Notice to US Food and Drug Administration of the Conclusion that the Intended Use of *Bifidobacterium lactis* IDCC 4301 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

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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 *Bifidobacterium lactis* IDCC 4301 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")

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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 *Bifidobacterium lactis* IDCC 4301 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
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Agent of the Notifier

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1.3 Name of the Substance

The name of the substance is *Bifidobacterium lactis* IDCC 4301.

1.4 Intended Conditions of Use

B. lactis IDCC 4301 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 USD A 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 GRAS status of *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of *B. lactis* IDCC 4301 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 GRAS conclusion 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 FD Aupon 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 GRAS notice 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 *B. lactis* IDCC 4301.

[redacted] May 10, 2022

Donghoon Oh — Date
Manager
Notifier

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

2.1 Taxonomy of *Bifidobacterium lactis* IDCC 4301

ILDONG's *B. lactis* IDCC 4301 was isolated from breast-fed infant feces and has been identified according to standard taxonomic guidelines. It is important to note that *Bifidobacteria* are typically included in the lactic acid bacteria (LAB) category due to their shared properties, but they are not phylogenetically related to the other LAB such as *Enterococcus*, *Lactobacillus*, and *Lactococcus*.[1] Therefore, *B. lactis* IDCC 4301 is referred to as a LAB throughout this report. It has been unequivocally identified genetically based on 16S rRNA sequences with confirmed 99% sequence homology to its type strain, *B. lactis* YIT 4121.

The taxonomic lineage of *Bifidobacterium* is:

Kingdom: *Bacteria*

Phylum: *Actinobacteria*

Class: *Actinobacteria*

Order: *Bifidobacteriales*

Family: *Bifidobacteriaceae*

Genus: *Bifidobacterium*

Species: *Bifidobacterium animalis* subspecies *lactis*

Strain: *Bifidobacterium animalis* subspecies *lactis* IDCC 4301

2.2 Manufacturing

2.2.1 Good Manufacturing Practice

ILDONG's *B. lactis* IDCC 4301 is manufactured in Korea at an FD Aregistered 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 22000 (FSSC 22000)
- Bureau Veritas Certification, Food Safety Management Systems (ISO 22000)

2.2.2 Raw Materials

ILDONG confirms that the raw materials used in the production of *B. lactis* IDCC 4301 are of appropriate food grade and are not genetically modified.

2.2.3 Manufacturing Narrative and Flowchart

The manufacturing flowchart shown in Figure 1 below applies to the production process for *B. lactis* IDCC 4301. 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 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.

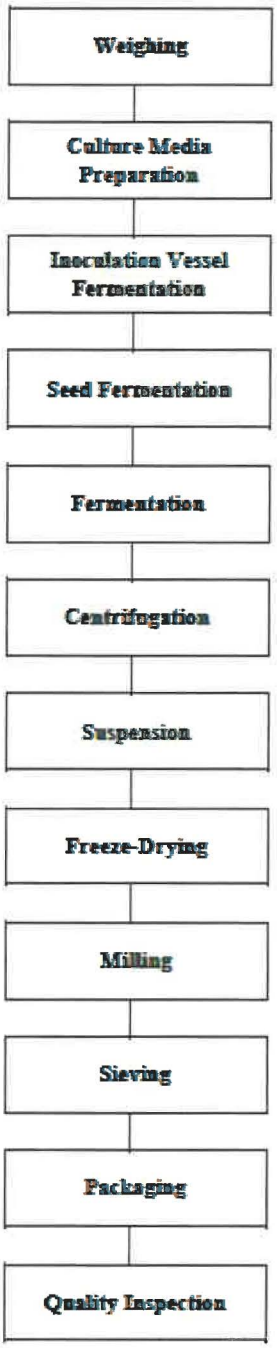

Figure 1. *Bifidobacterium lactis* IDCC 4301 Manufacturing Flowchart

2.3 Specifications

The specifications for the food-grade product *B. lactis* IDCC 4301, along with the specification methods, which have been validated for their stated purpose, are listed in Table 1 below.

Table 1. *Bifidobacterium lactis* IDCC 4301 Product Specifications

Tested Parameters	Limits/Specifications	Method
Appearance	White to light yellow powder	KFSC 8/1/1.1
Identification	*Bifidobacterium animalis* subsp. *lactis*	16S rRNA Sequencing
Cell count	$\geq 2.5 \times 10^{11}$ 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	KFSC 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 Batch Analysis

Production conformity and consistency of ILDONG's *B. lactis* IDCC 4301 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 marker compounds, microbial analyses, and heavy metals (see Table 2).

Table 2. *Bifidobacterium lactis* IDCC 4301 Batch Analyses

Tested Parameters	Specification	Lot #/Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0601 06/2019	Lot# IDK0901 09/2019
Appearance	White to light yellow powder	Conforms	Conforms	Conforms
Identification	*Bifidobacterium animalis* subsp. *lactis*	Conforms	Conforms	Conforms
Cell count	$\geq 2.5 \times 10^{11}$ CFU/g	2.5×10^{11} CFU/g	2.9×10^{11} CFU/g	2.84×10^{11} CFU/g
Particle size	95% Pass > 50 mesh	Conforms	Conforms	Conforms
Water activity (Aw)	< 0.15	0.0788	0.0643	0.0677

Tested Parameters	Specification	Lot #/Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0601 06/2019	Lot# IDK0901 09/2019
Microbiological Tests				
Coliforms	Negative/1 0g	Negative/1 0 g	Negative/1 0 g	Negative/1 0 g
Escherichia coli	Negative/1 0g	Negative/1 0 g	Negative/1 0 g	Negative/1 0 g
Yeast & Molds	<10 CFU/g	Conforms	Conforms	Conforms
Salmonella	Negative/1 0g	Negative/1 0 g	Negative/1 0 g	Negative/1 0 g
Staphylococcus aureus	Negative/g	Negative/g	Negative/g	Negative/g
Heavy Metals				
Lead[a]	< 1.0 mg/kg	0.01 mg/kg	0.01 mg/kg	0.00 mg/kg
Cadmium[b]	< 0.3 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Mercury[c]	< 0.1 mg/kg	0.02 mg/kg	0.00 mg/kg	0.00 mg/kg
Arsenic[d]	< 0.5 mg/kg	000 mg/kg	000 mg/kg	001 mg/kg

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 µg/kg
[d] Limit of Detection = 0.7 µg/kg

2.5 Stability Study

A real time stability test was performed on ILDONG's *B. lactis* IDCC 4301 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 twenty-four 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 *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301.

