JHeimbach LLC

October 6, 2022

Susan J. Carlson, Ph.D., Director
Office of Food Additive Safety (HFS-200),
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Dr., College Park, MD 20740

Dear Dr. Carlson:

Pursuant to 21 CFR Part 170, Subpart E, Danisco USA, Inc., a wholly owned subsidiary of International Flavors and Fragrances, Inc., through me as its agent, hereby provides notice of a claim that the addition of *Lactiplantibacillus plantarum* ATCC-202195 to infant formula, toddler foods, and conventional foods is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Danisco USA has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

A virus-free CD is enclosed containing Form 3667, the GRAS monograph, and signatures of members of the GRAS Panel in a zip directory produced through COSM.

If you have any questions regarding this notification, please feel free to contact me at 202-320-3063 or jh@jheimbach.com.

Sincerely,

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

1205 Prince Edward Street, Fredericksburg Virginia 22535, USA
tel. (+1) 804-742-5548 cell (+1) 202-320-3063 jh@jheimbach.com

GRAS DETERMINATION FOR THE USE OF *LACTIPLANTIBACILLUS PLANTARUM* ATCC-202195 IN INFANT FORMULA, TODDLER FOODS, AND CONVENTIONAL FOODS

Prepared for:

Danisco USA, Inc.

(A Wholly Owned Subsidiary of International Flavors and Fragrances, Inc.)

October 2022

Part 1. Signed Statements and Certifications

1.1. GRAS Notice Submission

In accordance with 21 CFR 170.255, Danisco USA Inc. (a wholly owned subsidiary of International Flavors and Fragrances, Inc.) submits this GRAS notice through its agent James T. Heimbach, president of JHeimbach LLC, for *Lactiplantibacillus plantarum* ATCC-202195.

Name and Address of Notifier
Danisco USA Inc., a wholly owned subsidiary of International Flavors and Fragrances, Inc.
DuPont Experimental Station – E353
200 Powder Mill Road
Wilmington DE 19803

Notifier Contact
Jayne Davies
Global Regulatory Affairs
Tel: 610-864-7219
jayne.c.davies@iff.com

Agent Contact
James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street #66
Port Royal VA 22535
Tel: 804-742-5543
jh@jheimbach.com

1.2. Name of Notified Microorganism

The notified microorganism is *Lactiplantibacillus plantarum* strain ATCC-202195 (formerly classified as "*Lactobacillus plantarum,*" and so referred to in supporting documents and scientific studies published prior to April 15, 2020), sold under the brand name *L. plantarum* (HOWARU Lp-202195™). It is a Gram-positive, obligate hetero-fermentative, lactic acid bacterium. Throughout this document, the strain will most often be referenced as *L. plantarum* ATCC-202195.

1.3. Intended Conditions of Use

Intended uses are non-exempt infant and toddler formulas based on milk (intact or partially hydrolyzed) or soy protein, extensively hydrolyzed exempt formula, conventional foods including foods for infants and young children, juice and drinks for infants and young children, milk products including flavored milk beverages, meal replacement and powdered drink mixes, milk product analogs including soy, soy products, processed fruits and fruit juices, confectionary snacks and baked goods. Addition to infant formula will not exceed 10^8 cfu/g powdered formula and addition to conventional foods will not exceed $2x10^{10}$ cfu/serving.

It is not intended to be used by certain individuals under medical supervision, including immune-compromised individuals, infants with marked carbohydrate malabsorption such as short bowel

syndrome or gastrointestinal bypass surgery, or patients with increased risk of developing small intestinal bacterial overgrowth, including those with gastrointestinal dysmotility, or long term use of proton pump inhibitor or opioid medication.

1.4. Statutory Basis for GRAS Status

Danisco has concluded that the notified microorganism, *L. plantarum* ATCC-202195, as described herein is generally recognized as safe (GRAS) under the conditions of its intended use. This GRAS conclusion was reached through scientific procedures and in concert with the views of a panel of experts who are qualified by scientific training and experience to evaluate the safety of microorganisms added to foods in accordance with 21 CFR 170.30(a) and (b).

1.5. Premarket Exempt Status

Based upon Danisco's GRAS conclusion as stated in Section 1.4 above, it is Danisco's view that the intended use of *L. plantarum* ATCC-202195 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.6. Data Availability

The data and information that are the basis for the determination of the GRAS status of the intended use of *L. plantarum* ATCC-202195 are available to FDA upon request. Such data and information may be sent to FDA either in electronic format or on paper or reviewed during customary business hours at the home office of JHeimbach LLC, located at 923 Water Street, Port Royal VA 22535.

1.7. FOIA Statement

None of the data and information in this GRAS notice is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.8. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of *L. plantarum* ATCC-202195.

1.9. FSIS Statement

Not applicable.

1 tifier or Agent

[redacted]

J C.N.
President
JHeimbach LLC
Agent to Danisco USA, Inc., a wholly owned subsidiary of IFF, Inc.

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

2.1. Identity/Identification

2.1.1. SCIENTIFIC NAME, TAXONOMY AND OTHER NAMES

The taxonomy of *L. plantarum* ATCC-202195 at the species level was established by 16S rRNA gene comparison to the reference strain *Lactobacillus plantarum* WCFS1, with 99.95% sequence identity.

Based on the results of the 16S rRNA comparison, *L. plantarum* ATCC-202195 has the following taxonomic lineage:

Kingdom: Bacteria

Phylum: Firmicutes (Gram positive spore forming bacteria)

Class: Bacilli

Order: Lactobacillales

Family: Lactobacillaceae

Genus: *Lactiplantibacillus*

Species: *plantarum*

2.1.2. DESCRIPTION/SOURCE INFORMATION AND GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF THE ORGANISM

L. plantarum ATCC-202195 was isolated from infant human feces (Wright et al 2020) and deposited to the American Type Culture Collection (ATCC) in January 1999 (Panigrahi et al 2000).

Genomic DNA was isolated using Invitrogen Purelink Genomic DNA Mini Isolation Kit following the standard protocol. Genome sequencing was carried out at the University of Illinois at Urbana-Champaign using 150 bp paired-end reads on a NovaSeq. Read quality was evaluated using FastQC (Andrews 2010). Reads were trimmed using Spades v 1.33 and subsampled to 1 million reads. Sequencing was also conducted on Oxford Nanopore and the reads were filtered using NanoFilt (De Coster et al. 2018). The hybrid assembly of Illumina and ONT reads was carried out using Unicycler (Wick et al. 2017). The assembled genome was then uploaded for annotation to PATRIC (PathoSystems Resource Integration Center, Davis et al. 2020), a NIH/NIAID-funded project developed at the University of Chicago and the Biocomplexity Institute and Initiative of the University of Virginia with the primary goal of providing access to genome sequences and analysis tools for studying microbes.

All 16S sequences and full genomes were either obtained from Danisco's culture collection or publicly available and sourced from PATRIC. 16S rRNA alignment was conducted using MUSCLE v.3.8.425 (Edgar 2004) as part of the Geneious software v.2019.2.3. The genomic average nucleotide identity (ANI) was calculated using OrthoANI (Lee et al. 2016). The phylogenetic tree was constructed using the service provided by PATRIC which uses RAxML to align 100 single copy genes found in all genomes with bootstrap values shown for 100 iterations.

The phylogenetic tree of select *Lactiplantibacillus* species (Figure 1) was constructed based on the alignment of 100 core genes using RAxML(Stamatakis 2014). *Bacillus subtilis* ssp. *subtilis* strain 168 was used as an outgroup. All *L. plantarum* strains, including *L. plantarum* ATCC-202195, cluster together separate from other *Lactiplantibacillus* species.

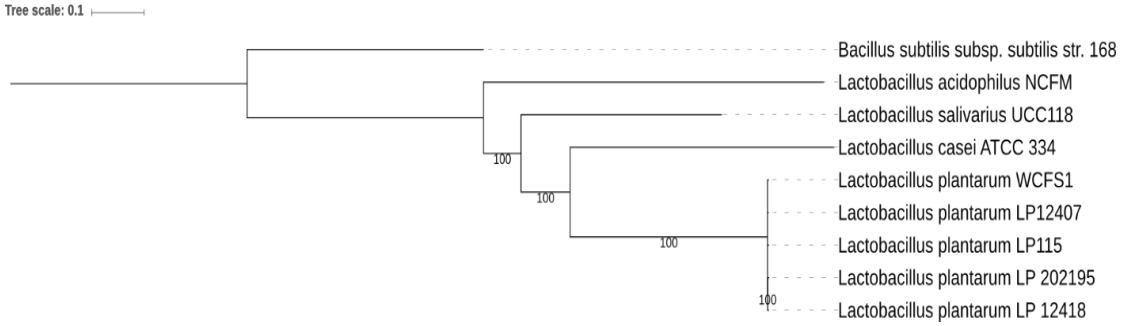


Figure 1: Phylogenic Tree of Select Lactiplantibacillus Species.

Using whole genome ANI, there is 99% similarity between *L. plantarum* ATCC-202195 and the reference strain, *L. plantarum* WCFS1, *L. plantarum* Lp-115 (DGCC 4715), and another commercially available live microbial strain *L. plantarum* JDM1 (Table 1). Using reference genomes for select species of *Lactiplantibacillus* and *L. plantarum* strains, a phylogenetic tree using 100 core genes using RAxML and 100 bootstraps was constructed (see Figure 1). All the *L. plantarum* strains cluster together on the tree separate from other species of *Lactiplanti-bacillus*, further supporting the 16S and ANI results that *L. plantarum* ATCC-202195 belongs to the *L. plantarum* species.

Table 1: Whole Genome Average Nucleotide Identity (ANI) of Lactiplantibacillus plantarum Strains.

	L. plantarum ATCC-202195	*L. plantarum Lp-115*	*L. plantarum WCFS1*	*L. plantarum JDM1*
L. plantarum ATCC-202195	100	99.1	99.1	99.1
L. plantarum Lp-115	99.1	100	99.2	99.1
L. plantarum WCFS1	99.1	99.2	100	99.1
L. plantarum JDM1	99.1	99.1	99.1	100

2.1.2.1. Genomic Comparison of *L. plantarum* ATCC-202195 with *L. plantarum* Lp-115

The genome of *L. plantarum* ATCC-202195 was compared with that of *L. plantarum* Lp-115, a generally recognized as safe (GRAS) acknowledged strain and established dietary substance (GRN 722; FDA 2018). *L. plantarum* Lp-115 was notified by DuPont and filed as GRAS in 2017 for use in conventional foods at a serving level of 10^{10} cfu and estimated daily exposure up to 10^{11} cfu. Along with a high average nucleotide identity (ANI), both *L. plantarum* ATCC-202195 and *L. plantarum* Lp-115 have similar genome organization, with minimal variations and unique regions, as seen in Figure 2. These distinctions are indicated by gaps within the colored blocks and are discussed in further detail below.

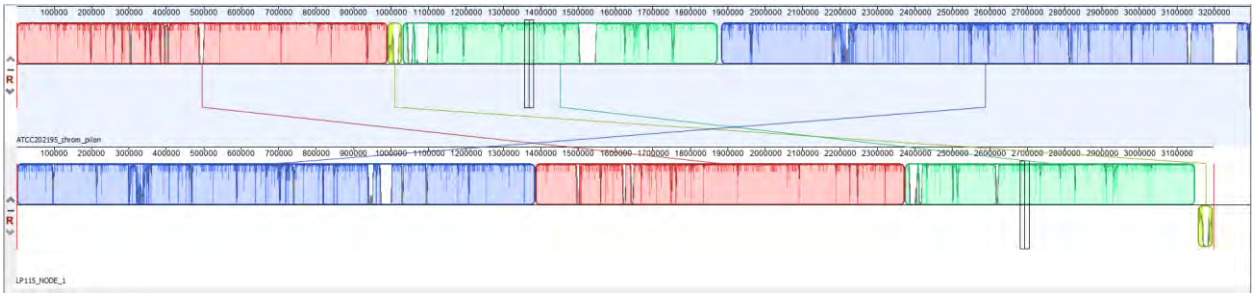


Figure 2: A Mauve Alignment Between *L. plantarum* ATCC-202195 (Top) and *L. plantarum* Lp-115 (Bottom).

The image shows regions of similarity as similarly colored blocks in both genomes with lines connecting similar regions. The breaks or lines within the colored blocks indicate non-homologous regions.

2.1.2.2. Genomic Comparison with *L. plantarum* LP12407 and *L. plantarum* LP12418

The genome of *L. plantarum* ATCC-202195 was compared with that of *L. plantarum* LP 12407 and *L. plantarum* LP 12418, both of which are recently acknowledged dietary substances based on NDI notification letters issued in September 2020 (FDA 2020a, 2020b). *L. plantarum* LP 12407 was notified by DuPont and filed for use in dietary supplements at a serving level of 5 x 10^{10} cfu/day for chronic consumption in adults and 1 x 10^{10} cfu/day in children for up to 90 days. *L. plantarum* Lp 12418 was notified by DuPont and filed for use in dietary supplements at serving level of 5 x 10^{10} cfu/day for chronic consumption in adults and 1 x 10^{9} cfu/day in children for chronic use.

ANI comparison demonstrates that *L. plantarum* ATCC-202195 shares 98.9% identity with *L. plantarum* Lp 12407 and 99.1% identity with *L. plantarum* LP 12418. Further, the genomes were used to align 100 core genes of *L. plantarum* ATCC-202195, *L. plantarum* LP 12407 and *L. plantarum* LP 12418 as well as those of *L. plantarum* Lp 115 and the type strain *L. plantarum* WCFS1. As demonstrated through the high degree of sequence homology, *L. plantarum* ATCC-202195 can be considered closely related to the NDI acknowledged strains *L. plantarum* LP 12407 and *L. plantarum* LP 12418.

2.1.2.3. Carbohydrate Analysis

The API 50 CH kit and associated 50 CHL medium provides 50 biochemical analyses for the study of carbohydrate metabolism in *Lactobacillus/Lactiplantibacillus* and related genera. Cultures of *L. plantarum* ATCC-202195 were grown overnight in defined fermentation medium under defined pH and temperature conditions and temperature. An aliquot of overnight broth was concentrated and centrifuged to produce a pellet. The sample was inoculated in API 50 CHL medium and the resulting suspension was pipetted into API 50 CH test wells. All tests were covered with mineral oil and incubated aerobically at 37ºC for 48 hours. Reactions were observed after 48-hour incubation and recorded; results are presented in Table 2.

Table 2: Results of the API 50 CH Assay of *L. plantarum* ATCC-202195.

Lactobacillus plantarum LP202195 DGCC12988 strain API 50 CH (37°C, 48 hrs)									
CONTROL	-	GALactose	+	a-Methyl-D-Mannoside	+	MELibiose	+	D TURanose	+
GLYcerol	-	GLUcose	+	a-Methyl-D-Glucoside	-	Sucrose	+	D LYXose	-
ERYthritol	-	FRUctose	+	N-Acetyl-Glucosamine	+	TREhalose	+	D TAGatose	+
D ARAbinose	-	MaNnosE	+	AMYgdalin	+	INUlin	-	D FUCose	-
L ARAbinose	+	SorBosE	+	ARButin	+	MeLeZitose	+	L FUCose	-
RIBose	+	RHAmnose	-	ESCulin	+	RAFfinose	+	D ARabitoL	-
D XYLose	-	DULcitol	-	SALicin	+	Starch	-	L ARabitoL	-
L XYLose	-	INOsitol	-	CELlobiose	+	GLYcoGEN	-	GlucoNaTe	+
ADOnitol	-	MANnitol	+	MALtose	+	XyLiTol	-	2-Keto-Gluconate	-
Beta Methyl-D-Xyloside	-	SORbitol	+	LACtose	+	GENtiobiose	+	5-Keto-Gluconate	-

2.1.2.4. RAST Analysis of Protein Coding Regions

To further characterize the genomic differences of each strain, the genomes were annotated using RAST (Rapid Annotations using Subsystems Technology) developed by Argonne National Laboratory, Argonne, Illinois (Overbeek et al. 2014). RAST is an automatic annotation server that uses the SEED system to allow for high quality annotation of microbial genomes. After annotation, a reciprocal sequenced-based comparison of protein coding regions between *L. plantarum* strain ATCC- 202195 and strains Lp-115 and WCFS1 was performed in RAST using the circular genome alignment tool (Aziz et al. 2008), which provides information on strain-specific phenotypic traits (i.e., metabolic and cellular physiology potential) and the genomic context for each protein as seen in Figure 3.

The colors indicate the percent similarity of Lp-115 and WCFS1 to *L. plantarum* ATCC-202195 with regions of high similarity shown in purple and regions with low similarity shown in red. The vast majority of protein sequences are homologous, but both strains contain unique protein sequences.

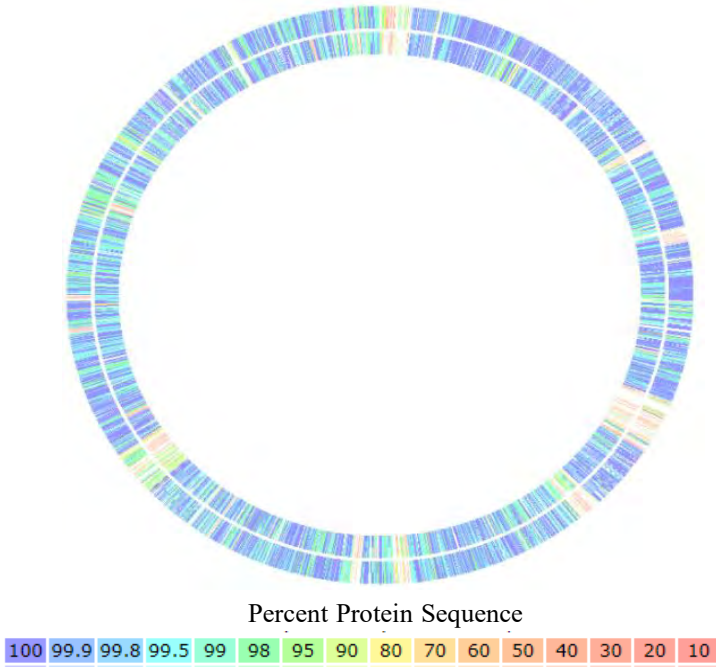


Figure 3: Reciprocal Protein Comparison of *L. plantarum* Lp-115 (outside) and WCFS1 (inside).

