* at its intended use level on blood cholesterol levels, such as physiologically-low cholesterol or hypocholesterolemia, including in individuals taking statins." As shown in Figure 4 of Zafar et al., there were no statistically significant intragroup differences in total cholesterol in treated animals after 28 days compared to baseline. This demonstrates, regardless of intragroup lipoprotein effects, that there were no physiologically-low cholesterolemic effects or hypocholesterolemia that resulted from any of the treatments. Also, as such effects are well known, and expected, with statins, especially given that the reported administered dose (10 mg/kg bw/day) of atorvastatin is above the maximum recommended human equivalent dose. This calls into question whether the applied statin dose was effectively administered/absorbed to result in a clinically relevant effect. This could also explain why intragroup differences in LDL-C over baseline were lesser in relative magnitude than the bacterial intragroup differences.
- Additionally, there were no study groups treated with both bacteria and the statin; therefore, it is unknown whether a potential for any additive (or subtractive) effects exists for any of the lipid parameters examined.
- Furthermore, as standards of care for treatment of hypercholesterolemia recommend, in most situations, that dietary intervention, precede treatment with statins (or other cholesterol-lower medications), should the diet include an *L. rhamnosus* strain, a statin prescription should ensue only after such dietary intervention failed reducing the likelihood of any potential overtreatment with statin initiation. Additionally, all patients prescribed a statin drug should be regularly monitored by the prescribing health care provider to ensure both adequate therapeutic effect and absence of overtreatment.
- It is important to also note that *Lactobacillus* species are prominent bacteria found in the digestive tract and have been consumed around the world as part of fermented foods since the earliest records of food preservations. Furthermore, a search for clinical studies evaluating the safety of statin drugs taken in conjunction with *L. rhamnosus* was conducted and no publications were found, indicating that it is not considered a safety concern. Further, studies by Zhang et al. (2021), Bhat et al. (2019) and Zhou et al. (2000),

discussed in Subpart 6.3.1, Table 5 (pages 26–27), did not show a significant change in cholesterol levels in rodents that consumed *L. rhamnosus* via gavage.[3-5]

- Importantly, there is no evidence that the consumption *L. rhamnosus* IDCC 3201 at its intended use level will have any such effect on blood cholesterol levels of humans on statins or otherwise.

References

1. Zafar H, Ain NU, et al. Lacticaseibacillus rhamnosus FM9 and Limosilactobacillus fermentum Y57 Are as Effective as Statins at Improving Blood Lipid Profile in High Cholesterol, High-Fat Diet Model in Male Wistar Rats. *Nutrients*. 2022;14(8)
2. Reis SA, Conceicao LL, et al. Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotics. *Nutr Res Rev*. 2017;30(1):36-49
3. Zhang B, Lynch B, et al. Lactobacillus rhamnosus MP108: Toxicological evaluation. *J Food Sci*. 2021;86(1):228-241
4. Bhat MI, Singh VK, et al. Adherence capability and safety assessment of an indigenous probiotic strain Lactobacillus rhamnosus MTCC-5897. *Microb Pathog*. 2019;130:120-130
5. Zhou JS, Shu Q, et al. Safety assessment of potential probiotic lactic acid bacterial strains Lactobacillus rhamnosus HN001, Lb. acidophilus HN017, and Bifidobacterium lactis HN019 in BALB/c mice. *Int J Food Microbiol*. 2000;56(1):87-96

Kaiping Deng
Staff Fellow/Regulatory Review Scientist
Regulatory Review Branch
Division of Food Ingredients
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 708-924-0622
kaiping.deng@fda.hhs.gov

May 1, 2023

Re: Amendment #3 to GRAS Notice Nos. GRN 1092 and GRN

Dear Dr. Deng,

We thank you for the video conference with the GRAS evaluation teams for GRN 1092 and GRN 1093 on April 26, 2023. During the conference, the GRAS team noted concerns with respect to specifications and batch analyses for heavy metal impurities in the notifier's ingredients, *Bifidobacterium lactis* IDCC 4301 (GRN 1092) and *Lacticaseibacillus rhamnosus* IDCC 3201 (GRN 1093) as follows:

FDA Query:
With respect to the statement, "The limits have been revised based on the upper end of the results range observed on analysis of historical batch analysis data of the commercial product" in amendment #2 of GRNs 1092 and 1093, FDA asked why, if some batch data, may be as high as the previously provided limits, is there such a large degree of batch-to-batch variation, given the low result levels of the CoAs provided for review with GRNs 1092 and 1093?

Notifier Response:
Heavy metal limits for *B. lactis* 4301 (GRN 1092) and *L. rhamnosus* IDCC 3201 (GRN 1093) were originally set according to the heavy metal standards as given in the Korean Health Functional Food Codex by the Ministry of Food and Drug Safety.

Heavy metal specification limits were amended to lower levels on April 20, 2023 (amendment #2 to GRN 1092 and GRN 1093). At this time, limits were set to provide a wide margin above batch results following a review of historical batch analysis data, which included very old data.

As noted in Subpart 2.2.1 of GRN 1092 (page 9) and Subpart 2.3.1 of GRN 1093 (page 10), *B. lactis* 4301 and *L. rhamnosus* IDCC 3201, respectively, are manufactured under strict adherence to GMP standards (which are independently certified) in an FDA

registered facility. As part of the notifier's commitment to quality, the manufacturing facilities, equipment, and laboratory analytical instruments have been continuously improved over the years and the ingredients are produced with stricter quality control levels under the current manufacturing processes relative to initial manufacturing processes.

As such, to further lower the heavy metal specification limits for the safety of U.S. consumers, the notifier has conducted additional statistical sampling of batches produced using the current manufacturing processes and determined that it is not necessary to maintain previous specification limits. Therefore, the lower limits as shown in the response below are justified.

FDA Query:
FDA noted that the magnitude of provided batch analyses heavy metal results for each ingredient below the specification limits (which are the same for both ingredients) of amendment 2 are large (at least 30 to 50 times lower) with respect to FDA's "Closer to Zero" initiative. FDA believes that a difference of ≤10-fold is a reasonable goal for ingredient manufacturers to target. Further, FDA noted they would be satisfied if specification limits for lead, cadmium, mercury, and arsenic were set to not more than 0.1 mg/kg (ppm) for each of the ingredients *B. lactis* IDCC 4301 and *L. rhamnosus* IDCC 3201.

Notifier Response:
Based on statistical sampling of batches produced under the current manufacturing processes (as described and shown in GRN 1092 and GRN 1093), we further amend the product specification for heavy metal limits as follows:

Table 1. Heavy metal specifications (amended April 27, 2023)

Tested Parameters	Limits	Method	Corresponding Internationally Recognized Methods
Lead	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.2	AOAC 2013.06
Cadmium	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.3	AOAC 2013.06
Mercury	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.6	AOAC 2013.06
Arsenic	< 0.1 mg/kg	KFSC 8/9/9.1/9.1.4	AOAC 2013.06

Abbreviations: AOAC, Association of Official Analytical Collaboration; KFSC: Korean Food Standards Codex. KHFSC