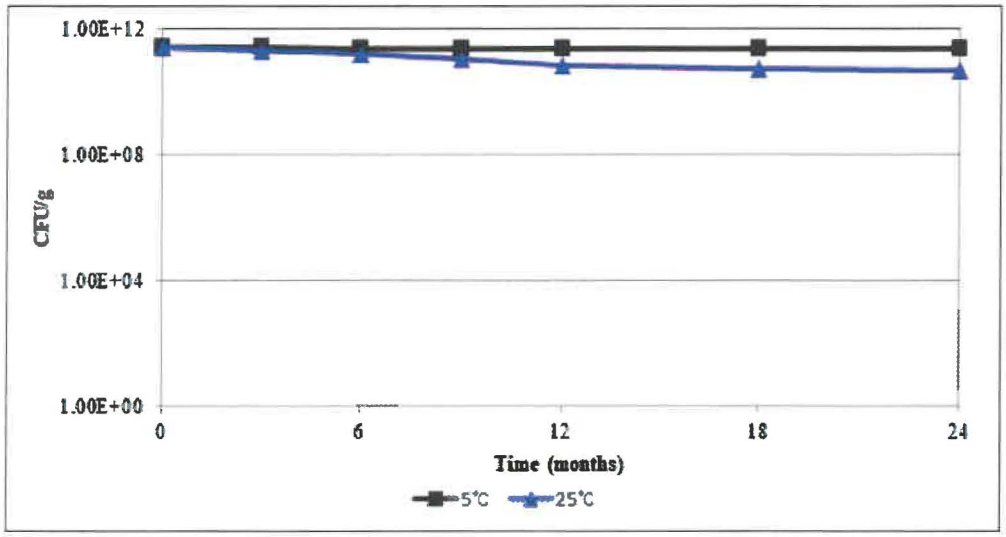

Figure 2. *Bifidobacterium lactis* IDCC 4301 Real-Time Stability Study

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.[2, 3] 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.[4] It is generally recommended that resistance to antibiotics be assessed in all probiotic strains prior to marketing.[2, 5-9]

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.[2, 10] 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 *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 is sensitive to all antibiotics included in EFSA's guidelines for the *B. lactis* species except for vancomycin. The genetic nature of the antibiotic resistance in these strains was evaluated and one antibiotic resistance gene was found.

Table 3. *Bifidobacterium lactis* IDCC 4301 Assessment of Antimicrobial Susceptibility

Antimicrobial Agent	Phenotypic MIC (mg/L)			*B. lactis* IDCC 4301 Genetic Resistance
	B. lactis IDCC 4301 (Observed)	*B. lactis* (EFSA Breakpoints)[10]	Assessment for *B. lactis* IDCC 4301	
Ampicillin	< 0.125	2	Sensitive	No
Gentamicin	16–32	64	Sensitive	No
Streptomycin	64–128	128	Sensitive	No
Kanamycin	64–256	n.r	n.r	No
Clindamycin	0.125–0.25	1	Sensitive	No
Chloramphenicol	1–2	4	Sensitive	No
Vancomycin	> 512	2	Resistant	No
Erythromycin	0.125–0.5	1	Sensitive	No

Antimicrobial Agent	Phenotypic MIC (mg/L)			*B. lactis* IDCC 4301 Genetic Resistance
	B. lactis IDCC 4301 (Observed)	*B. lactis* (EFSA Breakpoints)[10]	Assessment for *B. lactis* IDCC 4301	
Tetracycline	8	8	Sensitive	Yes

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.[10]

As summarized in Table 3 above, *Bifidobacterium lactis* IDCC 4301 was susceptible to all of the antibiotics recommended for testing by EFSA, with MIC values at or below the EFSA breakpoints, except for vancomycin. Several authors including Kheadr et al. (2007) and Charteris et al. (1998) have reported that *Bifidobacteria* are generally resistant to vancomycin.[11,12] Kheadr et al. (1998) found that *Bifidobacteria*'s resistance to vancomycin has been increasing.[11] While other authors have found that *Bifidobacteria* are sensitive to vancomycin, Charteris et al., (2008) attributed the different findings as being possibly related to "differing assay methodologies as vancomycin is reported to diffuse poorly in agar media".[13] While IDCC 4301 was found to have phenotypic antibiotic resistance to vancomycin, it was not found to contain antibiotic resistance genes in the genomic sequence that are known to be relevant to this antibiotic. The absence of genetic resistance in the genome of this strain implies that its resistance is likely to be intrinsic and not likely to be horizontally transferrable to other bacteria. 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."[14]

One antimicrobial resistance gene, *tet(W)*, was found on the chromosome of IDCC 4301, and is known to convey resistance to tetracycline. As the strain did not show phenotypic resistance to tetracycline over the EFSA breakpoint, resistance due to this gene is not considered of concern. However, discussion about the *tet(W)* gene is still provided below in order to be thorough.

Bifidobacterium spp. show a high prevalence and wide distribution of resistance to tetracylines.[15, 16] For example, Aires et al. (2007) found that 33% of *Bifidobacteria* showed tetracycline resistance.[15] In fact, generally all strains of *B. animalis* subsp. *lactis* described to date show medium level resistance to tetracycline.[17] Proteins that protect the ribosome from the action of tetracyclines, encoded by the family of *tet* genes, are commonly found in this genus, and the *tet*(W) gene is especially ubiquitous. Aires et al. (2007) found that 83% of the tetracycline-resistant isolates carried the *tet*(W) gene.[15] Additionally, there is precedent for this gene to be present in FDA GRAS strains of bacteria (e.g. strains *B. lactis* BB12, *B. lactis* UABIa-12, *B. lactis* R0421, *B. lactis* Bf-6, *B. lactis* BI-04, HN019, and B420, in GRNs 952, 872, 856, 855, 445, 377, and 049).