From the reciprocal comparison in RAST, a list of proteins that are unique to either *L. plantarum* ATCC-202195 or *L. plantarum* Lp-115 was extracted. For simplicity, WCFS1 was not included in these comparisons. There are no significant differences between *L. plantarum* ATCC-202195 and *L. plantarum* Lp-115 within the major central pathways for carbon metabolism or biosynthesis of amino acids, fatty acids, vitamins and cofactors, or purine and pyrimidine nucleotide biosynthesis. While both genomes have several prophages in common, each strain also contains two distinct prophages. Additionally, there are two PTS sugar transport systems that are distinct within each strain and roughly 30 systems that are common to both strains. Differences between these two strains also include rhamnose operons, a CRISPR system, and presence of a plasmid, as shown in Table 3.

Table 3: Predictive Protein Distinctions of *L. plantarum* Strains Lp-115 and ATCC-202195.

Protein (presence marked with "X")	LP-115	ATCC-202195
Prophage 1	X	
Prophage 2	X	
Prophage 3		X
Prophage 4		X
Plasmid		X
Rhamnose containing glycans	X	
Rhamnose utilization operon		X
CRISPR	X	
Sugar transport system	30 shared, 2 unique	30 shared, 2 unique

Based on the presence of a rhamnose synthesis gene cluster, it is probable that *L. plantarum* Lp-115 can synthesize rhamnose containing glycans while *L. plantarum* ATCC-202195 cannot. Rhamnose-containing glycans are often present in the capsule of Gram-positive bacteria or can be incorporated into cell wall-anchoring polysaccharides. These glycans are important structural characteristics used to distinguish different strains and play a role in the binding of phage and eukaryotic cells. Despite not being able to produce rhamnose containing glycans, *L. plantarum* ATCC-202195 does contain a rhamnose utilization operon that is absent from *L. plantarum* Lp-115. These results suggest that one strain can produce, but not metabolize L-rhamnose, while the other strain (*L. plantarum* ATCC-202195) is able to metabolize L-rhamnose, but not synthesize it.

Finally, *L. plantarum* Lp-115 contains a single CRISPR locus while *L. plantarum* ATCC-202195 appears not to have an annotated CRISPR system.

2.1.3. MOBILE ELEMENTS

RAST and ISFinder database were used to search the *L. plantarum* ATCC-202195 genome for mobile elements. No transposons, transposases, insertion sequences, phages, or plasmids of health concern were found.

2.1.4. ANTIBIOTIC RESISTANCE

The presence of antibiotic resistance genes was screened using PATRIC, which runs Victors (Sayers et al. 2019), CARD (Jia et al. 2017), and their own curated database of antibiotic resistance genes (Wattam et al. 2017).

Antimicrobial resistance in bacteria can be mediated by many different mechanisms that range from unknown and non-specific to fully understood and well-studied. In order to address the question of transferability of antibiotic resistance, it is best to define the two types of resistance. Intrinsic resistance reflects an organism's ability to thrive in the presence of an antimicrobial agent, is typical of the strains of a given species (Mathur and Singh 2005), and is not horizontally transferable. In contrast, when a strain is resistant to a drug that the species is typically sensitive to, it may be considered acquired resistance. Acquired resistance can be mediated by mutation of indigenous genes or by added genes (EFSA 2012). 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. Therefore, the focus has been on acquired resistance genes with the belief that they present a greater risk of transfer of resistance via horizontal gene transfer within and between species (Mathur and Singh 2005). Lactic acid bacteria (LAB) have been reported to have both intrinsic and acquired resistances to many classes of antibiotics, only some of which are known to be transferable (Nawaz et al. 2011; Zhang et al. 2011). There are three identified mechanisms of horizontal gene transfer (HGT) in bacteria: natural transformation, conjugation, and transduction. Some LAB species have these abilities, and some do not. In fact, strain level differences need to be evaluated in order to determine if HGT is possible (Ouoba et al. 2008; Marshall et al. 2009). Antibiotic resistance has been previously documented to be transferable on plasmids, transposases, and phages (Aires et al. 2007; Wang et al. 2006; Marshall et al. 2009; Colomer-Lluch et al. 2011). Therefore, the highest risk of an antibiotic gene being mobilized to another strain/species comes from these mechanisms of HGT, all of which have previously been reported in LAB in both *in vitro* and *in vivo* studies (Mathur and Singh 2005).

In each case, a whole genome sequence of the manufactured strain was obtained and analyzed for the mechanisms of HGT. Using the sequenced genome, screening for the presence of antibiotic resistance genes and mobile elements was completed. With this background, the antibiogram of *L. plantarum* ATCC-202195 was established using ISO 10932 IDF223 method and VetMIC Lact-1 and 2 micro-dilution plates that include all antibiotics that are recommended by the FEEDAP. Recorded MICs are displayed in Table 4. All MIC values are below or equal to the Microbial Break Points (MBPs) defined for *Lactobacillus plantarum* except for kanamycin (Rychen et al. 2018).

Intrinsic resistance to kanamycin is a common characteristic of *Lactobacillus* species (Campedelli et al. 2019). In most bacteria, including *Lactobacillus* (now *Lactiplantibacillus*), resistance to kanamycin is due to aminoglycoside-modifying enzymes such as acetyltransferases, nucleotidyltransferases, or phophotransferases. A recent publication found that most *L. plantarum* species have at least one nucleotidyltransferase which confers such resistance (Campedelli et al. 2019).

Like most others of its species, *L. plantarum* ATCC-202195 has a nucleotidyltransferase (*ant9*-la) that was found in the CARD database. This has been reported to provide resistance to aminoglycosides such as kanamycin. This gene is not located within 5000 bp upstream or downstream of any mobile elements (transposases, insertion sequences, etc) and is therefore not

considered a risk for transfer of antibiotic resistance genes.

Table 4: Antibiotic Susceptibility Profile for *L. plantarum* ATCC-202195.

Antibiotic	MIC Breakpoint (µg/ml)	*L. plantarum* ATCC-202195 MIC (µg/ml)
Gentamycin	16	4
Kanamycin	64	128
Streptomycin	NR	32
Tetracycline	31	32
Erythromycin	1	0.5
Clindamycin	4	0.5
Chloramphenicol	8	8
Ampicillin	2	0.25
Vacomycin	NR	>128

2.1.5. VIRULENCE ACTIVITY

The presence of virulence factors and toxins was screened using PATRIC running Victors, the Virulence Factors Database (VFDB; Liu et al., 2019), CARD, and their own curated database of virulence genes (Wattam et al. 2017). Other toxins were screened using annotation and protein sequence alignment to hemolysins, bacteriocins, and toxin-antitoxin systems. No virulence or toxin genes were identified.

Toxin-antitoxin systems are intracellular regulatory mechanisms that are thought to enable different functions such as gene regulation, growth control, and programmed cell death (Magnuson 2007). As such, they pose no danger to hosts. *L. plantarum* ATCC-202195 contains the toxin-antitoxin system *ydcDE*, an mRNA-degrading endonuclease that mediates programmed cellular death during stress (Engelberg-Kulka et al. 2006).

L. plantarum is known to produce a type of bacteriocin known as a plantaricin. Using sequence homology of plantaricins from the reference genome, five genes in *L. plantarum* ATCC-202195 were identified with sequence homology to plantaricins K, J, N, F, and E. While these proteins have been shown to have bactericidal activity, they pose no danger to human or other eukaryotic cells (Moll et al. 1999).

2.1.6. BIOGENIC AMINE PRODUCTION

Histamine: In lactic acid bacteria, production of histamine results from the catabolism of histidine by a histidine decarboxylase. A specific detection method for histidine decarboxylase genes has been developed internally based on the scientific literature and on the most updated genomic databases. Applied to *L. plantarum* ATCC-202195, the method failed to detect a histidine decarboxylase gene. Consequently, *L. plantarum* ATCC-202195 is unlikely to produce histamine.

Tyramine: In lactic acid bacteria, production of tyramine results from the catabolism of tyrosine by a tyrosine decarboxylase. A specific detection method for tyrosine decarboxylase genes has been developed internally based on the scientific literature and on the most updated

genomic databases. Applied to *L. plantarum* ATCC-202195, the method did not detect a tyrosine decarboxylase gene. Consequently, *L. plantarum* ATCC-202195 is unlikely to produce tyramine.

2.1.7. HEMOLYSIS

Some bacterial species can induce hemolysis through the production of hemolysin. There are three potential hemolytic outcomes of this assay including alpha (incomplete) hemolysis, beta (complete) hemolysis, and no hemolysis.

Cultures of *L. plantarum* ATCC-202195 were grown overnight in strain-specific medium and temperature. An aliquot of the overnight culture was streaked onto prepared blood agar plates and incubated at strain-specific temperature for 72 hours. Plates were observed for growth and hemolysis at 48 and 72 hours. *L. plantarum* ATCC-202195 induced alpha-hemolysis when cultured on sheep's blood agar plates.

Alpha-hemolysis is usually caused by the reduction of hemoglobin to methemoglobin due to hydrogen peroxide produced by bacteria. The cell membrane is still intact for alpha-hemolyzed red blood cells. There are multiple metabolic pathways that lead to hydrogen peroxide production in bacteria. *L. plantarum* ATCC-202195 has a protein, catalase KatE (EC 1.11.1.6), which converts $2\ H_2O_2 = O_2 + 2\ H_2O$. This protein limits but does not totally prevent the secretion of hydrogen peroxide (Archibald and Fridovich 1981; Guidone et al. 2013). During aerobic growth, *L. plantarum* and other gram-positive bacteria are known to secrete hydrogen peroxide and cause alpha-hemolysis (Archibald and Fridovich 1981; Barnard and Stinson 1996; Burnside et al. 2010; Guidone et al. 2013). Secretion of hydrogen peroxide does not pose a health risk and has been documented to limit enteric and vaginal pathogens (Atassi and Servin 2010; Beck et al. 2019; Sgibnev and Kremleva 2017).

2.1.8. ACID AND BILE SALT TOLERANCE

A variety of traits are believed to be relevant for surviving GI tract passage, the most important of which is tolerance to both the acidic conditions present in the stomach and concentrations of bile salts found in the small intestine. To demonstrate the tolerance of *L. plantarum* ATCC-202195 to acid and bile salts, an assay using a modified gastric juice was utilized to simulate contact with a moderately acidic stomach fluid environment. Bile tolerance was estimated by determining the % recovery on bile-containing agar medium compared to a non-bile-containing control medium.

Culture was obtained from seed vials, inoculated into strain-specific medium, and grown overnight. An aliquot of the overnight broth culture was pelletized, washed, and resuspended. An aliquot of the resuspended pellet was mixed with tempered gastric juice (hydrochloric acid and pepsin [0.32%] at pH 3.5). An aliquot was immediately diluted and plated in MRS agar with and without 0.3% ox-gall bile salt for a T0 control. The balance of sample in gastric juice was incubated for one hour at which time the final aliquot was taken for T1 plating using the same MRS media. Plates were allowed to solidify and incubated at 37ºC under anaerobic conditions (anaerobic jars) for 72 hours. *L. plantarum* ATCC-202195 exhibited 89% survival following exposure to a low pH pepsin solution and greater than 80% survival in bile salt solutions.

2.1.9. D(-)/L(+)-LACTIC ACID PRODUCTION

Lactic acid is the most important metabolic product of fermentation and a defining feature of lactic acid bacteria. Due to its molecular structure, lactic acid has two optical isomers, L(+)-lactic acid and its mirror image D(-)-lactic acid. Both isomers can be generated during pyruvate metabolism through two separate enzymes. L-lactate dehydrogenase (LDH; EC 1.1.1.27) converts pyruvate to L-lactate and D-lactate dehydrogenase (D-LDH; EC 1.1.1.28) converts pyruvate to D-lactate Additionally, some *Lactobacillus* strains contain the enzyme lactate racemase, LarA, (EC 5.1.2.1), which converts L-lactate to D-lactate and vice versa. The genome of *L. plantarum* ATCC-202195 contains all three enzymes, LDH, D-LDH, and LarA, indicating that this strain can produce both L(+)-lactic acid and D(-)-lactic acid.

An assay was performed by Danisco to determine the proportion of each isomer produced by *L. plantarum* ATCC-202195. Cultures of the strain were grown overnight in strain-specific medium and temperature. An aliquot of overnight broth culture was pelletized, and the supernatant was retained. The supernatant was inactivated at 80ºC for 15 minutes and diluted to achieve the desired total lactic acid concentration range. D(-)/L(+)-lactic acid detection was performed using a colorimetric measurement of lactate dehydrogenase enzyme activity for the respective isomers. Total lactic acid and isomer specific measurements were determined relative to control samples. *L. plantarum* ATCC-202195 produced both D(-)-lactic acid and L(+)-lactic acid isomers. Of the total lactic acid produced, 59.9% was D(-)-lactate and 40.1% was L(+)-lactate.

Concerns about D(-)-lactic acid-producing live microorganisms ingested by infants came from indications that ingestion may lead to an increase in D(-)-lactic acid levels in the blood, producing D-lactic acidosis at sufficiently high levels. However, research (e.g., Papagaroufalis et al. 2014) has demonstrated that healthy infants consuming live microorganism strains which produce D(-)-lactic acid do not exhibit clinically significant changes in D(-)-lactic acid in the blood. Papagaroufalis et al. (2014) compared urinary D(-)-lactate concentrations during the first 28 days of life of healthy infants given formula with or without D(-)-lactic acid-producing bacteria and found that, while levels were transiently raised (though not to a level deemed adversive), no increase was observed beyond 2 weeks. Lukasik et al. (2018) reviewed 5 randomized clinical trials covering 544 healthy infants as well as several case reports and experimental studies. They determined that "no clinically relevant adverse effects of d-lactic acid-producing probiotics and fermented infant formulas were described in healthy children," and concluded that "probiotics and fermented formulas did not cause d-lactic acidosis in healthy children." The gut microbiome of breast-fed infants already contains D-lactic-acid-producing bacteria such as *L. reuteri* and *L. plantarum* (Connolly et al. 2005; Martín et al. 2003; Vanderhoof et al. 1998). Based on this information, there is no safety concern in healthy children related to the ability of *L. plantarum* ATCC-202195 to secrete D(-)-lactic acid. It may be noted that FDA previously approved at least three D(-)-lactate producing strains for use in infant formula, including *L. reuteri* (GRN 254 and 410), *L. helveticus* Rosell®-52 (GRN 758), and *L. acidophilus* NCFM (GRN 865)

However, D-lactic acidosis may occur in individuals after jejuno-ileal bypass surgery, short bowel syndrome, or other causes of carbohydrate malabsorption, when large amounts of unabsorbed carbohydrates reach the colon and the colon is colonized with an appreciable number of D(-)-lactate-producing bacteria. The clinical presentation of the disorder is characterized by recurrent episodes of metabolic acidosis due to serum D-lactate concentrations >0.5 mmol/L that

may even reach levels >10 mmol/L, and encephalopathy with severe neurologic impairment (Thurn et al 1985; Ewaschuk et al 2005; Kang et al 2006).

In view of the potential risk of development of D-lactate acidosis and encephalopathy in infants and children with gastrointestinal conditions, products containing *L. plantarum* ATCC-202195 should be labelled with a statement indicating that the product should not be taken by patients with marked carbohydrate malabsorption such as patients with short bowel syndrome or gastrointestinal bypass surgery, and patients with increased risk of developing small intestinal bacterial overgrowth such as gastrointestinal dysmotility, or long term use of proton pump inhibitor or opioid medication.

Cautions regarding use of *L. plantarum* ATCC-202195 in immune-compromised individuals and others under medical supervision are similar to those for other administered bacteria and many food ingredients and do not compromise the GRAS status of the intended use of the strain.

2.1.10. SUMMARY AND CONCLUSIONS

Genomic sequencing confirmed the identity and taxonomy of *L. plantarum* ATCC-202195. The genome sequencing and assembly resulted in 12 contigs with a combined genome size of 3.24 MB and 44.3% GC, which is similar to other *L. plantarum* strains. Comparisons of protein coding genes between *L. plantarum* ATCC-202195 and *L. plantarum* strain Lp-115 (DGCC 4715) revealed gene differences that distinguish *L. plantarum* ATCC-202195 from *L. plantarum* Lp-115. However, it also revealed 99.1% similarity between *L. plantarum* ATCC-202195 and the GRAS strain *L. plantarum* Lp-115, the type strain *L. plantarum* WCFS1, and another commercially available strain, *L. plantarum* JDM1. Therefore, the identity of this strain was confirmed as belonging to the species *L. plantarum* with high confidence.

Further documentation of safety of *L. plantarum* ATCC-202195 comes from the antibiogram as well as evaluation of virulence and toxin genes. The antibiogram for this strain resulted in MIC values below or equal to the microbial break points defined by EFSA for *Lactobacillus plantarum/pentosus* with the exception of kanamycin, resistance to which is due to the presence of nucleotidyltransferase (*ant9*-la). This gene is genomically based and not located within 5000 bp upstream or downstream of any mobile elements (transposase, insertion sequences, etc.) and is therefore not considered a risk for transfer of antibiotic resistance genes. No virulence or toxin genes were identified based on a genome survey using the VFDB database and RAST. While a few genes were identified in *L. plantarum* ATCC-202195 as potentially associated with virulence (toxin-antitoxin system, bacteriocins, hemolysin), further analysis demonstrated that these elements were not associated with virulence and as such it was concluded that these genes do not present a risk for human health. Assessment of the toxin-antitoxin systems showed that they were associated with intracellular regulatory mechanisms. Similarly, the assessment of the genomes for the bacteriocins produced by this strain (known as plantaricins) indicated no danger to human or other eukaryotic cells. *L. plantarum* ATCC-202195 was demonstrated to promote alpha-hemolysis in culture conditions, which was determined to be most likely mediated through secretion of hydrogen peroxide. This does not pose a health risk. In summary, *L. plantarum* ATCC-202195 does not pose a risk of antibiotic resistance transfer, virulence, or toxicity and as such does not present a safety hazard or risk to human hosts.

Taken together, *L. plantarum* ATCC-202195 is well characterized to the strain level and shows close homology to a strain (*L. plantarum* Lp 115) with a documented history of use in food and recognized as GRAS by the FDA.