The primary concern of acquired resistance is not the acquisition of a gene or mutation that provides resistance, but rather the ability of that resistance to be horizontally transferred to commensal and/or pathogenic species. To date, there has not been any evidence demonstrating that the *tet*(W) gene has the ability to transfer resistance, and therefore poses no known risk of transfer. Wang et al. (2017) states that *Bifidobacteria* rarely harbor plasmids.[18, 19] Conjugation experiments were not able to produce any transfer to commensal bacteria of the chromosomal tetracycline resistance.[17]

ILDONG performed a mobile genetic element analysis of their strain, including a search for the presence of transposases, and prophage DNA segments within the *B. lactis* IDCC 4301 chromosome or, if present, plasmid/s. There were no plasmids or other replicons found and no antibiotic resistance genes were located in the prophage regions. Eight genes encoding transposases, which are generally common to *B. lactis* strains,[20] were identified, however none were closely flanking (within 10 kb) the *tet*(W) resistance gene suggesting the resistance gene will not be a "passengers" or "cargo" of the mobile genetic elements.[21, 22] As the strain did not show phenotypic resistance to tetracycline over the EFSA breakpoint, and mobile genetic elements were not identified in the strain, the possibility of gene transfer of the *tet*(W) gene to other organisms is considered low.

2.7 Genomic Analysis for Virulence and Pathogenicity

ILDONG evaluated the potential of *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 does not contain any genes that have been demonstrated to be virulent to hosts.

2.8 Hemolysis

ILDONG evaluated the hemolytic properties of *B. lactis* IDCC 4301. A *Staphylococcus aureus* strain was used as a positive control for beta hemolysis while *Lactobacillus 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 *B. lactis* IDCC 4301 showed a γ-hemolytic phenotype (no zone) on blood agar medium.

2.9 Biogenic Amine Formation

Some species and/or strains of LAB 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.[23] 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.[23] Biogenic amine formation in fermented foods has been reviewed by EFSA (2011)[24] and Spano (2010).[23] Histamine and tyramine are considered the most concerning with regard to food safety.[24]

Generally, detection of strains possessing amino acid decarboxylase deaminase activity is helpful to aid in mitigating the accumulation of these amines in food products.[23] Per assessment using HPLC (high performance liquid chromatography) analysis, *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301'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.[6] Some LAB as well as several other members of the intestinal microflora produce a mixture of L- and D-lactate.[25] 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.[26-32] 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).[28, 32-35]

B. lactis IDCC 4301 produces predominantly L-lactate (82.87%)and produces a less significant amount of D-Lactate (17.13% .) The results aid in the characterization of this strain.

2.11 Physical or Technical Effect

B. lactis IDCC 4301 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 *B. lactis* IDCC 4301, manufactured in accordance with GMP, is intended to be used as ingredients 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 *B. lactis* IDCC 4301 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.[36] 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.[37] 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 *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301.

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 *B. lactis* IDCC 4301 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 *B. lactis* is discussed in Section 6.

Part 6: Narrative

6.1 History of Consumption

6.1.1 *Bifidobacterium*

Bacteria of the genus *Bifidobacterium* are gram-positive, catalase-negative, anaerobic or microaerophylic, non-spore forming bacteria and have a high GC base pair content.[38-40] The name *Bifidobacterium* originated from the appearance of these microbes as "bifid" (branched or Y-shaped) rods, although various environmental influences can alter their shape.[39] A member of the genus was originally identified in the stool of breastfed infants in 1899 by Tissier, and others have since been isolated from a range of natural environments including the oral cavity, sewage, the insect gut, and the gastrointestinal tract of various mammals.[41] It has been classified as a distinct genus since 1973/4.[39, 42]

Bifidobacterium are among the first microbes to colonize the human gastrointestinal tract and are thought to represent 5–10% of total flora in children and adults and are found at a concentration of approximately 10^{9-11} CFU/g feces.[43] This genus is the predominant colonizer in infants until weaning, at which point *Bacteroides* and other bacterial groups surpass them in growth.[40] The presence of various *Bifidobacterial* species in the human gastrointestinal and vaginal tracts is often associated with the well-being of the host individual, and there appear to be specialized profiles of various *Bifidobacterium* species according to an individual's age.[39] Species of this genus have also been isolated from other mammals, birds, insects, food products, and sewage.[42]

The genomes of *Bifidobacterium* reflect their adaption to various regions of the human gastrointestinal tract. For example, *B. longum* found in the lower tract contain a large number of genes involved in breaking down complex dietary and host-derived carbohydrates, while *B. dentum* found in the oral cavity contain a number of genes specific to the metabolism of saliva-derived compounds.[39]

Since their discovery as dominant microbes in the feces of breast-fed infants, there have been numerous studies addressing their potential health benefits through their role in modulating gut microflora. Because of this, *Bifidobacteria* are frequently incorporated into foods as probiotic cultures.[44]

6.1.2 *Bifidobacterium lactis*

B. lactis IDCC 4301 is classified by ILDONG as *B. animalis* subsp. *lactis*.[45] This species is often utilized in the food industry worldwide (e.g., for milk fermentation) due to its good resistance to oxidative and other stressors that occur during aerobic manufacturing and storage conditions.[39] Strains of *B. lactis* have been documented as having a high survival capacity in the human gastrointestinal tract.[46] *B. lactis* is

listed in the IDF's 2018 Inventory, with food usages listed as dairy, beer, vegetable juice, and fruit juice.[47]

6.2 Regulatory Opinions

6.2.1 Europe

EFSA has 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.[48] EFSA regularly 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.[49] 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 EFSA, and published as Panel Statements. The most recent Panel Statement was adopted in June of 2021 and includes research published through March 2021.[50] As EFSA reviews safety literature pertinent to QPS units, clinical studies discussed in Subpart 6.3 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 as a qualification to the QPS status, safety is presumed at any reasonable dose, which is the case for the ILDONG taxonomic units.[51] 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.

B. lactis remains on the most recent EFSA QPS list.[50] As shown in Part 2, ILDONG has tested *B. lactis* IDCC 4301 for the qualification assessment of antimicrobial resistance according to EFSA guidelines for microorganisms used as feed additives or as production organisms, and identified no concerns in this regard.[10]

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. It was estimated in 2009 that approximately 40% of food enzymes marketed in Europe were produced by bacterial/fungal recombinant strains, and vitamins, amino acids, and polysaccharides are also obtained from recombinant strains.[52] Five GRAS notices related to *B. lactis* strains are listed in FDA's GRN inventory. Of these, four have received the no questions letter from FDA and one was ceased to be evaluated at the notifier's request. A brief summary of these FDA notifications is shown below in Table 4.

Table 4. FDA GRAS Notifications that Include *Bifidobacterium lactis* Strains

***B. lactis* Strain**	**FDA GRN**	**Status**	**Maximum Intended Use**	**Exposure Estimates by the Notifiers**
Strain AD011	GRN 952	NQ	Non-exempt infant formula for term infants—10^8 CFU/g of powdered formula Conventional food—10^{10} CFU/serving	Eaters-only dietary exposure—10^{10} CFU/day for infants 90th percentile eaters-only dietary exposure—2.7 x 10^{10} CFU/day for the US population
Strain AD011	GRN 875	Withdrawn		
Strain UABIa-12	GRN 872	NQ	10^{9-11} CFU/serving	The use of this strain will replace other *B. lactis* strains already present in foods and therefore, the dietary exposure to *B. lactis* will not increase.
Strain BB-12	GRN 856	NQ	5 x 10^{10} CFU/serving	Up to 10^{11} CFU/day plus any overage.
Strain R0421	GRN 855	NQ	Healthy infants 5 x10^7 CFU/g of infant formula	5 x 10^9 CFU/day

Abbreviations: CFU, colony forming units; EDI, estimated daily intake; NQ, no questions letter from FDA; yo, years old.

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.

B. lactis 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. There are 34 products containing *B. lactis* approved to be marketed under the Natural Health Products Regulations of Health Canada (NPNs 80011343, 80024350, 80012306, etc.). Unfortunately, the data that Health Canada relied upon to make their determination is not available to the public.

6.3 Safety Information

Toxicological studies have been published on various strains of *B. lactis* and are summarized in subpart 6.3.1. Additionally, human studies on *B. lactis* strains are discussed in subpart 6.3.2. There were no published human or toxicological studies located for *B. lactis* IDCC 4301 specifically as it is considered a novel strain. The studies reviewed do not suggest any concerns related to the safety of the strain. The literature search for the safety studies was conducted on December 17, 2021 and January 10, 2022.

6.3.1 Toxicological Studies on *Bifidobacterium lactis* strains

There were eight standard toxicological studies found in the published literature on various strains of *B. lactis*, which are summarized below.

Table 5. Summary of *Bifidobacterium lactis* Toxicological Studies

Author/ Guide-lines	Strain(s)	Study Type/ Duration	Animal Number (Strain)/ Group	Dose Groups/ Concentration	NOAEL/ Conclusion/ Findings
Lu et al., 2021[53] **FAO/WHO guidelines (2020)**	*B. lactis* BL-99, *L. paracasei* K56 & *L. paracasei* ET-22	AOTS (14-day) gavage	10 ICR mice/sex/ group (4 groups)	Dose—3 x 10^{12} CFU/kg bw	No mortalities or findings on gross and microscopic examination.
		28-day repeated dose intragastric	10 Sprague Dawley rats/sex/ group (10 groups)	Control—PBS HDG—5.25 x 10^{11} (K56), 2.62 x 10^{11} (ET-22) & 1.88 x 10^{11} (BL-99) CFU/kg bw MDG—(HDG/2)—2.65 x 10^{11}, 1.31 x10^{11} & 9.4 x 10^{10} CFU/kg bw LDG—(HDG/4)—1.31 x 10^{11}, 6.55 x 10^{10}, 4.7 x 10^{10} CFU/kg bw	NOAEL—5.25 x 10^{11} (K56), 2.62 x 10^{11} (ET-22) & 1.88 x 10^{11} (BL-99) CFU/kg bw, the highest doses tested.
		BRMA	*S. typhimurium* TA97, TA98, TA100 & TA102	Control—distilled water Dose groups— 8, 40, 200, 1000 & 5000 µg/dish ± S9	No mutagenicity.
Morovic et al., 2017[54] **Redbook 2000 IV. C.2**	*L. acidophilus* NCFM®, *L. paracasei* Lpc-37®, *B. lactis* Bl-04® & Bi-07® & their combination	AOTS gavage	F (Crl:CD® (SD)) rats/ (authors didn't state number of rats)	Dose groups—5000 mg/kg HOWARU® Restore (2.64 × 10^{12} CFU/kg bw), *L. acidophilus* NCFM® (1.72 × 10^{12} CFU/kg), *L. paracasei* Lpc-37® (3.35 x 10^{12} CFU/kg), *B. lactis* Bl-04® (4.05 × 10^{12} CFU/kg), *B. lactis* Bi-07® (3.07 × 10^{12} CFU/kg)	No acute toxicity noted. No deaths reported.
Miao et al., 2016[55]	*B. lactis* Bi-07 & *L.*	30-day oral dosing	10 Wistar rats/sex/	Control—deionized water	No mortalities, changes in body weight, food intake,

Author/ Guide-lines	Strain(s)	Study Type/ Duration	Animal Number (Strain)/ Group	Dose Groups/ Concentration	NOAEL/ Conclusion/ Findings
	acidophilus NCFM		group (5 groups)	Placebo—yogurt Dose groups—symbiotic-supplemented fermented milk 480 g/day with *B. lactis* (8.0×10^7 CFU/g) & *L. acidophilus* (6.6×10^7 CFU/g) Dose groups—1-fold, 10-fold and 20-fold bacteria levels stated above	or behavior. Findings included statistically significant elevation in serum total protein, hemoglobin, and albumin levels in the female rats in the LDG & MDG compared to the control but not with the yogurt group. There was a statistically significant decrease in serum glucose in male rats in the LDG. There were statistically significantly decreased serum triglyceride levels in both sexes (HDG in females & LDG & MDG in males). The authors concluded that these changes were not toxicologically relevant to the test article.
Salazar et al., 2011[56]	*B. lactis* IPLA R1 or *B. longum* IPLA E44	24-day gavage	8 Wistar rats/group (3 groups)	Placebo—100 µL of sterile skimmed milk Dose groups—10^9 CFU/day/strain in a volume of 100 µL in skimmed milk	No deaths, abnormal variations in food or water intake, unexpected behavior, or significant change in weight.
Zhou et al., 2000(a)[57]	*L. rhamnosus* HN001, *L. acidophilus* HN017, *B. lactis*	8-day gavage	8 M BALB/c mice/ group (6 groups)	Control—10% skim milk Dose group—10^{11}	No AEs related to feed intake, activity, live weight gain and general health status.