2.2. Manufacturing Process

2.2.1. FERMENTATION MEDIA

The materials for the production of *L. plantarum* ATCC-202195 include the seed culture and the fermentation media. The seed lot is fully characterized as *L. plantarum* ATCC-202195 to verify its identity prior to production. Ingredients utilized in the fermentation media for the production of *L. plantarum* ATCC-202195 are approved food-grade substances. These ingredients do not contain allergens (proteins) as per the Food Allergen Labeling and Consumer Protection Act (FALCPA), including protein derived from milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts, soybeans, or sesame seed. Furthermore, these ingredients are not derived from gluten containing grains (i.e. barley, oats, rye, wheat) nor grown on media derived from such grains.

2.2.2. FORMULATION INGREDIENTS

A mixture of approved food-grade non-allergenic (as per above do not contain allergens/proteins as specified in FALCPA) cryoprotectants, added to the fermentate prior to the freeze-drying process, are incorporated into the cell membrane to protect the cells from freeze-drying, allowing them to remain viable throughout the lyophilization process.

2.2.3. MANUFACTURING PROCESS

Fermentation begins with the culture working seed through large scale fermentation. The bacteria are then harvested and concentrated into pellet form, and then freeze-dried in a qualified facility. The manufacturing process for production of cultures is batch-type fermentation in which a blend of proteins, carbohydrates, and vitamins and minerals are blended with water, sterilized, and inoculated with the selected bacteria. Each fermentation product has defined growth medium and fermentation conditions (pH, temperature). *L. plantarum* ATCC-202195 is manufactured in compliance with the U.S. Food and Drug Administration's current Good Manufacturing Practice guidelines (21 CFR 117) in FDA regulated and inspected facilities. All ingredients utilized are food grade and approved for use by the FDA.

L. plantarum ATCC-202195, produced by Danisco as a single strain with no added excipients, does not contain allergens as determined by the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA), including protein derived from milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts, soybeans, and sesame seed. Allergen control practices are included in the above discussed Quality Systems. Equipment that has come in contact with potential allergens are managed by preventive controls. Cleaning is verified through visual examination and testing. Testing includes total organic carbon and allergen swabbing using 3M™ Clean Trace™ Surface Protein – Allergen Swabs. These allergen swabs are validated for a range of allergenic proteins, including egg, milk, gluten, soy, peanut, almond and buckwheat. A visual reading of a color change indicates the level of cleanliness by detecting as

little as 3µg of allergen on surfaces and in solution. Any corrective action is taken immediately by re-cleaning and re-testing.

Neither *Lactiplantibacillus* nor *L. plantarum* are known to be food allergens (Castellazzi, 2013) and there have been no reported allergenic responses in the *L. plantarum* clinical studies.

The manufacturing process is summarized below in Figure 4.

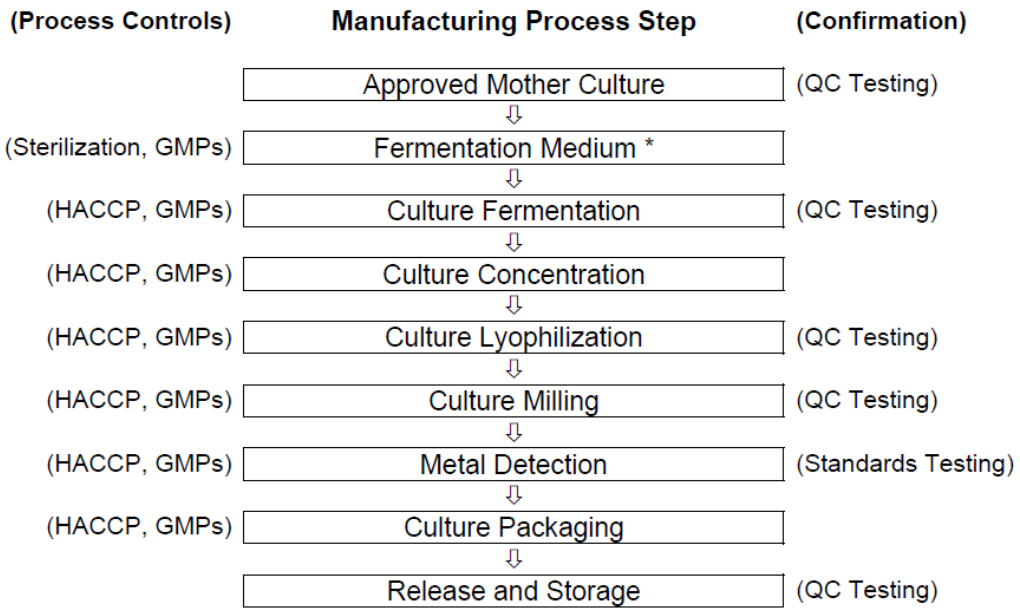


Figure 4: Live Microbial Ingredient Manufacturing Process with Process Controls.

2.2.4. MASTER SEED

The source organism used is *L plantarum* ATCC-202195, DGC#12988. A Master Seed repository is maintained for each bacterial strain at the Danisco Global Culture Center (DGCC) in Niebüll, Germany. The repository is a collection of purified, tested, and qualified Master Seed stocks derived from single strain isolates stored at -180°C in liquid nitrogen to maintain long-term cell viability. Danisco independently verifies the identity of each organism. Accordingly, each seed-lot in the culture bank is fully characterized to ensure the identity of the seed strains. From the seed vials, Danisco produces concentrated starter for the industrial fermentation.

Whole-genome sequencing is conducted to establish the identity of each master-seed batch to the genus, species, and strain level prior to preservation. The microbiological quality of the Master Seeds is determined by testing for microbiological contamination at the DGCC. These identity and purity specifications are absolute acceptance criteria for the Master Seeds. If a Master Seed vial lot fails any of the required tests, the lot is placed on QC hold to prohibit use and the lot is subsequently destroyed.

2.2.5. WORKING SEED

All Working Seeds are prepared under controlled conditions beginning with demonstration that the Master Seed stock meets established acceptance criteria and each new lot

of Working Seeds is held in "quarantine" pending QC testing (strain identity and purity as described for the Master Seeds) and release. If the Working Seed vial lot fails any of the required tests, the lot is placed on QC hold and destroyed. Qualified, tested Working Seed stocks are stored at -80°C until used in production fermentation.

The use of tandem Master and Working seed inventories reduces the risk of genetic drift over time due to excessive sub-culturing of strains and ensures the integrity of the strain collection. All steps in the preparation of Master and Working seed are documented in a specified database, allowing traceability of every seed preparation down to each single batch of raw material used.

2.2.6. FERMENTATION PROCESS

Fermentation begins by withdrawing one of the working seed vials. Scaling-up proceeds via a series of fermentations until a commercial size batch is complete. The fermentation process begins in a 100-ml vessel, transferred sequentially to a 6-L vessel, a 300-L vessel, and finally to the largest vessel, where fermentation is completed.

The fermentation production process is a closed system with no external exposure throughout seed inoculation to cell harvest. Prior to the start of each fermentation batch, all mixing tanks, heat exchangers, lines, fermenters, and centrifuges are cleaned via automated clean-in-place systems. Systems are then steam sanitized prior to product contact**.**

L. plantarum ATCC-202195 has an optimal pH and temperature for growth, both which are maintained in the large-scale fermenter. As each organism produces organic acids during metabolism, an ammonium hydroxide base is injected into the medium to maintain pH at the proper set point in order to maintain the optimum pH during growth.

Two methods are employed to measure growth in the fermenter. First, a flow meter on the ammonium hydroxide feed line to the fermenter measures the volume of base used to maintain optimum growth pH of the culture. The base addition rate is proportional to the acid developed in the fermentation, which is proportional to cell growth rates. Second, the pH in the fermenter is monitored on digital display and on recording charts. By consulting these charts, the growth characteristic of a given fermentation can be determined.

Fermenters are normally cooled to stop the fermentation when the pH and base addition data indicate that the fermentation has entered stationary phase. Cooled fermentate is pumped to continuous flow centrifuges and the bacteria are concentrated and cryoprotectants as described in Section 2.2.2 are added. The cooled concentrate and cryoprotectant mixture is pelletized by immersion of concentrate droplets in liquid nitrogen. These concentrate pellets are then freeze-dried in a qualified facility.

A schematic representation of the fermentation process is presented in Figure 5.

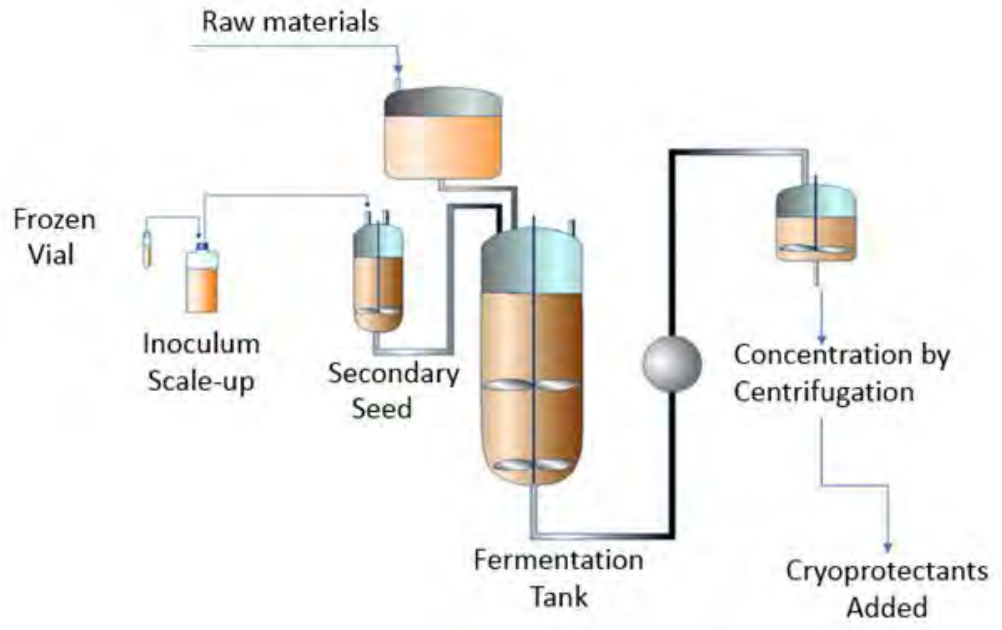


Figure 5: Fermentation Process for *L. plantarum* ATCC-202195.

2.2.7. MILLING

The freeze-dried pellets are milled according to standard procedures utilizing a Fitzpatrick mill fitted with a mesh screen operating at 2000 rpm. Production batch records contain mill charge and appropriate operator sign-off. A schematic overview of the freeze-dry and milling process is presented in Figure 6.

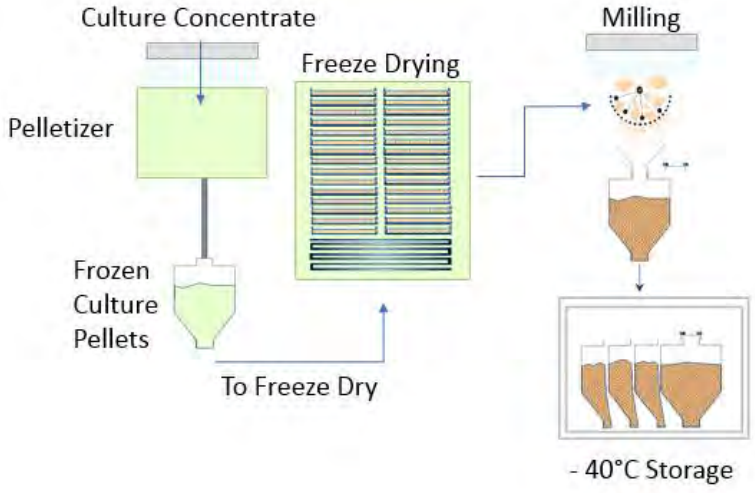


Figure 6: Freeze Drying and Milling Process Diagram for *L. plantarum* ATCC-202195.

2.2.8. BLENDING

The blending process is performed under 21 CFR 111 and 21 CFR 117 cGMPs. Blending can occur by blending in Marion and/or V-blender mixers, or by utilizing Intermediate

Bulk Containers. The processes are slightly different but are used interchangeably depending on available resources. Milling and ingredient addition is performed in a controlled environment. The ingredients added to the blender, the milled pellets and excipient, are documented on production batch records containing traceability information and appropriate operator sign off.

Milled pellets, along with the excipient, are added to the blender and allowed to mix for an established amount of time prior to packaging to ensure homogeneity. Product is dispensed out of the blender and through a metal detector prior to packaging.

2.2.9. PACKAGING

Bulk packaging of the product is carried out in a controlled environment. The HVAC system consists of an air-handling unit with air-cooled direct expansion type condenser including ducted heater for reheating. Pressure relief dampers operate in conjunction with the fresh-air intake system maintaining the whole area at a positive pressure to prevent contaminant infiltration to the packaging room. The area design conditions include maintaining a dry bulb temperature of 72°F and a relative humidity of ≤35%.

2.2.10. QUALITY SYSTEMS

The manufacturing plants have fully implemented HACCP plans, Standard Operating Procedures, and Quality Control programs to ensure the quality of each product and possess ISO FSSC 22000 food safety certification. A quality control laboratory is maintained on site. Quality control (QC) personnel are qualified by training and experience to test products and to release product based on specifications. In addition, an external, third-party laboratory with ISO 17025 certification performs QC testing under contract.

The Quality Control unit utilizes a SAP computer quality control system for the specification, quality control data entry, and product release. No product can be released for use without acceptance by the Quality Control unit according to specified acceptance criteria. Each bacterial fermentation product must meet specifications and have a confirmation of identity (compared to the Master Seed) by 16S rDNA sequence analysis or RiboPrinter analysis for approval of release of the product. Microbiological testing is performed using standard methods.by trained QC microbiologists at the manufacturing facility and by the abovementioned certified external laboratory.

Cleaning and quality testing including allergen control of the process rooms and equipment are under the control of Manufacturing and Quality Assurance, following the appropriate Standard Operating Procedures (SOPs). Fermentation rooms are isolated from the freeze-drying processes and access is controlled. Materials cannot enter the milling and blending process areas prior to cleaning, sanitation, and subsequent surface testing for cleanliness via ATP testing. Room access is controlled by appropriate signage. Operator sign-off for cleanliness, sanitation, and testing is required on the lot batch ticket.

Process rooms are segregated from other manufacturing areas with appropriate closures. Room air quality is controlled via HEPA air filtration of incoming air and maintenance of positive pressure in the process rooms relative to adjacent processing areas. HEPA filtration operation is monitored for performance; air quality is monitored monthly by Quality Assurance. Operators may not bring materials into process areas where HEPA filtration is not functioning to

specification. Operators sign-off on the lot batch ticket for temperature and humidity.

Rooms and equipment used in manufacturing are approved for production only after cleaning, sanitization, and quality testing. Prior to qualification of the process room for production, as specified in the appropriate SOP, the blending room is sprayed from ceiling to floor with 145-160°F water. All large equipment having any product contact surfaces is thoroughly scrubbed, foamed with a neutral detergent cleaner, rinsed with cold water, sanitized with an acid/iodine-based sanitizer at 50 ppm, and re-rinsed with cold water. The floor is sanitized with an acid/iodine sanitizer at NLT 50 ppm.

Process rooms and equipment are tested and released via sign off by Quality Assurance following cleaning and sanitation for microbial contamination and test results are entered on the batch tickets. ATP and Microbiological swabs are taken after cleaning and sanitation. Room and equipment surfaces must be negative by test in order to qualify for use in production. Batch records are maintained as per Standard Operating Procedures and are provided to Quality Assurance for each lot produced. Quality Assurance is responsible for batch ticket review. The Hazard Analysis and Critical Control Point (HACCP) Flow Diagram is presented in Figure 7.

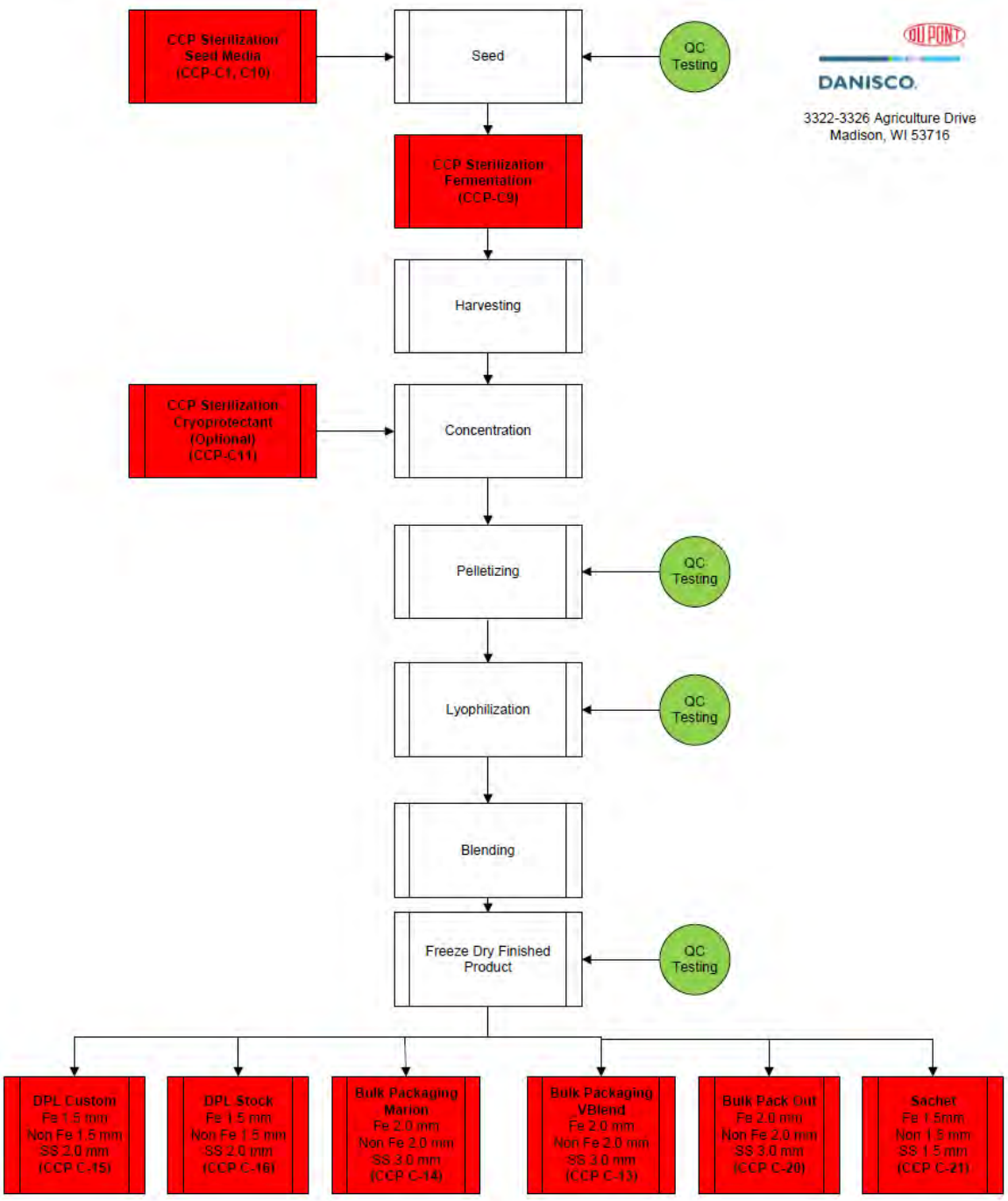


Figure 7: Hazard Analysis and Critical Control Point (HACCP) Flow Diagram.