Author/ Guide-lines	Strain(s)	Study Type/ Duration	Animal Number (Strain)/ Group	Dose Groups/ Concentration	NOAEL/ Conclusion/ Findings
	HN019, *L. acidophilus* LA-1 & *L. rhamnosus* GG (the latter 2 were used as reference strains)			CFU/strain/mouse/ day	
Zhou et al., 2000(b)[58]	*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 (5 groups)	Control—10% SMP Dose groups—2.5 x 10^9, 5 x 10^{10} or 2.5 x 10^{12} CFU/kg bw/day per strain (mice were inoculated with 1 of the 4 LAB strains at 3 different doses)	No toxicity up to the highest dose test. No significant findings in clinical chemistry, macroscopic examination, feed intake, or growth.

Abbreviations: AEs, adverse effects; AOTS, Acute Oral Toxicity Study; BRMA, B acterial Reverse Mutation Assay; bw, body weight; CFU, colony forming units; HDG, high dose group; LAB, lactic acid bacteria; LDG, low dose groups; M, male; MDG, medium dose groups; NOAEL, no observed adverse effects level; OECD, Organization for Economic Cooperation Development; SMP, skim milk powder; subsp., subspecies.

6.3.2 Human Studies

The safety of *B. lactis* IDCC 4301 has not been formally investigated in healthy adult subjects. However, many recent human clinical studies have been and continue to be published on various other *B. lactis* strains. There were five human studies relevant to *B. lactis* published since the most recent QPS review by EFSA and they are summarized in the table below. These studies ranged from 15 days to eight weeks and the maximum number of participants was 192 infants. The maximum dose in the studies was 2 x 10^{11} CFU, orally administered.[59] There were no significant adverse effects in any of the studies and they do not suggest any concern for safety of this species. This search was conducted on January 10, 2022.

Table 6. Summary of Recent *Bifidobacterium lactis* Human Clinical Trials

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
Anania et al., 2021[60]	To evaluate 2 strains, 1 of which was *B. lactis* BB12 DSM 15954. Prospective double-blind RCT Dose—2 x 10^9 CFU/day of strain BB12.	3 months	250 children ages 6–17 yo	Authors reported that there were no clinically related AEs from intervention.
Chen et al., 2021[61]	To evaluate the effects of *B. lactis* BB-12®. Double-blind, placebo-controlled RCT Dose—1 x 10^9 CFU/day	3 weeks	192 full-term infants < 3 mo diagnosed with infant colic	No AEs related to intervention were reported during the study.
Czajeczny et al., 2021[62]	To evaluate 2 strains, 1 of which was *B. lactis* BS01. Single-blind, RCT Dose—2 x 10^9 CFU/day for strain BS01	6 weeks	53 women	Authors reported there were no AEs during the study.
Haghighat et al., 2021[63]	To examine effects of 4 strains, 1 of which was *B. bifidum* BIA-6—2.7 x 10^7 CFU/g. Three-arm parallel design, placebo-controlled, double-blind, RCT Dose—5 grams of powder dissolved in water 4 times/day (1.1 x 10^8 CFU/g including all strains).	12 weeks	75 HD patients	There were no significant AEs. AEs that occurred were determined to be unrelated to study participation, including headache, fatigue, & breathing problems.
Mageswary et al., 2021[64]	To evaluate the effects of *B. lactis* Probio-M8. Prospective, double-blind, RCT Dose—2 x 10^{10} CFU/day	4 weeks	120 RTI-hospitalized children	Authors stated there were no AEs or any reported health implications related to the test article.
Makela et al., 2021[59]	To evaluate the effects of a product containing *B. lactis* 420 (B420).	8 weeks, divided in 4 phases.	50 healthy adult volunteers, 20–40 yo	243 AEs were reported in 45 participants. Most common AE was

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	Double-blind, RCT Dose—10^{11} CFU ⁄ap twice/day, 12 hours between doses	Phase #1—run-in phase (consumption of concomitant treatments were prohibited) for 14–20 days Phase #2—treatment or ph cebo was consumed Phase #3—treatment or ph cebo was co-administered with NSAID for 14 days Phase #4—2-week fu monitoring for AE & fecal samples were col ected.		headache. 105 AEs were considered relt ed to the study products, 82 were mil , 21 were moderate & 2 were severe. The 2 severe AEs includ ed severe abdominal pain lst ing for 5 hours, 1.5 hrs aff er taking B420 on day 4 of phase #2. The other AE includ ed severe abdominal pain for 2 hrs starting 11 hrs aff er B420 & NSAID on the 3rd day of phase #3. Both AEs resolved. Additionally, 4 in the intervention group & 5 in placebo group reported GI symptoms in phase #2. 15 participants had GI symptoms in phase #3. No serious AEs or other significant AEs occurred.
Piatek et al., 2021[65]	To examine effects of 8 strains, 1 of which was *B. lactis* BI0-04. Open-label, two parallel treatment group study Dose—10^9 CFU/day (equal amounts per strain)	4 weeks	87 infants 3–6 weeks old with infantile colic	Authors stated that there were no AEs reported.
Quero et al., 2021[66]	To examine effects of 3 strains, 1 of which was *B. lactis* CBP-001010 & vitamins including zinc & selenium. Triple-blinded, RCT Pilot study	30 days	27 male participants, 13 professional soccer plays & 14 sedentary students	There were no AEs discussed by the authors.

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	Dose—10^9 CFU/day including all strains			
Tavares-Silva et al., 2021[67]	To examine effects of 5 strains, 1 of which was *B. lactis* BL-G101. Double-blind, placebo-controlled RCT Dose—1 billion CFU/day/strain	30 days before a marathon	14 healthy male marathon runners	The probiotic group presented a higher number of symptoms the first two days. There were no differences observed between groups on the third day & a reduction in symptoms in the treatment group on the fifth day. AEs were not further discussed.
Tavasoli et al., 2021[68]	To evaluate the effects of a product containing 4 species, 1 of which was *B. lactis*. Double-blind RCT Dose—1.8 x 10^9 CFU/cap twice/day (including all strains with 1:1:1:1 ratio)	4 weeks	100 adults with ≥ 2 radiopaque stone episodes & hyperoxaluria (24 hr urine oxalate ≥ 40 mg/24 hr)	No severe AEs.
Tsilika et al., 2021[69]	To evaluate the effects of a product containing 4 species, 1 of which is *B. lactis* BB-12. Multi-center RCT Dose—1.75 x 10^9 CFU twice/day for strain BB-12 (1 through a nasogastric tube & 1 spread on the oropharynx)	15 days	112 adults with recent trauma involving head injury & ≥ 1 organ system; intubation & expected to require MV either in the ambulance or the ED & likelihood that the duration of MV or >10 days & life expectancy >15 days	No major differences were found between groups with regard to AEs.
Zheng et al., 2021[70]	To evaluate 4 strains, 1 of which was *B. lactis* LPL-RH (CGMCC No. 14007).	Duration is unclear.	100 adults with gastric cancer	Authors did not discuss AEs.

Author	Purpose, Dose & Description	Duration	# of Subjects	Comments (results)
	RCT Dose—10^9 CFU/cap for strain LPL-RH (up to 3 caps/day)			

Abbreviations: AE, adverse event; ED, emergency department; f/u, follow-up; GI, gastrointestinal; hr, hour; hrs, hours; MV, mechanical ventilation; NSAID, non-steroidal anti-inflammatory drug; RCT, randomized control trial; RTI, acute respiratory tract infections; yo, years old.

6.3.3 Opportunistic Infections

Infections caused by LAB have been described in the literature (e.g., sepsis and endocarditis) but for the most part occur at very low rates.[49] Infections associated with *Bifidobacterium* almost always occur in immunocompromised patients, those who have suffered surgical or accidental insult, or have a serious underlying illness.[49] 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.[1, 49] Bacteria, usually from the host's own commensal microflora, generally enter the bloodstream and adhere to the heart valves.[1] The vast majority of all infections occur from commensal bacteria, and the ingestion of LAB does not seem to be of additional concern with regard to infection possibilities.[1, 49] In summary, the potential to cause infection is generally in individuals with compromised immune systems or other significant predisposing conditions, and specific strain-associated virulence factors have not been noted.[38]

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. *B. lactis* IDCC 4301 is grown in culture medium that contains soy components. Thus, food products that contain this strain may be required to declare the allergen in their labeling per FALCPA. Otherwise, *B. lactis* IDCC 4301 does not contain gluten, milk, celery, mustard, sulfur dioxide and sulfites, lupin, or mollusks.

It is worth noting that the literature suggests there is an inverse relationship between LAB consumption and allergies and atopic eczema.[71]

6.5 Past Sales and Reported Adverse Events

ILDONG has been selling *B. lactis* on all continents, mainly for human consumption, since 2016. According to the company, nearly 45,931 kilograms of the *B. lactis* IDCC 4301 represented in this report were sold over the six years from January 1, 2016, to November 31, 2021. Commercial products in which *B. lactis* IDCC 4301 is currently sold are shown below.

- BIOVICHEON Premium
- BIOVITA Family
- BIOVITA Family mini tablet
- gQlab 10B Alive Probiotics Gold (gQlab S 10 Billion live probiotics)
- gQlab Bifido plus (gQlab Bifido Postbiotics, gQlab Bifido Multibiotics)
- gQlab Daily
- 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 Good Morning Probiotics)
- MICROBIOME PROBIOTICS
- PROBIO500 TRIPLE

Related to this six-year sales period, complaints and non-serious adverse events were registered. There were approximately 108 complaints over this period. 94

complaints are for products currently being sold, which are shown in the list above. These products all contain *B. lactis* IDCC 4301, along with other strains and excipients. The majority of complaints, 77%, 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 three cases of vomiting. 18% were minor skin reactions including itch, facial flushing, and a mild rash. There were two complaints of dizziness and headaches. Finally, there were three complaints of allergy. There was one complaint of a cold sore.

As described above, ILDONG's *B. lactis* has a long history of safe consumption by humans and animals.[47] 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 "*Bifidobacterium lactis*" returned 3755 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 *B. lactis*. All information was accessed from the databases on December 17, 20201.

FAERS

FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System (FAERS AE) revealed 73 cases of relevant adverse events which included one death. These events are summarized in the table below.

Table 7. Adverse Events reported for *Bifidobacterium lactis* 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
73 cases in total 2021 (13) 2020 (17) 2019 (20) 2018 (13) 2017 (6) 2016 (1) 2015 (3)	18–64 (32) 65–85 (3) Not specified (38)	Gastrointestinal disorders (64) Respiratory, thoracic & mediastinal disorders (62) Nervous system disorders (53) Injury, poisoning & procedural complications (22) General disorders & administration site conditions (15) Infections & infestations (10) Investigations (9) Metabolism & Nutrition Disorders (8) Vascular Disorders (7) Neoplasms benign, malignant & unspecified (6) Psychiatric Disorders (5) Skin & subcutaneous tissue disorders (4) Musculoskeletal & Connective Tissue Disorders (4) Renal & Urinary Disorders (2) Endocrine Disorders (1) Eye Disorders (1) Immune system disorders (71 Cardiac disorders (1) Blood & Lymphatic System Disorders (1) Pregnancy, puerperium & perinatal conditions (1)	1 death—female 68 yo, (2020) Suspect products listed (note that it does not mean they are the cause, and it was unclear if they were taking this concomitantly)— Micardis; Novalgin (Metamizole Sodium)

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 *B. lactis* IDCC 4301 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 rRNA sequence as well as complete genome sequencing, with confirmed 99% sequence homology to its type strain sequence, *B. lactis* YIT 4121;
- The analyses and resulting data showing *B. lactis* IDCC 4301 lacks resistance to clinically relevant antibiotics per European Food Safety Authority (EFSA) minimal inhibitory concentration cut-offs and guidelines, with the exception of vancomycin, and the presence of one antimicrobial resistance gene, *tet(W)*, where further investigation by ILDONG showed that the resistance did not correlate with any related genetic sequences, and/or is not expected to be transferrable;

- The lack of potential of *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301;
- The intended use as an ingredient in foods at an addition level of up to 1 x 10^{11} CFU per serving, which is in line with addition levels for other GRAS microbial ingredients (including *B. lactis* in GRNs 872 and 856);

6.6.2 Data and Information that are Corroborative of Safety

- *B. lactis'* EFSA QPS 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;
- The documented long history of safe human consumption of *B. lactis* as a common bacterial species in fermented foods,[47] such as dairy, beer, vegetable juice, and fruit juice, over decades without known concerns for safety;[47]
- The lack of serious adverse events reported in clinical trials using *B. lactis* at daily dosages up to 2 x 10^{11} CFU/day;
- Published toxicology studies on other *B. lactis* 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.[72-74]

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 *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 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 on December 17, 2021 and January 10, 2022.