2.3 Product Specifications and Compositional Variability

2.3.1 PRODUCT SPECIFICATIONS

Danisco has established specifications to assure that each batch of *L. plantarum* ATCC-202195 produced is of food-grade quality.

Table 5. Product Specifications for *L. plantarum* ATCC-202195.

Parameter	*L. plantarum* ATCC-202195	
	Specification	**Reference Method**
Total viable count/ Assay (cfu/g)	4.0-7.5x10^{11}cfu/g	ISO 7889/IDF 117
Arsenic	<100 µg/kg	AOAC methods*, 2011.19 and 993.14
Cadmium	<200 µg/kg	AOAC methods*, 2011.19 and 993.14
Lead	<500 µg/kg	AOAC methods*, 2011.19 and 993.14
Mercury	<50 µg/kg	AOAC methods*, 2011.19 and 993.14
Tin	<5000 µg/kg	AOAC methods, 2011.19 and 993.14
Cronobacter sakazakii	Absent in 25 g	ISO 22964:2017
Enterobacteriaceae	Absent in 10 g	ISO 21528-1
Salmonella spp.	Absent in 25 g	AOAC 2004.03
Sufite-reducing bacteria	<10 cfu/g	ISO 15213:2003(E)
Yeast and mold	<10 cfu/g	USP 61
Bacillus cereus	<100 cfu/g	AOAC 980.31
Coliforms	<3 cfu/10 g	AOAC 966.24
Escherichia coli	<3 cfu/10 g	AOAC 966.24
Listeria monocytogenes	Absent in 25 g	AOAC 2004.06
Staphylococcus aureus	<10 cfu/g	AOAC 975.55
* Paquette et al. 2011		

2.3.2 COMPLIANCE WITH SPECIFICATIONS

Three non-consecutive batches of *L. plantarum* ATCC-*202195.* were analyzed and the results compared with food-grade specifications. As shown in Table 5, all tested batches were in compliance, demonstrating that the production process is in control.

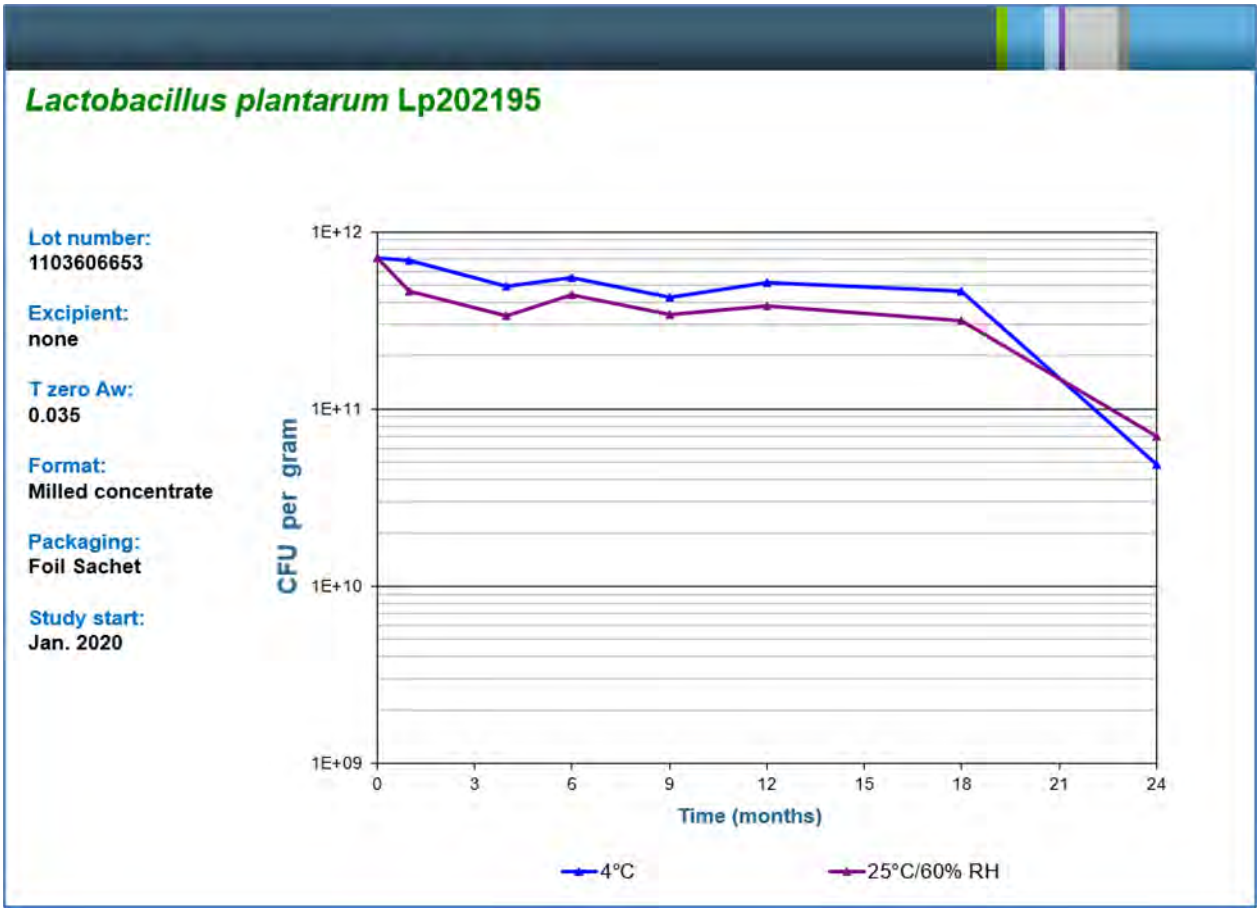
Table 6. Analysis of Compositional Variability of *L. plantarum* ATCC-202195.

Parameter	Specification	Batch		
		1103606642	**1103606653**	**1103606649**
Total viable count/Assay (cfu/g)	4.0-7.5x10^{11} cfu/g	5.60x10^{11} cfu/g	5.53x10^{11} cfu/g	4.83x10^{11} cfu/g
Arsenic	<100 µg/kg	24.3 µg/kg	63.3 µg/kg	44.9 µg/kg
Cadmium	<200 µg/kg	<10.0 µg/kg	<10.0 µg/kg	<20 µg/kg
Lead	<500 µg/kg	<10.0 µg/kg	24.3 µg/kg	33 µg/kg
Mercury	<50 µg/kg	<10.0 µg/kg	<10.0 µg/kg	<10 µg/kg
Tin	<5000 µg/kg	14.6 µg/kg	40.3 µg/kg	27.9 µg/kg
Cronobacter sakazakii	Absent in 25 g	Negative	Negative	Negative
Enterobacteriaceae	Absent in 10 g	Negative	Negative	Negative
Salmonella spp.	Absent in 25 g	Negative	Negative	Negative
Sufite-reducing bacteria	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Yeast and mold	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Bacillus cereus	<100 cfu/g	<100 cfu/g	<100 cfu/g	<100 cfu/g
Coliforms	<3 cfu/10 g	Negative	Negative	Negative
Escherichia coli	<3 cfu/10 g	Negative	Negative	Negative
Listeria monocytogenes	Absent in 25 g	Negative	Negative	Negative
Staphylococcus aureus	<10 cfu/g	Negative	Negative	Negative

2.4 Shelf-Life Stability

Stability studies under controlled conditions have been conducted for *L. plantarum* ATCC-202195. The results of most recent data (September 2020) are presented below in Figure 14. The stability of *L. plantarum* ATCC-202195 was analyzed at refrigerated (4°C) and ICH Zone 2 conditions (25°C/60%RH) in a foil sachet over at 24-month period. Viability cell counts were evaluated at regular intervals during storage. The data below shows acceptable stability to allow deliverability of a target amount of live culture throughout shelf-life of the final product.

Figure 14. Stability Curve for *L. plantarum* ATCC-202195



Part 3: Intended Use and Dietary Exposure

Intended uses are non-exempt infant and toddler formulas based on milk (intact or partially hydrolyzed) or soy protein, extensively hydrolyzed exempt formula, conventional foods including foods for infants and young children, juice and drinks for infants and young children, milk products including flavored milk beverages, meal replacement and powdered drink mixes, milk product analogs including soy, soy products, processed fruits and fruit juices, confectionary snacks and baked goods. It is not intended to be used by certain individuals under medical supervision, including immune-compromised individuals, infants with marked carbohydrate malabsorption such as short bowel syndrome or gastrointestinal bypass surgery, or patients with increased risk of developing small intestinal bacterial overgrowth including those with gastrointestinal dysmotility, or long term use of proton pump inhibitor or opioid medication.

Food Category	Food Use	Max	Min
Formula for infants and young children- milk, soy, partially hydrolyzed Extensively Hydrolyzed exempt infant formula	Infant formula (0-12 months), including extensively hydrolyzed exempt formula	10^8 cfu/g provides 11B ($1.1x10^{10}$) cfu/d	10^6 cfu/g to provide 1.1 $x10^8$ cfu/d
Formula for infants and young children	Toddler formula (9 mo and above)	10^8 cfu/g provides 11B ($1.1x10^{10}$) cfu/d	10^6 cfu/g to provide 1.1 $x10^8$ cfu/d
Foods for infants	Cereal and grain products, dry ready-to-eat cereals, puffs/melts, fruit and vegetable purees	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Foods for young children	Cereal and grain products, dry ready-to-eat cereals	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Juice and drinks for infants and young children	Juice/drinks/Dry-blended beverages	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Milk products	Yogurt, spoonable and drinkable, smoothies	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Milk product analogs including soy	Smoothies, high-protein beverages, yogurts (non-dairy)	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Soy products		$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Powdered meal replacement or nutritional beverages		$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Processed fruits and fruit juices	Fruit juices and nectars (including fruit-based beverages)	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving

Confectionary snacks	Candies	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving
Baked Goods	Cereal and Nutrition Bars	$2x10^{10}$/250 g serving ($8 x10^7$ cfu/g)	1 x 10^9 cfu/250g serving

DuPont proposes the use of *L. plantarum* ATCC-202195 in non-exempt infant formulas, extensively hydrolyzed exempt infant formulas, and "toddler formulas" (the latter referring to products intended for infants $\geq$ 9 months and young children from 12 months of age and older) at a level of 1 x 10^8 cfu/g powdered formula for consumption by term infants and toddlers from the time of birth through 2 years of age. This level of *L. plantarum* ATCC-202195 is intended to ensure a minimum concentration of 10^6 cfu/g throughout the 12-18 month shelf life of the infant formula powder.

Dietary exposure in infants and young children

Assuming infant formula is consumed as the sole source of nutrition for infants from birth to 6 months of age, the estimated daily intake (EDI) of *L. plantarum* ATCC-202195 can be determined from estimated formula intake, an average of 800 ml/day for infants 0-6 months of age. As reported in the 2005-2012 NHANES database, mean intake of infant formula by this age group was 834 ml (Grimes et al 2017). Using this intake volume and dilution of infant formula prepared according to label instructions (i.e., 13.5 g powder/100 ml formula), Danisco estimates that the daily intake of *L. plantarum* ATCC-202195 microorganism would not exceed $1.1x10^{10}$ cfu per day . Assuming an approximate average body weight at birth of 3.55 kg and at 6 months of 7.6 kg (WHO), the maximum EDI is $2.8x10^9$ cfu/kg bw/day for newborns and $1.3x10^9$ cfu/kg bw/day for older infants. These exposures are well within the levels shown to be safe.

Children up to 2 years of age (also referred to as toddlers) are the population with potentially the highest dietary exposure to *L. plantarum* ATCC-202195 because they may consume both toddler formula and conventional foods. A conservative estimate of cumulative dietary exposure to *L. plantarum* ATCC-202195 by children up to 2 years of age can be determined from daily food intake data from 2007- 2016 NHANES. As reported in the 2020 Dietary Guidelines, toddlers at two years of age consume approximately six servings (50-250 g) of food and 2 servings of beverage as milk (1 cup, 250 ml) a day (USDA and HHS 2020). Assuming half of these foods and beverages contain added *L. plantarum* ATCC-202195 at an addition level of 2 $x10^{10}$ cfu per serving, total dietary intake from conventional foods and beverages would be $8x10^{10}$ cfu per day. Using the average weight at 2 years of 11.85 kg, this would be $6.8x10^9$ cfu/kg bw/day. If total beverage intake (i.e., 2 -1 cup servings) were replaced with a toddler formula containing added *L. plantarum* ATCC-202195 (addition level of 10^8 cfu/g) and half of the foods contained the added strain, total dietary intake would be $6.7x10^{10}$ cfu per day, $5.7X10^9$ cfu/kg bw/day.

The above demonstrates that with the addition of *L. plantarum* ATCC-202195 to infant and toddler formula, cumulative intake does not appear to increase in comparison to intake of conventional foods with addition of this strain.

Dietary exposure in the general population

The average individual consumes only about 20 servings/day of all foods combined (Millen et al., 2005), and assuming half of these foods would be supplemented with the strain, a conservative estimate of the total EDI at 2 x 10^{10}cfu /serving times 10 servings/day would be a maximum intake of 2x10^{11} cfu/person/day of conventional food or 2.9 x10^{9} cfu/kg bw/day based on average adult bodyweight of 70 kilograms. It is unlikely that a consumer would consume 10 servings of foods containing the strain. Furthermore, the number of cfu will decline over the shelf-life of the food.(Kailaapathy and Chin, 2000) for a variety of reasons. The incorporation of microbial cultures into processed food products and subsequent storage can be stressful for the bacterial cells, and their viability may decrease due to the food matrix chosen, water activity, and pH of the final product (Min et al., 2017). Accordingly, it is expected that the maximum ingestion would be less than the 2x10^{11} cfu/day (2.9 x10^{9} cfu/kg bw/day) and well within the levels that have been shown to be safe.

Part 4: Self-Limiting Levels of Use

There are no self-limiting levels of use of *L. plantarum* ATCC-202195 in foods or infant formula.

Part 5: Experience Based on Common Use in Food

The statutory basis for Danisco's conclusion of the GRAS status of the intended use of *L. plantarum* ATCC-202195 is scientific procedures rather than common use in food prior to 1958.

Part 6: Narrative

6.1. History of Consumption of *L. plantarum*

L. plantarum has been included as one of the microorganisms intentionally added to food that should be regarded as safe based on EFSA's comprehensive assessment of safety that resulted in the system designated "Qualified Presumption of Safety" (QPS). A list of qualifying microorganisms was compiled to represent those that meet the criteria of QPS and do not raise safety concerns (EFSA, 2007). This QPS list is frequently reviewed by the EFSA Panel on Biological Hazards (BIOHAZ) and the *L. plantarum* listing has remained unchanged since the QPS list was compiled.

L. plantarum is a natural health product (NHP) in Canada and is included in Health Canada's probiotics monograph, which requires documentation of safety.

FDA acknowledged the strain *L. plantarum* ATCC-202195 in New Dietary Ingredient Notification (NDIN) #198 from Kups International and formerly referred to as NDIN #171, submitted February 4, 2003, as a dietary ingredient under subpart (E) of section 201(ff)(1) of the FD&C Act, following review of an NDI notification in 2003. The notification described the inclusion of the dietary ingredient *L. plantarum* ATCC-202195 at a daily serving level of up to 1.6×10^{11} cfu/day for use in dietary supplements. *L. plantarum* ATCC-202195 has been lawfully marketed in the US as a dietary substance intended for the human diet for over 17 years.

Subsequently, the FDA acknowledged the strain *L. plantarum* ATCC-202195, submitted as HOWARU Lp-202195™ in NDIN #1198 from Danisco USA, Inc, submitted February 10, 2021. The notification described the inclusion of the dietary ingredient intended for chronic daily use in infants aged less than 1 year, older children aged 1-2 years, and adolescents aged 13-17 years at a serving size of up to 1×10^9 cfu/day, and in adults at a serving size of 500 billion cfu/day for adults.

Numerous strains of *L. plantarum* have been determined to be GRAS for food use with FDA indicating that it had no questions regarding those determinations. These include *L. plantarum* 299v (GRN 685, closed October 31, 2017), *L. plantarum* Lp-115 (GRN 722, closed February 16, 2018), *L. plantarum* ECGC 13110402 (GRN 847, closed September 30, 2019), *L. plantarum* DSM 33452 (GRN 946, closed February 5, 2021), and *L. plantarum* CECT 7527, CECT 7528, and CECT 7529 (GRN 953, closed February 5, 2020).

L. plantarum ATCC-202195 shares 99.1% sequence identity with *L. plantarum* Lp-115, the microorganism notified as GRAS in GRN 722 for use in conventional foods at a serving level of up to 10×10^9 cfu/day.

6.2. Safety of *L. plantarum* Strains: Oral Toxicity and Genotoxicity Studies

6.2.1. TOXICITY STUDY OF *L. PLANTARUM* STRAIN ATCC-202195

A subchronic oral toxicity study of *L. plantarum s*train ATCC-202195 was reported, but not published, by Bauter (2020)[1]. The study was conducted in compliance with OECD guideline

[1] Since this study is unpublished, it is regarded solely as corroborating information to the data that are generally available.