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

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April 4, 2023

Re: Responses to FDA's GRN 1092 Questions

Dear Dr. Deng,

Please find responses to FDA's questions concerning *B. lactis* 4301 (GRN 1092) 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 *B. lactis* IDCC 4301 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 2.2.2 (Raw Materials, page 10 of 46) of GRN 1092 is amended to include the following statement: All processing aids used in the manufacture of *B. lactis* IDCC 4301 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 12), you provided the results from the analyses of three non-consecutive batches of *B. lactis* IDCC 4301, including the results for heavy metals. We note that the batch analyses show that the results for lead, cadmium, mercury and arsenic are significantly lower than the corresponding specification limits.
    - We recommend that you lower the specification limits for 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
Mercury	< 0.1 mg/kg	< 0.1 mg/kg

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

- 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 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:

Heavy Metals				
Tested Parameters	New Specification	Lot #/Month of Manufacture		
		Lot# IDK0201 02/2019	Lot# IDK0601 06/2019	Lot# IDK0901 09/2019
Lead[a]	< 0.5 mg/kg	0.01 mg/kg	0.01 mg/kg	< LOD
Cadmium[b]	< 0.3 mg/kg	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg
Mercury[c]	< 0.1 mg/kg	0.02 mg/kg	< LOD	< LOD
Arsenic[d]	< 0.3 mg/kg	< LOD	< LOD	0.01 mg/kg

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 *B. lactis* IDCC 4301 is intended to be used as an ingredient. Please specify a serving size for each food category (or food subcategory if necessary) and provide the reference that was used as the basis for determining the serving size. In addition, please confirm that the maximum use level is 1.02 x 1011 CFU/serving (i.e., including the 2% overage)/serving regardless of the food category and that the intended food uses exclude alcoholic beverages.
    - Response, part a: As stated in the GRN notice, *B. lactis* IDCC 4301 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 *B. lactis* IDCC 4301 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:
    - The maximum numbers of servings/day provided in the 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 *B. lactis* IDCC 4301, nor are other whole food products such as vegetables and fruits, making our exposures estimates all the more conservative.
    - 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.

    - 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 recommendation 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 *B. lactis* IDCC 4301 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.
        - Response: 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 *B. lactis* IDCC 4301 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 x 10^{10} CFU/kg bw/day.

5. In Section 2.1, you identified *B. lactis* IDCC 4301 taxonomically according to standard taxonomic guidelines.
    - 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-2848.

6. In Section 2.2, you stated that the preculture is prepared by inoculating the frozen samples of the preserved strain.
    - 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).

    - 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 2.3 (page 12 of 46) of the submitted GRAS notice (GRN 1092), we included a statement that the methods cited in Table 1 had been validated for their stated purposes; therefore, we are confused by this question.

          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 in table below:

Table 1. *Bifidobacterium lactis* IDCC 4301 Product Specifications

Tested Parameters	New Limits/Specifications	Method	Corresponding Internationally Recognized Methods
Appearance	White to light yellow powder	KFSC 8/1/1.1	
Identification	*Bifidobacterium animalis* subsp. *lactis*	16S rRNA Sequencing	
Cell count	$\geq 2.5 \times 10^{11}$ 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	KFSC 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?
        - Response: 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. In Section 6.3 of the Safety Narrative, you summarized eight toxicological and five human clinical studies on *B. lactis*.
    - Since none of the cited safety studies was on the strain *B. lactis* IDCC 4301, please describe any study where *B. lactis* IDCC 4301 and the other *B.*

lactis strains were compared or how the test articles used in these studies compare quantitatively and qualitatively to your proposed ingredient. Most of these studies used levels of $\leq 10^9$ CFU/day. Please explain how these studies will determine safety at an estimated dietary exposure higher than what was used in the human studies.

- Response, part a: While it is unclear precisely how the strains listed in Section 6.3 are related to *B. lactis* IDCC 4301, it is important to note that there are reports in the literature that no pathogenic genes have identified in the *Bifidobacterium* species, implying that the genus has low concern with regard to safety.[2] Additionally, there are numerous publications regarding safety of microbial species and strains, such as Pariza et al. (2015) and more recently, Roe et al. (2022).[3, 4] Roe et al. (2022) states that "if sufficient history of safe use is known for oral consumption of a specific bacterial species, and the strain of interest has been properly identified to the strain level, its genome properly sequenced, annotated and shown to not contain genes of concern, and intended use of the ingredient falls within an exposure considered to be safe, phase 1 clinical study safety studies are likely not needed for use by generally healthy humans. If there are limitations on the history of safe use of the strain and/or the species exists on the EFSA QPS list for example, then some limited testing may be necessary. This should include a search of phylogenomic databases using the whole genome sequencing to determine presence of various antibiotic resistance, virulence and toxin genes and phenotypic testing for antibiotic resistance should be conducted according to standard to antibiotic screens."[3] As stated in the GRAS notice and summarized in Subparts 6.6.1 and 6.6.2 on pages 38–38 of 46, the *B. lactis* species has a long history of safe use, the strain has been properly identified to the strain level, its genome has been properly sequenced and annotated, demonstrating that it does not contain genes of concern. Further, a complete genome sequencing was completed, demonstrating that there are no antibiotic resistance, virulence or toxin genes.
- Response, part b: The human studies listed in the dossier are not considered pivotal data; the intent of this information was corroborative and intended to show recent information that is present in the public domain related to the species. While indeed studies used lower doses than the estimated exposure to the *B. lactis* IDCC 4301 strain and, thus, do not directly support the intended use, they did not suggest any concerns related to the safety of *B. lactis* at the applied dosages.

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 10, 2022. The search engines utilized included PubMed and Medline. The search terms included "*Bifidobacterium lactis*" and "*B. lactis*".

11. In Section 6.1.1, you cited a sentence from the Abstract of reference #44 to imply health benefits of the strain: "Since their discovery as dominant microbes in the feces of breast-fed infants, there have been numerous studies addressing their potential health benefits through their role in modulating gut microflora. Because of this, *Bifidobacteria* are frequently incorporated into foods as probiotic cultures.44" This reference is a comparative genome analysis of nine strains of *Bifidobacteria* to reveal their potential capacities to adapt to their habitats. It is improper to quote it here to imply health benefit of "probiotics". 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 information. We agree that this was not an appropriate statement to make from the data shown in this paper, and that overall the notice should focus on safety of the ingredient and not any purported benefits. 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 *B. lactis* strains from the FDA's GRN inventory. Please note that GRN 875 was withdrawn. For each of the other three successful GRAS notices, please provide a brief paragraph summarizing the information pertaining to safety.
        - Response: Please note that we summarized four GRAS notices in GRN 1092 (not three as stated above) that received a "no question" letter from the FDA, briefly below.

- A GRAS notice to FDA (GRN 855) for *B. lactis* CBS-118529 received a no questions letter from FDA for use as an ingredient in non-exempt powdered milk-based infant formula for healthy term infants at an addition level of 8 x 10^7 CFU/g of powder. In FDA's no questions letter dated on February 5, 2020, they summarized the GRN safety narrative for the ingredient, including the following. The notifier describes "*Bifidobacteria* as commensals within the digestive tract of humans and describes the history of safe use of *B. lactis* in fermented foods. They relied on publications that support the safe consumption of *B. lactis* CBS-118529, including peer-reviewed scientific journals, governmental reviews, and product approvals. Additionally, support comes from published clinical trials in which infants, children, and adults were fed *B. lactis* CBS-118529 and no significant adverse effects were noted in any of these studies."

- A GRAS notice to FDA (GRN 856) for *B. lactis* strain DSM 15954 received a no questions letter from FDA for use as an ingredient in foods at an addition level of up to 5 x 10^{11} CFU/serving. Foods include milk and dairy products such as yogurt and other fermented milk products; dairy alternatives (plant-based (oat, soy, almond, coconut, pea, etc.) fermented milk and yogurt products; beverages such as juice and protein shakes; shelf-stable products such as bars (granola, protein, meal replacement bars), confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings); and breakfast cereals (ready-to-eat (RTE) and hot). In FDA's no questions letter dated on December 9, 2019, they summarized the GRN safety narrative for the ingredient, including the following. The notifier states "*Bifidobacteria* are widely consumed in fermented foods and have a long history of safe use of *B. animalis* subsp. *lactis* in fermented foods. They explain that *Bifidobacteria* may cause opportunistic infections in immunocompromised patients, however, this is not relevant under their intended conditions of use. The notifier cites publications in peer-reviewed scientific journals that support the safe consumption of *B. animalis* DSM 15954. Additionally, they describe published clinical trials in which infants, children and adults were fed *B. animalis* DSM 15954 and state that no significant adverse effects on participants were noted in any of these studies."

- A GRAS notice to FDA (GRN 872) for *B. lactis* 30334, received a no questions letter from FDA for use as an ingredient in foods generally (excluding infant formula and foods under the

jurisdiction of the USDA) at an addition level of up to $10^{9–11}$ CFU/serving. In FDA's no questions letter dated on December 9, 2019, they summarized the GRN safety narrative for the ingredient, as follows. The notifier "discusses the long history of safe use of lactic acid bacteria in foods and how *B. animalis* subsp. *lactis* has been safely used in fermented foods. They cite publications that support the safe consumption of *B. animalis* subsp. *lactis*, including peer-reviewed scientific journals, governmental reviews, and product approvals. Additionally, the notifier describes published clinical trials in which infants, children and adults were fed *B. animalis* NCIMB 30334 and state that no significant adverse effects on participants were noted in any of these studies."

- A GRAS notice to FDA (GRN 952) for *B. lactis* AD011, received a no questions letter from FDA for use as an ingredient in foods at an addition level of up to 10^{10} CFU/serving and in non-exempt infant formula for term infants at an addition level of up to 10^{8} CFU/g of powered infant formula. Foods include fermented milk, including buttermilk and kefir, flavored milk beverage mixes, dried milk powder, imitation milk, yogurt, powdered baby cereals and foods, meal replacement and nutritional drink mix powders, and powdered sugar substitutes. In FDA's no questions letter dated on March 17, 2021, they summarized the GRN safety narrative for the ingredient, as follows. The notifier "discusses published and publicly available information to support safety of *B. lactis* AD011. They state that *B. lactis* AD011 genome does not contain regions with significant homology to known toxigenic or pathogenic genes. Further, the notifier discusses published studies that provide evidence that *B. lactis* AD011 exhibits antibiotic susceptibility, does not contain plasmid capable of transmitting antibiotic resistance genes, does not show hemolytic and mucolytic activities, and does not produce clinically significant levels of biogenic amines and ammonia. They state that the fate and the safety profile of orally consumed *B. lactis* AD011 is not expected to be significantly different from what are observed after consumption of other *Bifidobacterium* species. Moreover, the notifier describes supportive published clinical studies in which infants, children, or adults consumed *B. lactis* AD011 (or other *Bifidobacterium* or *Lactobacillus* strains) and state that no adverse effects were reported."

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. Sanders ME, Akkermans LM, et al. Safety assessment of probiotics for human use. *Gut Microbes*. 2010;1(3):164-85
3. Roe AL, Boyte ME, et al. Considerations for determining safety of probiotics: A USP perspective. *Regul Toxicol Pharmacol*. 2022;136:105266
4. 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

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April 12, 2023

Re: Responses to FDA's GRN 1092 Second Round of Questions

Dear Dr. Deng,

Please find responses to the second round of FDA's questions concerning *B. lactis* 4301 (GRN 1092) 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 4/10/2023	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 *B. lactis* IDCC 4301 per serving, the maximum estimated dietary exposure from the intended use of *B. lactis* IDCC 4301 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. As your response to Question 11, please amend the GRN to remove the quoted sentence from the Abstract of reference #44.
    - We amend GRN 1092 to strike, as shown below, from the notice, the statement (including its citation #44 in Subpart 7.2, page 43 of 46) found as the last paragraph of Subpart 6.1.1 on page 24 of 46:

        "~~Since their discovery as dominant microbes in the feces of breast-fed infants, there have been numerous studies addressing their potential health benefits through their role in modulating gut microflora. Because of this, *Bifidobacteria* are frequently incorporated into foods as probiotic cultures.~~[44]"

        ~~44. Lee JH and O'Sullivan DJ. Genomic insights into bifidobacteria. *Microbiol Mol Biol Rev*. 2010;74(3):378-416~~

4. We appreciate your response to Question 12 by providing brief summaries for the four successful GRAS notices related to *B. lactis* strains.
    - You are most welcome.

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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