No. 408 for testing of chemicals and food ingredients as well as the FDA's toxicological principles for the safety assessment of food ingredients (Red Book). Forty adult Sprague-Dawley CD® IGS rats (20 males and 20 females) were equally distributed into two groups (10/sex/group). The rats used on test were randomly distributed, stratified by body weight, among the dose and control groups on the day of study commencement. Group 1 animals served as the vehicle control group and those in Group 2 were administered 2000 mg/kg bw/day of *L. plantarum* ATCC-202195. The test substance was administered daily via gavage for 90 days at a dose volume of 10 ml/kg bw/day with the test article dissolved in distilled water at a concentration of 200 mg/ml w/v. The dose was selected based on a proposed clinical dose that was not expected to cause marked toxicity, and the stability, homogeneity, and viability were assessed at the beginning, middle, and end of the dosing phase.

Prior to study initiation and towards the end of the study, the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. The animals were observed for viability, signs of gross toxicity, and behavioral changes at least once daily during the study, and weekly for a battery of detailed clinical observations. Motor activity and functional observational battery were performed towards the end of the study.

Animals were group-housed and given tap water and rat chow *ad libitum*. Body weights were recorded twice during acclimation, including prior to test initiation (Day 1), weekly thereafter, and prior to sacrifice. Feed consumption measurements per cage were taken to correlate with body weight measurements. Urine and blood samples were collected on Day 92/93 (for males and females, respectively) for coagulation, urinalysis, hematology, and clinical chemistry determinations.

The hematological evaluation include assessment of erythrocyte count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, absolute reticulocyte count platelet count, total white blood cell and differential leukocyte count, and mean corpuscular hemoglobin concentration. The clinical chemistry analysis included assessment of serum aspartate aminotransferase, serum alanine aminotransferase sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, blood creatinine, total cholesterol, triacylglycerol, fasting glucose, total serum protein, albumin, globulin, calcium, inorganic phosphorus sodium, potassium, chloride, high-density lipoprotein, and low-density lipoprotein.

Rats that died in the course of the study were examined for the cause of death on the day the observation was made. Rats were evaluated for gross lesions. At the end of the study, gross necropsies and histological evaluation of selected organs and tissues were performed on all study animals. Histological examination was performed on the preserved organs and tissues of the animals from the control and dose groups as well as from any animal that died during the course of the study. In addition, gross lesions of potential toxicological significance noted in any test groups at the time of terminal sacrifice were also examined.

Potential translocation of the test organism into systemic circulation and all organs was investigated. A sample of whole blood was collected, and sections of liver and mesenteric lymph nodes were excised and maintained on ice. Aliquots of selected homogenized and/or diluted samples were plated on MRS plates and incubated at 38°C under anaerobic conditions for 72 hours or until colony growth was adequate for counting. All plates were visually inspected after incubation and individual colonies were counted.

The achieved dose of *L. plantarum* ATCC-202195 was $8.71x10^{11}$ cfu/kg bw/day. There were no mortalities or detailed clinical observation findings attributable to the oral administration of *L. plantarum* ATCC-202195. Two male rats were found dead on day 60 of the study; these deaths were attributable to gavage error. All clinical observations were considered incidental and of no toxicological significance. There were no test substance-related functional observational battery findings in male and female rats. All observations were noted similarly in controls, considered to be incidental, or present in a non-dose-dependent manner. Motor activity measurements for male and female rats administered *L. plantarum* ATCC-202195 were comparable to control. There were no test substance-related changes in hematology, coagulation, clinical chemistry, or urinalysis parameters. In addition, there were no adverse changes in body weight parameters, feed consumption, feed efficiency, macroscopic, or microscopic observations in male and female rats attributed to the oral administration of *L. plantarum* ATCC-202195. Statistically significant increases observed in male absolute weight over the course of the study generally corresponded to increases in mean daily food consumption and were considered to be non-adverse and of no toxicological significance. Increased sorbitol dehydro-genase activity in treated females was considered an incidental change as it was not associated with any microscopic changes in the liver. All other changes in clinical chemistry endpoints occurred sporadically, were within historical control ranges, and considered to be due to biological variance among rats.

Significant increases were observed in absolute kidney and liver weights in males and females, along with significant increases noted for the spleen and testes in males. Corresponding increases were noted in relative liver to terminal body weight in females, as well as for the liver, kidney, and testes relative to brain weight in males. With no clinical pathology or histopathological correlates, all significant changes noted in absolute and relative organ weights in male and female rats were determined to be non-adverse. The findings at terminal sacrifice were considered incidental and of no toxicological significance.

In the translocation analysis, there were no bacterial cells detected in the blood samples taken in either group. Lactobacilli and other facultative anaerobes were detected in the liver of one female from each of control and *L. plantarum* groups. Also, translocated bacteria were found in the mesenteric lymph nodes of rats of both sexes in the two groups. The difference in the number of translocated bacteria was not significant between *L. plantarum* and control groups. The fact that there were no bacteria detected in the blood and the highest number of detected bacteria were found in the lymph nodes indicates that the lymph node barrier was efficient in trapping bacteria and preventing them from spreading elsewhere in the body (Garcia-Tsao et al. 1995).

Under the conditions of the study and based on the toxicological endpoints evaluated, the no-observed-adverse-effect level (NOAEL) for the oral administration of *L. plantarum* ATCC-202195 was determined to be 2000 mg/kg bw/day, the single dose tested, equivalent to $8.71x10^{11}$ cfu/kg bw/day.

6.2.2. TOXICITY STUDIES OF OTHER STRAINS OF *L. PLANTARUM*

L. plantarum AF1

In a study reported by Lee et al. (2012), doses of *L. plantarum* AF1 at 0, 0.625, 1.25, 2.5, or 5.0 g/kg bw were administered by gavage to male and female ICR mice four times within a

24-hour period to assess its acute toxicity. Treated mice were observed for 14 days for mortality and signs of morbidity, including changes in body weight, feed intake, and other clinical findings. As there were no significant changes in the body conditions, body weights and other clinical signs as well as absence of gross lesion in the major organs, the LD_{50} for *L. plantarum* AF1 was estimated to be greater than the highest dose ingested (5 g/kg bw; the authors did not report exposure in cfu).

L. plantarum HK006 and HK109

The safety of *L. plantarum* HK006 and HK109 was assessed in a bacterial mutagenicity assay and in a 28-day study of repeated-dose oral toxicity in Wistar rats (Tsai et al. 2014). In the mutagenicity study, strains TA98 and TA1535 of *Salmonella typhimurium* were incubated with the test organisms in the presence or absence of metabolic bioactivation, using relevant positive controls for different mutagenicity endpoints. After an incubation period of 2-3 days at 37 °C, revertant colonies were counted. The study revealed that *L. plantarum* HK006 and HK109 did not induce any changes in revertant colonies in comparison with the negative control. The authors concluded that these strains of *L. plantarum* are not mutagenic under the conditions tested.

In the oral toxicity study, 4-week-old male and female Wistar rats were allocated to 3 groups (8 rats/sex/group) and were given placebo or *L. plantarum* at doses of $9x10^9$or $4.5x10^{10}$ cfu/kg bw/day daily for 28 days, during which the activity, behavior, and hair luster of each rat were observed and recorded daily. Water and feed intake and bodyweight were recorded. At the end of the supplementation period, animals were necropsied and blood samples were collected for assessment of hematological and biochemical parameters.

There were no noticeable changes in the general behavior of the animals, nor in body weight, feed and water intake, hematological parameters (all of which were within their normal ranges), or clinical chemistry parameters except for significant reductions in alkaline phosphatase and K^+ in both doses of the test products given to the female groups. Also, there were increases in glucose in both doses in comparison with controls while a significant decrease was reported in cholesterol and creatinine. Liver aspartate aminotransferase values were also significantly reduced in a dose-dependent fashion in female rats. However, the authors concluded that none of these was of any toxicological significance. Therefore, the oral NOAEL for *Lactobacillus plantarum* HK006 and HK109 was reported to be the highest tested dose of $4.5x10^{10}$ cfu/kg bw/day (Tsai et al. 2014).

L. plantarum KABP-031 and *L. plantarum* KABP-032

An acute oral toxicity study in the rat (Bosch et al. 2012a) was performed for the *L. plantarum* KABP-031 and KABP-032 strains. Eighteen 9-week-old Wistar rats, 9 of each sex, were equally divided into three groups (placebo, KABP-031, and KABP-032) and dosed in the morning on two consecutive days at a level of 5×10^{10} cfu/kg bw/day with the bulk powder strain suspended in water or just water and excipient powder in the placebo group. Clinical observations were made for 5 additional days after dosing. Individual body weights were recorded on Day 1, 2, 3, 5, and 7. Water and feed consumption were monitored.

At the end of the observation period, the animals were sacrificed with carbon dioxide. All animals were subjected to gross necropsy and mesenteric lymph node samples were taken to determine whether bacterial translocation had occurred. No signs of systemic toxicity were noted

during the observation period and all animals showed expected gains in body weight; no differences in consumption of feed or water were observed. No abnormalities were noted at necropsy. No enterobacteria translocation occurred in any group. The authors concluded that "the results indicate that the administration of the probiotics under the experimental conditions does not have adverse effects on the health of the animals."

L. plantarum Lp-115

In an unpublished report cited in GRAS notification GRN722, the toxicity of *L. plantarum* strain Lp-115 was evaluated in an up-and-down acute oral toxicity study in Sprague-Dawley rats (FDA 2018). A single dose of of the strain ($4.2x10^{12}$ cfu/kg bw) was orally administered to overnight-fasted 10-week-old female Crl:CD(SD) rats at serving levels equivalent to $8.62x10^{11}$ to $9.05x10^{11}$ cfu/animal. The rats were monitored for 14 days after dosing followed by necropsy for gross examination of tissue and/organ damage. Study revealed no incidents of mortality, clinical abnormalities, or loss of body weight. Further, there were no gross lesions in organs or tissues. As there were no deaths at the only dose tested, the acute oral LD_{50} for *L.plantarum* Lp115 in Sprague-Dawley rats was greater than $4.2x10^{12}$ cfu/kg bw.

Daniel et al. (2006) investigated the role of three *Lactobacillus* species, *L. plantarum* Lp-115, *L. salivarius* Ls-33, and L. *acidophilus* NCFM (as well as *L. paracasei*, which served as the control) on intestinal inflammation and bacterial translocation in mice. Gastrointestinal survival of *lactobacilli* was simulated *in vitro* by testing resistance to pepsin, pancreatin, and bile, and adhesion to Caco-2 cells. There was a high level of resistance to pepsin and bile in *L. plantarum* Lp-115 in comparison with other live microbial strains tested. In the animal-study phase, healthy BALB/c mice were orally dosed with $1x10^{10}$ cfu of each bacterial strain (n=5/strain) daily for 4 consecutive days followed by collection of fecal samples which were cultured for the evaluation of live organisms in the GI tract. Recovery of *L. plantarum* Lp-115 from the fecal samples was demonstrated up to 13 days after the cessation of daily administration of the organism. There were no reports of adverse effects in animals. Cultures of mesenteric lymph nodes (MLN), spleen, liver, and kidneys did not test positive for *L. plantarum* Lp-115, indicating that that there was no translocation of the organism.

L. plantarum MTCC 5690

The safety of *L. plantarum* MTCC 5690 and *L. fermentum* MTCC 5689 was assessed in a murine model (Pradhan et al. 2019). The authors investigated the genotoxicity of these strains using the *in vivo* chromosomal aberration and micronucleus assays. They also carried out a series of *in vivo* oral toxicity studies in Swiss Albino mice – 14-day repeated-dose oral toxcity, 28-day subacute oral toxicity, and 90-day subchronic oral toxicity study. In the 14-day study, animals were orally dosed with $4x10^{10}$ cfu/kg bw/day of the test strain or vehicle control after which body weight, feed intake, and other safety parameters were evaluated. In the subacute toxicity study, animals were dosed with $4x10^{7}$ cfu/kg bw/day for 28 days. Animals were observed for signs of toxicity and distress every 48 hours. Feed and water intake were also determined at the same intervals. At the end of the study, all animals were sacrificed and processed for histological examination. In the subchronic study, mice were orally gavaged with $4x10^{7}$ cfu/kg bw/day of the bacterial strain or vehicle control for 90 days (n = 8 mice per group). Animals were observed daily for signs of morbidity and for mortality. Bodyweight and food and water consumption were measured every other day. At the end of the study, animals were sacrificed and prepared for histology. Blood samples from mice in all three studies were processed for hematological and

biochemical analyses.

Results from this study indicated no test-article-induced effect on clinical parameters. Neither of the two strains exhibited induced genotoxicity and there was no effect on selected organs. Hematological and biochemical analyses revealed no abnormalities. The authors of this article concluded that the results support the historical safety of these two strains.

L. plantarum CECT-7527, CECT 7528, and CECT 7529

Bosch et al. (2014) reported a 7-day oral toxicity study in Wistar rats for the *L. plantarum* CECT-7527, CECT 7528, and CECT 7529 strains, discussed in GRN 953. Twelve 9-week-old Wistar rats (sex not reported) were divided into two groups (placebo and equal combination of the 3 strains) and dosed at $5x10^{10}$ cfu/kg bw/day on 2 consecutive days. Clinical observations were made for 5 additional days. Individual body weights were recorded on Days 0 and 1, and then on everyother day. At the end of the observation period, the animals were euthanized with carbon dioxide. Animals were subjected to gross necropsy; mesenteric lymph node samples were taken to detect any bacterial translocation. No signs of toxicity were noted and all animals showed similar gains in bodyweight; no differences in feed or water intake were observed. No abnormalities were recorded at necropsy or histopathological examination. Bacterial translocation to the mesenteric lymph nodes and liver were similar between groups. The authors concluded that, "The results of the toxicity assay showed that *L. plantarum* CECT 7527, CECT 7528 and CECT 7529 were safe since they did not affect the animals' well-being and did not facilitate bacterial translocation even when administered at a high dose."

Kim et al. (2014) reported on an 8-week repeated-dose oral study of the combination of CECT-7527, CECT 7528, and CECT 7529 on cholesterol metabolism in 5-week-old male Sprague Dawley rats, also discussed in GRN 953. Forty rats were divided into four groups (n = 10/group). Hyperlipidemia in the rats was induced with a high-fat diet consumed throughout the study. Five rats consumed a control diet to confirm that the hyperlipidemia was due to the high fat diet. The 4 groups were control, low-dose ($0.6x10^9$ cfu/day), medium-dose ($1.2x10^9$ cfu/day), and high-dose ($2.4x10^9$ cfu/day) administered daily by gavage. Dietary intake was measured every 2days and bodyweight was measured weekly. Blood lipids, blood glucose, liver lipids, and organ weights were analyzed at the end of the experiment. The high-dose group had significantly lower feed intake and weight gain. All test groups had significantly less gain in liver weight than the control group. The medium- and high-dose groups had significantly lower blood serum levels of total cholesterol and LDL cholesterol. The high-dose group had significantly lower liver levels of total lipids and total cholesterol and lower serum leptin. No adverse effects of intake of the combination of CECT-7527, CECT 7528, and CECT 7529 were reported.

Mukerji et al. (2016), discussed in GRN 953, reported an OECD- and FDA Redbook 2000-compliant 90-day oral toxicity study of *L. plantarum* strains CECT 7527, CECT 7528, and CECT 7529 in 7-week-old male and female Crl:CD®(SD) rats. Three groups of rats (n = 10/sex/group) were individually housed, provided feed and water *ad libitum*, and gavaged daily for 90 days with PBS solution, $5.5x10^{10}$ cfu/kg bw/day of an equal blend of the 3 strains, or $1.85x10^{11}$ cfu/kg bw/day of the blend of strains. Ophthalmologic examinations were conducted prior to initiation of dosing and near the end of the study. Bacteria were enumerated at the beginning, middle, and end of the study. Mortality and morbidity were assessed at twice daily, and a clinical examination was conducted 1-3 hours aftereach gavage. Bodyweights and food consumption were measured weekly and clinical observations were conducted weekly. Fecal

samples for bacterial and chemical analysis were collected monthly, starting prior to initiation of dosing. At the end of the dosing period, rats were fasted in metabolism cages overnight for urine collection. On the day of euthanasia, blood was drawn for hematology, clinical chemistry, and coagulation parameters. Urinalysis results were tabulated for volume, specific gravity, pH, total protein, and urobilinogen.

The external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities were evaluated. Absolute and relative organ weights were determined for the liver, kidneys, adrenal glands, thymus, brain, spleen, heart, and reproductive organs. Tissues from the following organ systems were preserved in fixative, processed to slides, and stained with hematoxylin and eosin:

> Digestive system (liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands, and pancreas), urinary system (kidneys and urinary bladder), respiratory system (lungs, trachea, nose, larynx, and pharynx), cardiovascular system (heart and aorta), hemato-poietic system (spleen, thymus, mandibular lymph node, mesenteric lymph node, bone marrow, and Peyer's patches), endocrine system (pituitary, thyroid, parathyroid, and adrenal glands), nervous system (brain, spinal cord and sciatic nerve), musculoskeletal system (skeletal muscle, femur/knee joint, sternum), reproductive system of males (testes, epididymides, prostate, and seminal vesicles) and females (ovaries, uterus, mammary glands, and vagina), Harderian glands, skin, and eyes (including retina and optic nerve).

Tissues collected from animals in the control and high-dose groups were evaluated microscopically. Samples of blood, liver, and mesenteric lymph nodes were collected at necropsy and incubated; bacterial colonies on anaerobic plates were evaluated for identification of the test strains. Each colony was assessed morphologically for shape, margin, elevation, size, texture, appearance, pigmentation and optical properties. Wet fecal samples were collected and analyzed for bacterial identification and enumeration and for primary and secondary bile acids, neutral sterols, short-chain fatty acids, branched-chain fatty acids, and lactic acid.

One animal died due to a gavage error; all other animals survived to euthanasia. Body weight, weight gain, feed consumption, and feed efficiency showed no effects from the test article. The authors stated that there were no effects or abnormalities in the hematology, clinical-chemistry, coagulation, and urinalysis parameters associated with the bacterial strains. Macroscopic observation at necropsy showed no treatment-related abnormalities. The test strains were not translocated to the blood of any animal while translocation to the liver and mesenteric lymph nodes was reported at low to moderate numbers. The authors concluded that this was consistent with previous publications that showed low levels of lactobacilli translocation. This did not pose a safety concern at the low levels seen and the clinical, hematological, and microscopic findings were indicative of safety. The NOAEL in male and female Sprague-Dawley rats was determined to be 1.85×10^{11} cfu/kg bw/day, the highest dose level evaluated.

6.3. Human Studies of *L. plantarum* Strains

6.3.1. HUMAN STUDIES OF *L. PLANTARUM* STRAIN ATCC-202195

Panigrahi et al. (2008 and 2017) reported on a pilot study followed by a very large prospective, randomized, double-blind, placebo-controlled trial enrolling 4,556 apparently healthy Indian neonates. These two studies are summarized in Table 8. Frozen stool samples taken on Day 7 and Day 60 from 11 infants participating in Panigrahi et al. (2008) were

subjected to 16S rRNA gene sequencing of the fecal bacterial community (Chandel et al. 2017). All infants showed changes in bacterial diversity between Day 7 and Day 60. Firmicutes and Proteiobacteria were predominant in all samples, but actinobacteria and Bacteroidetes were low on Day 7. The infants receiving L. plantarum ATCC-202195 and fructooligosaccharide showed a 10-fold increase in Bacteroidetes by Day 60 as well as increasing colonization by *Enterococcus, Lactobacillus*, and *Bifidobacterium*. The authors concluded that "Synbiotic treatment induced an increase in overall taxa and Gram-positive diversity."

Table 8. Human Studies of *L. plantarum* Strain ATCC-202195.

Reference	Study Design & Objective	Subjects	Strain & Daily Dose	Duration	Safety-Related Results
Panigrahi et al. 2008	Prospective, randomized, double-blind, placebo-controlled pilot study of the colonizing ability, tolerance, and effect on GI microbiota of *L. plantarum* ATCC-202195	31 healthy term neonates (1-3 days of life delivered by C-section; 19 in test group, 12 in placebo group)	10^9 cfu of *L. plantarum* ATCC-202195 + 150 mg fructooligo-saccharide in 5% dextrose saline	7 days test-article ad-ministration; fecal samp-ling at base-line & days 3,7, 14, 21, & 28, and months 2, 3, 4, 5, & 6	100%, 94%, 88%, 56%, and 32% of infants receiving *L. plantarum* remained colonized at months 2, 3, 4, 5, and 6, respectively. Weight gain in the 2 groups was equal at day 7, but greater in the test group on day 28 and months 2, 3, and 6. The authors reported that "the supplement was tolerated well."
Panigrahi et al. 2017	Prospective, randomized, double-blind, placebo-controlled trial of the effects of *L. plantarum* ATCC-202195 + fructo-oligosaccharide on development of sepsis	4,556 healthy term neonates (1-4 days of life) delivered by C-section at high risk of sepsis (2,314 M & 2,242 F); 2,278 each in synbiotic and control groups	10^9 cfu of *L. plantarum* ATCC-202195 + 150 mg fructooligo-saccharide & maltodextrin as excipient	7 days test-article ad-ministration, 60 days observation	Incidence of death or sepsis w/l 60 days was reduced by 40%. Total of 10 deaths, none attributable to the intervention. None of the blood cultures drawn from septic infants were + for *Lactobacillus*. Investigators reported on the safety and tolerability of the investigational product. "This study was monitored tightly in the field and all AEs were recorded and reported, whether related to study intervention or not. All hospitalizations (including 319 cases of sepsis) were considered SAEs. Several unreal-ted events were re-corded as expected in the population, including one case each of hydrocephlus, biliary atresia and laryngo-malacia, and two non-fatal cases of neonatal malaria. GI AEs were surprisingly low, with only six cases of abdominal distention (five in the placebo group and one in the treatment group), and the preparation was well tolerated."

6.3.2. HUMAN STUDIES OF OTHER STRAINS OF *L. PLANTARUM*

A large number of clinical studies have been reported with a variety of strains of *L. plantarum*. These studies have enrolled infants, children, and adults; they have included healthy subjects and subjects suffering from conditions that might make them more vulnerable to adverse effects; have covered ingestion of single doses and durations ranging from 2 days to 6 months. These studies are summarized in Table 9.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.

Reference	Study Design & Objective	Subjects	Strain & Daily Dose	Dura-tion	Safety-Related Results
Barreto et al. 2013	Prospective, randomized, placebo-controlled trial of effects of *L. plantarum* Lp-115 on glycemia and homocysteine in post-menopausal women	12 postmenopausal women (mean age = 67 years) with metabolic syndrome, 12 each in test and control groups	$1.25x10^7$ cfu/ day of *L. plan-tarum* Lp-115	90 days	Reductions were seen in LDL cholesterol, homocysteine, and glucose in the group receiving the microbes. No AEs were reported.
Bengtsson et al. 2016	Prospective, randomized, double-blind, placebo-controlled trial of the effects of *L. plantarum* 299v and *Bifidobacterium infantis* Cure 21 on patients with poor ileal pouch function	32 patients with impaired pouch function, 24 men and 8 women, aged 27-70 years (median age = 50 years) Lp299v: 16	10^{10} cfu each of *L. plantarum* 299v and *B. infantis* Cure 21	21 days	There was no difference on any measures between the test and placebo groups. There was no discussion of any AEs of the treatment.
Berggren et al. (2003)	Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of a fermented oat product containing *L. plantarum* 299v on children's intestinal function and microbiota	69 apparently healthy children aged 6 months to 3 years. n = 33 in the test group	$1.4x10^{11}$ cfu *L. plantarum* 299v	3 weeks	*L. plantarum* 299v was present in the feces of all but one member of the test group and in none of the controls. Product-related AEs were reported for 5 children, 4 in the test group and 1 control: 3 test group children developed constipa-tion and one had re-gurgitations (which had begun before feeding commenced); one placebo-group child had softer than normal stools. No differences were seen between groups in stool frequency or consistency, flatulence, vomiting, or intestinal pain. The authors concluded that "the children tolerated the fermented oat product well."

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Bering et al. (2006)	Prospective, randomized, double-blind crossover-design study to assess the effect of *L. plantarum* 299v on absorption of non-heme iron from a phytate-rich meal	24 apparently healthy women with a mean age of 25 years. All received Lp299v.	$1.1x10^{11}$ cfu *L. plantarum* 299v	2 days	Iron absorption was found to be significantly higher with ingestion of *L. plantarum* 299v than with any other condition. No AEs were reported.
Bering et al. (2007)	Prospective, randomized, double-blind, placebo-controlled crossover study to test the effect of lyophilized *L. plantarum* 299v on absorption of non-heme iron	18 apparently healthy women with a mean age of 22 years. All received Lp299v.	10^{11} cfu viable lyophilized *L. plantarum* 299v	2 days	Iron absorption was found to be no higher with ingestion of viable lyophilized *L. plantarum* 299v than without. The authors suggested that the lack of effect of the bacteria could be explained by the bacteria not being in an active state. No AEs were reported.
Bukowska et al. (1998)	Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of *L. plantarum* 299v on markers of CVD	30 apparently healthy males with a mean age of 42.6 years Lp299v: 15	$1x10^{10}$ cfu *L. plantarum* 299v	6 weeks	Fibrinogen, total and LDL-cholesterol level decreased significantly in the test group. No AEs were reported from the ingestion of $1x10^{10}$ cfu/day of *L. plantarum* 299v.
Chong et al. 2019a	Randomized, double-blind, placebo-controlled trial to evaluate the microorganism's effects on upper respiratory tract infections and immune parameters.	One hundred twenty-four recruited subjects (18-60 years) with 53 in the placebo and 56 in the intervention arms completing the study with sufficient compliance for data analysis. Malaysia	(a) *L. plantarum* DR7 consumed as a 2g sachet at $1x10^{9}$ cfu/day and 95% maltodextrin (b) 2g 100% Maltodextrin in sachet	Daily for 12 weeks	3 subjects in the placebo arm and 2 in the intervention arm dropped out of the study. The authors noted the lack of toxicity of DR7 to HepG2 cells and insignificant changes of most CBC para-meters at week 12 as supportive of the safety of DR7.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Chong et al. 2019b	Randomized, double-blind, placebo-controlled trial to evaluate the microorganism's effects on stress and anxiety in adults based on the DASS-42 questionnaire.	One hundred twenty-four recruited subjects (18-60 years) with 55 in the placebo and 56 in the intervention arms completing the study with sufficient compliance for data analysis. Malaysia	(a) *L. plantarum* DR7 consumed as a 2g sachet at $1x10^9$ cfu/day and 95% maltodextrin (b) 2g 100% Maltodextrin in sachet	Daily for 12 weeks	Plasma cortisol level was reduced among DR7 subjects. Reduced plasma pro-inflammatory cytokines, such as inter-feron-γ and transforming growth factor-α and increased plasma anti-inflammatory cytokines, such as interleukin 10, were observed. The authors noted the lack of toxicity of DR7 to HepG2 cells.
Costa et al. 2014	Open-label study of the fate of ingested *L. plantarum* Lp-115	61 apparently healthy adults (22M, 39F) aged 17-50+ years	$2x10^{11}$ cfu of *L. plantarum* Lp-115	Varying time periods (15, 30, 45, 60, or 90 days)	*L. plantarum* was detected in all subjects during ingestion. At 15 and 45 days after discontinuing, numbers of lactobacilli were reduced to the baseline level. There were no reported AEs.
Costabile et al., 2017	Randomized, prospective, placebo-controlled trial to evaluate the microorganisms' effect on blood lipid profiles.	46 adults with mild hypercholesterolem ia with 23 (5M, 18F; 52.3±10.7 years) in the intervention arm and 23 ((M,14F; 52.0 ±8.4) in the placebo arm.	*L. plantarum* ECGC 13110402 at 2×10^9 cfu/capsule (4×10^9 cfu/day)	2x daily for 12 weeks	Some subgroup analysis showed improvements in lipids or blood pressure. No findings of clinical significance were identified in proinflam-atory biomarkers or in bowel parameters. No AEs were reported and the authors concluded that daily oral ingestion of the strain was well tolerated and safe.

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Cunningham-Rundles et al. (2000)	Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on the symptoms of AIDS	15 immunocom-promised children (5 males and 10 females) with HIV, aged 11.5 months to 14 years. All received Lp299v.	$2x10^{10}$ cfu *L. plantarum* 299v	About 1 month	No patient experienced symptoms of intolerance, and none had to be withdrawn. Although residence of *L. plantarum* 299v was established, no bacteria were detected in rectal swabs by the end of the first month after ces-sation of administration. Mononuclear cells isolated from peripheral blood showed a natural immune response to *L. plantarum* 299v in 60% of the children. The authors concluded, "The data suggest that *L. plantarum* 299v may be given safely to the immunocom-promised host and may indeed have a positive effect on immune response."
Del Piano et al. 2010	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* LP01 on symptoms of evacuation disorder	110 adult males and females with evacu-ation disorders & hard stools, other-wise apparently healthy	$2.5x10^{9}$ cfu/day of *L. plantarum* LP01	30 days	Significant improvement in number of bowel movements and ease of expulsion; reduction in abdominal bloating, anal itching, burning, or pain with no reported AEs.
Ducrotte et al. 2012	Prospective, randomized, double-blind, placebo-controlled, multi-center, parallel study of *L. plantarum* 299v in reduction in the frequency of abdominal pain episodes in IBS	214 patients (151 men and 63 women; mean age 37.5±12.6 years) with IBS	10^{10} cfu of *L. plantarum* 299v (n = 108) or potato starch placebo (n = 106)	4 weeks	3 test-group patients and 7 controls withdrew for non-treatment-related reasons. The authors reported that, "No significant side-effect was reported in any group during the 4 wk of treatment. The only AE reported was a transient vertigo onset by one of the patients who received *L. plantarum* 299v (DSM 9843). No change in blood parameters was detected throughout the study."

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Espadaler et al., 2019	An abstract for a presentation at the 10th Workshop of the Spanish Society of Probiotics and Prebiotics (SEPyP 2019) describes a prospective, observational study of subjects initiating consumption of KABP-011, KABP-012, and KABP-013.	343 subjects (median age of 55 years, 63% female)	*L. plantarum* KABP-011, KABP-012, and KABP-013 in a 1:1:1 ratio were consumed as a capsule at $1.2x10^9$ cfu/day	Daily for 12 weeks	Concurrent consumption of medications such as statins, fenofibrates, antihypertensive, antidiabetic, and antiplatelet medications was 46%, 5%, 57%, 32%, and 20%, respectively. 17% of patients reported tolerability issues but none of them was considered severe. The tolerability issues correlated with antiplatelet use only.
Fuentes et al., 2013, 2016	Prospective, randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on cholesterol and blood lipids in hypercholesterolemic subjects	60 subjects (34M and 26F, 51.8 ± 7.2 years) with moderately high cholesterol; 30 in the placebo and intervention arms.	*L. plantarum* KABP-011, KABP-012, and KABP-013 [CECT 7527, 7528, and 7529] in a 1:1:1 ratio were consumed as a capsule at $3.01x10^9$ cfu/day at the start of the study which had been reduced to $1.28x10^9$ cfu/day at the end of the study	Daily for 12 weeks 4-week follow-up	All subjects completed the study. At 12 weeks, the intervention group had significantly larger reductions in LDL cholesterol, total cholesterol, oxidized LDL, and triglycerides. HDL cholesterol was significantly increased. Body weight and BMI decreased from baseline for both groups. Assayed blood glucose, creatinine, GOT, GPT, GGT, and liver enzymes stayed within normal physiological limits and were not significantly different than baseline values. There were no treat-ment related AEs observed.

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Goossens et al. 2003	Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on gut ecology and microbiota	20 apparently healthy adults (9 males and 11 females); mean age = 32.9 years). Lp299v: 10	$2x10^{11}$ cfu *L. plantarum* 299v	4 weeks	No side effects were reported attributable to the intervention. Individuals consuming *L. plantarum* 299v all had the strain in their feces, but it could be recovered from only one person a week after the end of ingestion. The test group also had increased total lactobacilli, but there were no differences in total aerobes, total anaerobes, enterobac-teriaceae, spore-forming clostridia, *Enterococcus* spp., or *Bacteroides* spp., β-glucosidase or β-glucuronidase activity, endotoxin concentrations, SCFA concentrations, or pH. The authors concluded that "A fermented oatmeal drink containing *L. plantarum* 299v increases the number of lactobacilli in the faeces of healthy volunteers, but has no influence on other bacterial counts or on metabolic activities… The observed effect of *L. plantarum* 299v on the intestinal flora appears within 1 week after the start of consumption of the probiotic drink and disappears completely 1 week after cessation of consumption of the drink."

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Goossens et al. (2005)	Prospective, randomized, double-blind, placebo-controlled study of the survival of *L. plantarum* 299v in the GI tract and its effects on fecal microbiota, with and without gastric acid inhibition.	29 apparently healthy volunteers (9 males and 20 females, mean age = 28.5 years). All received Lp299v.	$2x10^{11}$ cfu *L. plantarum* 299v	2 weeks	No side effects were reported and there were no differences between groups in defecation frequency, stool consistency, fecal pH, or concentrations of short-chain fatty acids. The administered strain was detected in the feces of all participants at the end of administration, but only in one 4 weeks later. The authors concluded *that L. plantarum* 299v survives passage through the gastrointestinal tract irrespective of gastric acidity. There were no reports of AEs.
Goossens et al. (2006)	Prospective, randomized, double-blind, placebo-controlled study to assess the effect of ingestion of *L. plantarum* 299v on fecal bacterial ecology and mucosal adhesion of bacteria	29 apparently healthy patients (16 males and 13 females with a mean age of 56.9 years) undergoing colonoscopic examination for polyps. Lp299v: 15	$2x10^{11}$ cfu *L. plantarum* 299v	2 weeks	No side effects were reported, and no differences were seen between groups in reported defecation frequency or stool consistency. Feces from the test group showed increases in clostridia, total lactic-acid bacteria, and lactobacilli, but lactobacilli could be cultured in rectal and ascending-colon biopsies from only 3 and 2 patients, respectively, in the test group. No AEs were reported. The authors concluded that "*L. plantarum* 299v survives passage through the gastrointestinal tract… [and] the probiotic strain did colonize the colonic mucosa to a minor extent."

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Håkansson et al., 2019	Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' ability to suppress ongoing celiac disease in at risk children.	78 children with celiac disease with 40 receiving intervention (3-7 years of age).	a) 1g sachet of maltodextrin and *L. plantarum* HEAL9 / *L. paracasei* 8700:2 at 10^{10} cfu/sachet b) 1g sachet of maltodextrin	6 months	3 children in the intervention group and 4 children in the placebo group reported AEs of pain, flatulence, or diarrhea. 1 in each group reported GI symptoms.
Han et al. (2012)	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* on atopic dermatitis	118 children aged 1-13 years with atopic dermatitis	$2.5x10^9$ cfu/day of *L. plantarum* CJLP1333	12 weeks	Inflammation symptoms improved with no reported AEs.
Hoppe et al. 2015	2 prospective, randomized, single-blind, placebo-controlled, cross-over trials to assess the ability of *L. plantarum* 299v to improve iron absorption	22 apparently healthy Swedish women of reproductive age, 11 in each trial	10^9 or 10^{10} cfu *L. plantarum* 299v in trials 1 and 2	4 days	Iron absorption and retention was significantly higher with either 10^9 or 10^{10} cfu of *L. plantarum* 299v than with the control fruit drink (28.6±12.5 and 29.1±17.0% vs. 18.5±5.8 and 20.1±6.4%, respectively), but there was no significant difference in iron retention with the two doses. No AEs were reported at either dose level.
Huang et al., 2018	Randomized, double-blind, placebo-controlled trial to evaluate the microorganism's ergogenic effect on endurance performance.	16 healthy males aged 20-40 years with no professional athletic training. Eight were in each arm.	1×10^{11} cfu *L. plantarum* TWK10 as a capsule	Daily for 6 weeks	Significantly higher endurance performance and glucose content in a maximal effort treadmill running test were observed. There was no report of AEs..

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Johansson et al. (1993)	Open-label study of mucosal colonization of various strains of *Lactobacillus*	13 apparently healthy volunteers (4 males and 9 females) aged 31-56 years. All received Lp299v.	$5x10^8$ cfu of each of 19 bacterial strains: *L. plantarum* 299v; 2 additional *plantarum* strains; 2 strains each of *L. Sali-varius, L. reuteri, L. jenseni,* and *L. rham-nosus*; one strain each of *L. casei, L.acidophilus,* and *L. agilis*; and 5 *Lacto-bacillus* strains not classified as to species	10 days	5 strains were re-isolated from mucosa 1 and 11 days after cessation of ingestion—*L. plantarum* 299v, one other *L. plantarum*, and one each of *L. agilis, L. reuteri*, and *L. rhamnosus*. Total lactobacilli increased in the jejunum, but there were no other changes in the jejunal microecology. The rectal mucosa failed to show increases in lactobacilli but did have decreases in total anaerobic bacteria and gram-negative anaerobic bacteria. These changes were detected both one and 11 days after the end of administration. No AEs were reported.
Johansson et al. (1998)	Prospective, randomized, double-blind, placebo-controlled study of the effects of *L. plantarum* 299v on metabolic endpoints and fecal bacteria	48 apparently healthy adults (11 males and 37 females) with a mean age of 37 years. Lp299v: 26	$2x10^{10}$ cfu *L. plantarum* 299v	21 days	No participants withdrew and there were no differences in AEs reported by the 2 groups. 5 individuals in each group reported transient nausea or abdominal discomfort. Those receiving the fermented oats and live bacteria had a significant increase in stool volume and a decrease in flatulence. They also had significant increases in fecal levels of total carboxylic acids, particularly acetic, propionic, and lactic acid, but no significant change in fecal pH. *L. plantarum* 299v was found in large numbers in the feces of the test group at weeks 1 and 3, but in only 5 of the 26 individuals 8 days after cessation of ingestion.

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Jones et al. 2013	Prospective, randomized, double-blind, placebo-controlled pilot study of effects of *L. plantarum* 299v on obstructive jaundice during billary drainage	17 billary-drainage patients (12 men, 5 women aged 48 to 75 years [median = 52 years]) Lp299v: 5	The daily dose of *L. plantarum* 299v was not reported	7 days	While trends toward reduced intestinal permeability and reduced TNF p55 receptors with administration of *L. plantarum* 299v were reported, neither effect reached statistical significance. The findings of the hematological and biochemical analyses were not reported, suggesting that the findings were not re-markable. There were no reports of AEs.
Kingamkono et al. (1999)	Prospective, randomized, double-blind, placebo-controlled study of the effects of fermented and unfermented cereal gruel with *L. plantarum* 299v on the presence of fecal enteric bacteria	151 apparently healthy children aged 6 months to 5 years. N = 50 in the test group	The daily dose of *L. plantarum* 299v was not reported	13 days	The results from the test and control cereals did not differ significantly. The proportion of children in the test and control groups harboring enteric bacteria (campylobacter, salmonella, shigella, *E. coli* O157, and enterotoxigenic *E. coli*) did not differ. There were no reported AEs.
Klarin et al. (2005)	prospective, randomized, unblinded study of the ability of *L. plantarum* 299v to adhere to the gut mucosa of critically ill patients	15 critically ill patients admitted to the ICU, 8 males and 9 females aged 33 to 84 years (mean = 64.6 years). Lp299v: 8	$2x10^{11}$ cfu *L. plantarum* 299v/day	Duration of stay in the ICU—4-37 days; median = 11 days	All patients tolerated total or partial enteral feeding, and there were no differences in diarrhea, bloating, illness severity, hospital mortality, length of stay in the ICU, 6-month mortality, or levels of C-reactive protein or leukocyte count. 3 of 8 patients receiving *L. plantarum* 299v tested positive for the strain in the samples of rectal mucosa taken during the treatment. The authors concluded that "*L. plan-tarum* 299v administered to critically ill, antibiotic-treated patients can survive and colonise the gut mucosa, and repeated administra-tion of the bacteria is necessary to obtain this effect." No AEs were reported.

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Klarin et al. (2008)	Prospective, randomized, double-blind, placebo-controlled study of the capacity of *L. plantarum* 299v to reduce *Clostridium difficile*-associated disease in critically ill patients	44 ICU patients (26 males and 18 females aged 18-89 years; mean age = 64.7 years) receiving antibiotic therapy. N = 22 in the test group	$1.6x10^{11}$ cfu (later $8x10^{10}$ cfu) *L. plantarum* 299v/day	Duration of stay in the ICU—2.5-22 days; mean = 5.5 days	2 patients from each group died in the ICU; 1 patient from the test group died in the hospital, and 4 patients from the control group died within 6 months. There were no differences between the groups in sequential organ failure, length of ICU stay, or days on ventilators. In 71 fecal samples from the test group, none tested positive for *C. difficile*, while 4 emergent cases were found in the 80 samples from control group patients. Control group patients also harbored a number of potential pathogens not found in the test group. There were no differences in C-reactive protein, TNF-α, IL-1β, or IL-6; IL-10 and white blood cell counts were higher in the control group than in patients receiving *L. plantarum* 299v. Gut permeability was higher in the control group than in the test group. The study product was well tolerated and the authors stated that “We found no adverse impact of the given probiotic preparation.”

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Krag et al. 2012	Prospective open-label study of treatment of ulcerative colitis with *L. plantarum* 299v	39 ulcerative colitis patients (15 males and 24 females aged 19-50 years [median age – 35 years]) Lp299v: 39	$2.5x10^{10}$ cfu for 2 days, then $5x10^{10}$ cfu thereafter	Up to 176 days	The treatment reduced severity by 56.5%. The authors reported: "No major AEs were reported and there were no dropouts due to AEs. An increased number of bowel movements were reported by 11 patients (28%), bloating by four (10%) and an increased number of bowel movements and bloating by three (8%). All AEs were self-limiting or managed by dose adjustments. For example, if a patient experienced a presumable AE during the introduction of Profermin®, the period with the low Profer-min® dose was pro-longed for up to 2 wk. None of the 8 drop-outs or 4 excluded patients left the trial due to deterioration in UC symptoms."
Krag et al. 2013	Prospective randomized single-blind two-arm study comparing Profer-min® and Fresubin as treatments for ulcerative colitis	73 ulcerative-colitis patients (33 males and 40 females aged 20-78 years; mean age 41 years) Lp299v: 32	Median = $4.89x10^{10}$ cfu of *L. plantarum* 299v	8 weeks	The authors reported that, "No major AEs were reported, but 3 patients experienced AEs. In the Fresubin group, one experienced an 'obvious weight gain' and one felt it induced vomiting. In the Profermin group, one suffered from rumbling and bloating." They concluded that, "Supplementation with Profermin is safe, well tolerated, palatable."
Kujawa-Szewieczek et al. 2015	Retrospective open-label study of the use of *L. plantarum* 299v to reduce the incidence of *Clostridium difficile* infection	356 organ trans-plant patients, 174 before *L. plantarum* 299v and 182 after	10^9 cfu *L. plantarum* 299v	Not reported	Of these patients, 21 in the first year and 2 in the second year were diagnosed with *C. difficile* infection, infection rates of 12.1 and 1.1%, re-specttively. No AEs were reported due to treatment.

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Ladas et al. 2016	Prospective open-label multi-center pilot study of safety and efficacy of prophylactic use of *L. plantarum* 299v in children and adolescents undergoing hematopoietic cell transplantation	30 children and adolescents (16 males and 14 females aged 7.7±4.7 years; the age range was 2.2 to 17.3 years). N = 30 in the test group	10^8 cfu *L. plantarum* 299v/kg bw/ day	21 days (7 days prior to surgery & 14 post-surgery days)	The incidence of graft-versus-host disease was 30%, less than is usually encountered. No episodes of *L. plantarum* bacteremia were observed. The authors reported that, "We did not observe any SAEs or unexpected AEs attributed to [*L. plantarum*] in any patient enrolled to the study." The authors concluded that, "Our study provides preliminary evidence that administration of [*L. plantarum*] is safe and feasible in children and adolescents undergoing [hematopoietic cell transplantation]."
Lee et al. 2015	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* HY7714 on skin health	110 apparently healthy adult women (61 test group and 49 control group)	10^{10} cfu/day of *L. plantarum* HY7714	12 weeks	Incidence of AEs was monitored, but none was reported.
Lew et al. 2019c	Prospective, randomized, double-blind, placebo-controlled trial of the effect *of L. plantarum* P8 on stress in adults	103 apparently healthy adults	$2x10^{10}$ cfu of *L. plantarum* P8	12 weeks	Blood samples taken for assessment of hematological parameters revealed no AEs.
Ligaarden et al. 2010	Prospective, randomized, double-blind, placebo-controlled cross-over trial in adults with IBS	16 adults with diagnosed IBS	10^{10} cfu/day of *L. plantarum* MF1298	3 weeks each with strain & placebo; 1 week washout	13 patients were more satisfied with placebo than test bacteria, but there was no specific difference in symptoms. There were 3 AEs and one SAE, judged to be unrelated to the treatment.

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Lim et al. 2018	Randomized, double-blind, placebo-controlled trial to evaluate the synbiotics effects on constipated adults.	85 constipated adults per Rome-III standards. 36F and 7M, aged 29.5±8.34 years in the n=43 intervention arm.	1 x 10^{10} cfu/day of *L. plantarum* LP01, *B. lactis* BB12, and inulin-oligofruc-tose as a 2.5g sachet	Daily for 12 weeks	There was no statistically significant improvement in the intervention arm versus the placebo for all measures of functional constipation. There was no discussion of AEs.
Lonnermark et al. 2010	Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on GI symptoms during antibiotic therapy	239 patients (93 males and 146 females; median age = 45 years) receiving antibiotic therapy for infectious disease. Lp299v: 80	1x10^{10} cfu *L. plantarum* 299v/day	Until 7 days after the end of antibioti cs	76 patients withdrew or were excluded from the study, 38 each from the test and placebo groups; reasons for withdrawal did not differ between the groups. Diarrhea was infrequent (5 and 6 patients in the place-bo and test groups, respectively). The incidence of loose or watery stools but not meeting the criteria for diarrhea and the incidence of nausea were lower in the group receiving *L. plantarum* 299v than in the control group. The authors reported that “No side effects of the treatment were recorded.”
Lonnermark et al. 2015	Prospective, randomized, double-blind, placebo-controlled, multi-center study of the ability of *L. plantarum* 299v to treat *Salmonella* infection	149 patients with *Salmonella* infections (40 males and 109 females aged 5 to 68 years; median age = 36 years) Lp299v: 77	5x10^{10} cfu *L. plantarum* 299v	Median of 26 days	The authors reported a non-statistically significant tendency for a greater number of GI symptoms to be reported by patients consuming *L. plantarum* 299v. The conclusion of the authors was that, “Our results give little support for positive effects of *L. plantarum* 299v treatment in nontyphoid salmonellosis.” No AEs were reported.

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Lorenzo-Zúñiga et al., 2014	Randomized, double-blind, placebo-controlled, multi-center clinical trial to determine the dose related effects of the microorganisms on irritable bowel syndrome (IBS) symptoms measured with IBS-QoL, Visceral Sensitivity Index (VSI), and global symptom relief questionnaires.	84 subjects aged 20-70 years and 53F/31M with IBS according to Rome-III criteria. 28 in the high dose arm, 27 in the low dose arm, and 29 in the placebo arm. Spain	*Pediococcus acidilactici* KABP-021, *L. plantarum* KABP-022, and *L. plantarum* KABP-023 (1:1:1) at 1-3 x 10^{10} cfu/day (high dose) or 3-6 x 10^{9} cfu/day (low dose)	Daily for 42 days	Improvement in IBS-QoL and VSI were significantly improved compared to placebo in both the high- and low-dose groups. No adverse drug reactions or rescue medications were observed or required during the study. The dropout rates of 3 subjects in each of the intervention group were not significantly different than the 5 subject dropouts in the placebo group. There was a small increase in the level of liver enzymes observed in 4 subjects: 2 in the high dose group, 1 in the low dose group, and 1 in the placebo group.
Madempudi et al., 2019	Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects in patients with Type 2 diabetes.	79 subjects with Type 2 diabetes with 40 receiving intervention (62M, 17F, mean age of 52.4 years).	a) *L. salivarius* UBLS22, *L. casei* UBLC42, *L. plantarum* UBLP40, *L. acidophilus* UBLA34, *B. breve* UBBr01, and *B. coagulans* Unique IS2, 30 billion cfu and fructo-oligosaccharide, 100 mg) b) placebo capsules of maltodextrin	12 weeks	2 participants experienced mild flatulence or moderate constipation assessed as likely unrelated to the intervention. No other SAEs or deaths occurred during the study.

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Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Malik et al. 2018	Open lable study of the effect of *L. plantarum* 299v on endothelial function in men with coronary artery disease	20 men aged 40-75 years with stable coronary artery disease	$2x10^9$ cfu/day of *L. plantarum* 299v	6 weeks	Stool microbiome analysis found increased numbers of L*actobacillus* genus, but no changes in bacteria of other classes. There were no reports of adverse reaction to *L. plantarum* 299v supplementation.
Mane et al., 2011 Bosch et al., 2011 Bosch et al., 2012b	Prospective, randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on systemic immunity, blood chemistry, bowel movements, and influenza specific antibodies in the elderly	50 institutionalized subjects with 47 completing the study (26M and 21F >65 years), 19 in the high-dose arm, 13 in the low-dose arm, and 15 in the placebo arm. Spain	(a) *L. plantarum* KABP-031 and KABP-032 at 5 x 10^8 cfu/day in 20g of powdered skim milk diluted into 200 mL water (b) *L. plantarum* KABP-031 and KABP-032 at 5 x 10^9 cfu/day in 20g of powdered skim milk diluted into 200 mL water (c) 20g of powdered skim milk diluted into 200 mL water	Daily for 12 weeks 12 weeks follow-up	Significant differences in blood leukocyte phenotypes in the high-dose group were observed in T-suppressor (CD8+ CD25+) and NK (CD56+ CD16+) cells. The low-dose group had significant differ-ences in T-helper lymphocytes (CD4+ CD25+), B lympho-cytes (CD19+), and antigen presenting cells (HLA- DR+). Plasma TGF-β1 con-centration was de-creased in both groups. Subjects who experienced less than 3 bowel movements a week decreased when consuming the bacteria. Influenza specific IgA was increased in both the low- and high-dose groups. IgG antibodies were increased in the high-dose group. There were no signif-icant changes in the BMI, Barthel Index, and routine laboratory tests (albumin, glu-cose, total cholesterol, triglycerides, creatin-ine, AST, ALT, ALP, GGT, total bilirubin, hemoglobin, leuko-cytes, and platelets) between the groups during the treatment or follow-up periods.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Mangell et al. 2012	Prospective, randomized, double-blind, placebo-controlled trial of the effect of prophylactic *L. plantarum* 299v on pathogenic bacteria, translocation, and cell proliferation in colon surgery	64 patients (36 males and 28 females aged 64 to 80 years; median age = 72 years) referred for colonic resection Lp299v: 32	10^{11} cfu *L. plantarum* 299v	14 days	No benefit was obtained from administration of *L. plantarum* 299v to colon surgery patients; there were no differences between groups in the incidence of enteric pathogenic bacteria, bacterial translocation, or postoperative complications. The authors noted that, "No adverse effects were recorded after the administration of high doses of *L. plantarum* 299v."
McNaught et al. (2002)	Prospective, randomized, unblinded study to test if *L. plantarum* 299v administered before and after abdominal surgery reduces the incidence of sepsis	129 patients (75 males and 54 females with median age = 68 years). Lp299v: 64	$2.5x10^{10}$ cfu *L. plantarum* 299v/day	median = 2 weeks	No differences were seen between the test and control groups in bacterial translocation to the lymph nodes or ileal serosa, gastric colonization, C-reactive protein levels, septic complications, or mortality. The authors concluded that "preoperative administration of the probiotic *Lactobacillus plantarum* 299v for two weeks has no effect [either beneficial or adverse] on the human gut mucosal barrier … and the systemic inflammatory response."

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
McNaught et al. (2005)	Prospective, randomized, unblinded study of the effect of *L. plantarum* 299v on gut barrier function and systemic inflammatory response in critically ill patients	103 patients (58 males and 45 females aged 28-90 years; median age = 71 years) within 24 hours of admission to the ICU. Lp299v: 52	10^{10} cfu *L. plantarum* 299v/day	Until discharg e from the hospital —3-17 days; median = 9 days	There were no differ-ences between the 2 groups in intestinal permeability or in IgM or C-reactive protein, but IL-6 levels were lower in the test group than in the controls. The mortality rate was 35% in both groups. 68 septic complica-tions occurred; there were no differences in incidence, causes, or severity between test patients and controls. The authors reported that there were no changes in GI micro-flora, endotoxin ex-posure, intestinal permeability, septic morbidity, or mortality.
McNicholl et al., 2018	Prospective, randomized, double-blind, placebo-controlled study of bacteria in *Helicobacter pylori* therapy	209 adult patients with *H. pylori* infection; 103 in the intervention arm	1 x 10^9 cfu/day each of *P. acidilactici* and *L. plantarum*	Daily for 10 days	No differences in compliance or in eradication rates. Side effects at the end of the treatment were the primary outcome, but no differences between the test and placebo groups were reported.
Montero et al., 2017	Double-blind, randomized, placebo-controlled, parallel-group trial to evaluate microorganism's effects on gingival inflammation (GI), plaque index (PII), angulated bleeding core (AngBS), and microbial composition.	59 apparently healthy adults (31.7± 12.8 years) with 30 in the intervention arm.	*L. brevis* CECT 7480, *L. plantarum* CECT 7481, and *P. acidilactici* CECT 8633 (1:1:1 ratio) 2.0 x 10^9 cfu/day (1.0 x 10^9 cfu/dose) in chewable tablets.	2x daily for 6 weeks	One subject from the test group and 6 from the control group withdrew, none due to intervention-related AEs. There was no difference in compli-ance or in gingival inflam-mation between groups. Concentrations of *A. actinomycetemcomitans* decreased significantly in both the test and control group while those of *T. forsythia* decreased significantly only in the test group. 4 patients in the intervention arm and one patient in the placebo arm reported AEs, most often abdominal pain due to increased intestinal motility (possibly due to sorbitol in the tab-lets); no SAEs were reported.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Nabhani et al., 2018	Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms and FOS effects on pregnant women with diabetes.	90 pregnant women with gestational diabetes with 45 receiving intervention (mean age 30.3±5.6 years).	*L. acidophil-lus* (5×10^{10} cfu/g), *L. plantarum* (1.5×10^{10} cfu/g), *L. fermentum* (7×10^{9} cfu/g), *L. Gasseri* (2×10^{10} cfu/g) and 38.5 mg of FOS as a capsule	Daily for 6 weeks	The supplement did not show any significant effects on glycemia and insulin resistance/ sensitivity indices. The authors note that “none of the participants have reported specific side effects of synbiotic supplements.”
Nam et al. 2020	Open-label study of the effect of *L. plantarum* HY7714 on intestinal health	13 apparently healthy females aged 23-67 years	10^{10} cfu *L. plan-tarum* HY7714/ day	8 weeks	The authors reported a decrease in zonulin; inflammatory markers TNF-α, IL-6, IL-10, TSLP, and eotaxin; and MMP-2 and MMP-9. There were no reports of adverse reactions to *L. plantarum* HY7714 supplementation.
Naruszewic z et al. (2002)	Prospective, randomized, double-blind, placebo-controlled trial of the ability of *L. plantarum* 299v to reduce symptoms of CVD risk factors in smokers	36 apparently healthy 25-45-year-old smokers (18 of each sex). Lp299v: 18	$2x10^{10}$ cfu *L. plantarum* 299v/day	6 weeks	No AEs were reported; the test group had lower systolic blood pressure compared with before intake. No differences were apparent in total cholesterol, triacylglycerol, or lipoprotein(a), but HDL levels increased in the test group while leptin and insulin concentrations decreased; only the leptin change was statistically significant. There were decreases in F2-isoprostanes, IL-6, and fibrinogen concentrations among smokers ingesting *L. plantarum* 299v, as well as the adherence capability of monocytes. All of the biochemical changes attributed to the intervention were regarded as beneficial; no adverse changes were observed.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Niedzielin et al. (2001)	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* 299v on IBS patients	40 IBS patients (8 males and 32 females aged 27-63 years, mean = 45 years). Lp299v: 20	$2x10^{10}$ cfu *L. plantarum* 299v/day	4 weeks	The patients receiving *L. plantarum* 299v showed significantly greater improvement in their IBS symptoms than did the placebo group, and the authors noted that "No treatment related side-effects were observed."
Nobaek et al. (2000)	Prospective, randomized, double-blind, placebo-controlled trial of the effect of attempted alteration of the gastrointestinal microecology of IBS patients with *L. plantarum* 299v	52 adult patients with IBS.	$2x10^{10}$ cfu *L. plantarum* 299v/day	4 weeks	*L. plantarum* 299v was found in the fecal samples from 84% of the test group and in 32% of their rectal biopsies, but there were no changes or differences between test and control groups in other bacterial counts. The authors noted that the products were well tolerated and no treatment-related AEs were reported from ingestion of $2x10^{10}$ cfu/day of *L. plantarum* 299v for 4 weeks.
Olek et al., 2017	Randomized, double-blind, placebo-controlled trial to evaluate microorganism effect on side effects of outpatient antibiotic treatment of children.	438 children (1-11 years) with 218 (113M, 105F; mean age 5.1±2.6 years) in the intervention arm and 220 (122M, 98F; 5.2±2.9 years) in the placebo arm.	*L. plantarum* 299v at 1 x 10^{10} cfu/day as a capsule	Daily for 15-28 days	No beneficial effect was observed related to the incidence of loose/ watery stools, mean number of loose/watery stools, or the incidence of abdominal symptoms. 155 AEs were reported in 99 children by the parents. Children with AEs were significantly more common in the placebo arm (27.3% vs. 17.9%). The most frequent AEs were pyrexia, headache, rash, anorexia, cough viral infection, and ear pain. There were no SAEs reported.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Onning et al., (2003)	Prospective, randomized, double-blind, placebo-controlled study of the effect of a test beverage with *L. plantarum* 299v on plasma antioxidant capacity and fecal bacteria	98 volunteers with a high working pace (39 men and 59 women aged 21-61 years [mean age = 35 years]. n = 50 in the test group	$2.2x10^{10}$ cfu *L. plantarum* 299v/day	4 weeks	There was no difference between the test and control groups in the incidence or nature of AEs and there were none that could reasonably be attributed to ingestion of *L. plantarum* 299v.
Oudhuis et al., (2011)	Prospective randomized open-label trial comparing the effects of *L. plantarum* 299/299v and decontamina-tion of the digestive tract in reducing infection rates in ICU patients	254 ICU patients (157 males and 97 females aged 17 to 90 years; mean age = 62.7 years). n = 130 in the test group	$5x10^{9}$ cfu *L. plantarum* 299v/day	Duration of the stay in the ICU; mean = 11 days	There were no differences between the 2 groups in length of ICU or hospital stay, need for mechanical ventilation, or mortality. No AEs were reported from the treatment.
Paineau et al. 2008	Prospective, randomized, double-blind, placebo-controlled trial of the effect of microbial products on immune response to cholera vaccine.	83 apparently healthy adults aged 18-62 years	$2x10^{10}$ cfu/day of *L. plantarum* Lp-115	21 days	*L. plantarum* Lp-115 induced an increase in serum IgG and IgM response with no SAEs.
Park et al. 2020	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* Q180 on post-prandial lipid metabolism	70 apparently healthy men and women (35 each in test and placebo groups) aged ≥20 years with triacyl-gycerol <200 mg/dl.	$4x10^{9}$ cfu/day of *L. plantarum* Q180	12 weeks	No AEs were reported and the authors concluded that, "LPQ180 ingestion ameliorated postprandial lipid metabolism and maintained a healthy intestinal environment."
Pons et al., 2018	Double-blind, randomized, placebo-controlled pilot trial to evaluate microorganism's effects on swelling, pain, and eating difficulty after third molar extraction.	37 apparently healthy teenagers and young adults with 20 in the intervention arm.	*L. brevis* CECT 7480 and *L. plantarum* CECT 7481 (1:1 ratio) 1.0×10^{9} cfu/day (0.5×10^{9} cfu/dose) in chewable tablets.	2x daily for 1 week	There was no difference between groups in swelling at the extraction site, but patients receiving the intervention reported non-significantly reduced pain and significantly reduced eating difficulty. There were two infections at the site of tooth removal in each arm, but no other AEs were reported.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Ribeiro and Vanderhoof (1998)	Prospective, randomized, single-blind placebo-controlled trial of the ability of *L. plantarum* 299v to reduce the incidence of infective diarrhea among children	143 children aged 6 months to 3 years attending daycare in a region of Brazil with a high incidence of infectious diarrhea. n = 71 in test group.	10^{10} cfu *L. plantarum* 299v/day	3 months	Reductions in the incidence of diarrhea and respiratory infections were seen in both the test and control groups, with no differences between groups. The authors speculated that colonization of half of the children in the daycare setting with *L. plantarum* may have reduced the dissemin-ation of infectious diseases. No AEs were reported.
Sawant et al. (2010)	Prospective, randomized, double-blind, placebo-controlled, multi-center study of the capacity of *L. plantarum* 299v to reduce symptoms of IBS	200 IBS patients (141 males and 59 females; mean age = 37.8 years). N = 98 in test group	10^{10} cfu *L. plantarum* 299v/day	4 weeks	Patients ingesting the bacteria showed improvement in all assessed symptoms as compared with the control group. No changes were observed in pulse or respiratory rates, blood pressure, or body temperature, and no side effects were reported.
Sen et al. (2002)	Prospective, double-blind, placebo-controlled crossover trial of the effect of *L. plantarum* 299v on colonic fermentation of IBS patients	12 (1 male and 11 female aged 23-61 years, mean age = 40.6 years) gastroenterologic IBS outpatients. All received Lp299v.	$6.3x10^9$ cfu *L. plantarum* 299v/day	4 weeks	All subjects started with the placebo product. No difference was seen between the groups on any measure: exhalation of hydrogen and methane during calorimetry, breath hydrogen after lactulose ingestion, or daily symptom scores. The authors concluded that "*Lactobacillus plantarum* 299v in this study did not appear to alter colonic fermentation." No AEs were reported.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Stevenson et al. 2014	Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* 299v on IBS patients	81 IBS patients (2 males and 79 females aged 47.9±12.1 years); 54 received the strain and 27 received placebo	$2x10^{10}$ cfu *L. plantarum* 299v/day	8 weeks	Patients in both groups showed significantly reduced reported pain, but there was no difference between groups. There was no difference between groups in compliance and "the rate of AEs was very low. The tolerability of the test product was good."
Stjernquist-Desatnik et al. (2000)	3 open label experiments on the ability of *L. plantarum* 299v to colonize tonsilar epithelia	1st experiment: 6 adults aged 33-42 years (1 man and 5 women, mean age = 38 years); 2nd experiment: 2 women aged 41 and 42 years; 3rd experiment: same 2 women All received Lp299v.	1st experiment: 1 dose of $2x10^{11}$ cfu *L. plantarum* 299v; 2nd experiment: 1 dose of 10^{11} cfu *L. plantarum* 299v; 3rd experiment: 1 dose of 10^{10} cfu *L. plantarum* 299v	Single doses	In the 1st experiment, all 6 volunteers had detectable levels of *L. plantarum* 299v on their tonsillar epithelia after gargling and ingestion and all 6 had the bacteria present at 4 hours; however, only 1 person still had detectable levels at 8 hours after intake. Both women had *L. plantarum* 299v on their tonsillar epithelia after ingesting fermented gruel mixed with fruit juice and for 4 hours thereafter, but only intake of 10^{11} cfu resulted in detectable levels remaining at 80 hours. The authors concluded that, "the bacteria under inves-tigation may possess the capacity to adhere to tonsillar cells." No AEs were reported.
Vanderhoof et al. (1998) Case 1	Case study of short-bowel patient with small-bowel bacterial overgrowth	7-year-old boy. Received Lp299v	10^{10} cfu *L. plantarum* 299v/day	2 months	Within 2-3 weeks stool consistency improved, primarily in reduction of water content. No AEs were noted due to the therapy. No indication of D-lactic acidosis was reported in this short-bowel patient receiving *L. plantarum*.

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Vanderhoof et al. (1998) Case 2	Case study of short-bowel patient with small-bowel bacterial overgrowth	16-year-old boy Received Lp299v	10^{10} cfu *L. plantarum* 299v/day	Not reported	Replacement of the antibiotic with *L. plantarum* 299v produced good therapeutic response with no reportedAEs. No indication of D-lactic acidosis was reported in this short-bowel patient receiving *L. plantarum*.
Vanderhoof et al. (2000)	Open-label study	15 HIV-positive children aged 11.5 months to 14 years	$2x10^{10}$ cfu *L. plantarum* 299v/day	4 weeks	No reports of AEs.
Woodcock et al. (2004) [further analysis of participants in McNaught et al. (2002)]	Prospective, randomized, unblinded study of the effect of *L. plantarum* 299v on gut immune function of patients receiving abdominal surgery	22 patients (10 males and 12 females with median age = 69 years) undergoing small-bowel resection Lp299v: 11	$2.5x10^{10}$ cfu *L. plantarum* 299v/day	median = 2 weeks	There were no differences between the test and control groups in numbers of plasma cells or either IgA- or IgM-positive cells, or in mucosal-surface IgA levels, but the concentration of IgM was reduced in the group receiving *L. plantarum* 299v. The authors concluded that there is no evidence that administration of the strain has any effect on gut-associated lymphoid tissue. No AEs were reported.
Wullt et al. (2003) Wullt et al. (2007)	prospective, randomized, double-blind, placebo-controlled study of the ability of *L. plantarum* 299v to reduce the likelihood of further recurrent episodes of *Clostridium difficile*-associated diarrhea	21 patients (1 male and 20 females; mean age = 63.8 years) testing positive for *C. difficile* toxin and having a history of previous *C. difficile*-associated diarrhea. Lp299v: 12	$5x10^{10}$ cfu of *L. plantarum* 299v/day	38 days	There was a statistically insignificant reduction in the risk of recurrence among the patients receiving *L. plantarum* 299v, and the authors noted that “Treatment with the lactobacilli had no apparent side-effects.”

Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.					
Reference	**Study Design & Objective**	**Subjects**	**Strain & Daily Dose**	**Dura-tion**	**Safety-Related Results**
Zhang et al. 2013	Retrospective cohort study of the effect of fiber alone or with live microbials in the management of liver transplant patients.	34 liver transplant patients, 19M and 15F, mean age = 57 years; matched group (n = 33) that previously consumed fiber alone.	$5x10^9$ cfu/day of *L. plantarum* Lp-115	16 days	The live microbial group had a lower incidence of bacterial infections and spent less time on antibiotic therapy. *L. plantarum* Lp-115 was not implicated in any infections. AEs included abdominal cramps and diarrhea which were transient and likely due to fiber. No AEs were associated with *L. plantarum.*

6.4. Meta-Analyses of Human Studies of *L. plantarum*

In a large and comprehensive evidence-based review and meta-analysis, Hempel et al. (2011) addressed several key safety questions for live microbials, focusing on *Lactobacillus, Bifidobacterium, Saccharomyces, Streptococcus, Enterococcus,* and *Bacillus* species. Adverse events and safety parameters were evaluated in 622 studies of the more than 11,000 intervention studies they initially identified. While 235 of the studies reviewed did not define specific adverse events, making only general statements (e.g., "well tolerated"), 387 studies reported either the presence or absence of specific adverse events. The authors note that while not all studies were designed to assess adverse events and reports of rare adverse events were difficult to assess, they determined that the data found no infections caused by microbial ingredient consumption and no statistically significant increase of relative risk of adverse events was identified. When the studies that evaluated children or elderly populations were analyzed, the authors found no evidence of increased adverse events associated with microbial ingredient use, even in very young children <2 years old (Hempel et al. 2011).

Of the 67 studies that evaluated *L. plantarum*, 11 were in study populations that included children (infants-teens) and 14 were in study populations that included elderly subjects. Dose ranges that were reported for studies that included elderly subjects were $9x10^8$–$5x10^{13}$ cfu per day for *L. plantarum.* Dose ranges that were reported for children were $2x10^8$–$4.5x10^{11}$ cfu per day for *L. plantarum* (Hempel et al. 2011).

In discussion of the studies using *L. plantarum*, the authors highlighted two studies in which adverse events experienced were similar between live microbial and control groups (e.g. transient abdominal discomfort, nausea, or flulike symptoms experienced), and further noted that these two studies found *L. plantarum* did not cause abnormal changes in urinalysis, serum biochemical parameters, or allergic symptoms.

In a 2015 review of live microbial safety, Doron and Snydman provide additional commentary on the Hempel et al. (2011) study, noting that the totality of evidence should be taken into account (including history of safe use together with data from clinical trials, animal

and *in vitro* studies), and highlighting the importance of strain specific investigations to evaluate antibiotic resistance and toxin production. The authors report cases of bacteremia, sepsis, and endocarditis associated with *Lactobacillus* species, however, *L. plantarum* was not implicated (Doron and Snydman 2015). *L. plantarum* species were also noted as having been studied in immunocompromised and elderly populations without safety concerns.

6.5. Decision Tree

The safety of *L. plantarum* ATCC-202195 has also been established using a decision tree for determining safety of microbial culture to be consumed by humans or animals (Pariza et al. 2015)

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? **YES**

2. Has the strain genome been sequenced? **YES**

3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? **YES**

4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? **YES**

5. Does the strain produce antimicrobial substances? **NO**

6. Has the strain been genetically modified using rDNA techniques? **NO**

7. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? **NO—ISOLATED FROM HUMAN FECES**

8. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? **NO**

Conclusion: The strain is "deemed to be safe for use in the manufacture of food, probiotics, . . . for human consumption" (Pariza et al. 2015).

6.6. Safety Assessment and GRAS Determination

This section presents an assessment that demonstrates that the intended use of *L. plantarum* ATCC-202195 is safe and is GRAS based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of *L. plantarum* ATCC-202195 is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of consumers to *L. plantarum* ATCC-202195 under its intended conditions of use is not harmful. In the second step, the intended use of *L. plantarum* ATCC-202195 is determined to be GRAS by demonstrating that its safety under its intended conditions of use is generally recognized among qualified scientific experts and is based on generally available and accepted information.

The regulatory framework for establishing whether the intended use of a substance (or microorganism) is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components:

1. Data and information relied upon to establish the scientific element of safety must be generally available; and
2. There must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the intended use of *L. plantarum* ATCC-202195 is safe and is GRAS.

6.6.1. EVIDENCE OF SAFETY

Genomic analysis of *L. plantarum* ATCC-202195 established that it harbors no antibiotic resistance genes flanked by mobile elements, no confirmed virulence genes and none flanked by mobile elements, and no genes encoding toxin production. Phenotypic analysis shows an absence of antibiotic resistance above ECOFF levels and no production of biogenic amines. No evidence of pathogenicity has been reported, and the species is generally regarded as non-pathogenic as well as non-toxigenic. No indications of toxicity were found in acute and repeated-dose studies of oral toxicity or in genotoxicity assays in strain *L. plantarum* ATCC-202195 or other strains of *Lactiplantibacillus plantarum*, and no adverse effects were reported when the microorganisms were administered to humans. All of these findings support the conclusion that the intended use of *L. plantarum* ATCC-202195 is safe.

6.6.2. CONCLUSION OF THE EXPERT PANEL

The intended use of *L. plantarum* ATCC-202195 has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was shown by genomic analysis of the strain, a record of safe ingestion of numerous strains of *Lactiplantibacillus plantarum*, toxicity studies of *L. plantarum* ATCC-202195 and other strains, and research in humans, concluding that the expected exposure to *L. plantarum* ATCC-202195 is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the intended use of *L. plantarum* ATCC-202195 has been made through the deliberations of a GRAS Panel consisting of Berthold Koletzko, M.D., Ph.D., Michael Pariza, Ph.D., and Stephen Taylor, Ph.D., who reviewed this monograph prepared by Danisco, as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to *L. plantarum* ATCC-202195 anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on *L. plantarum* ATCC-202195 that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of *L. plantarum* ATCC-202195.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach a similar conclusion regarding the safety of *L. plantarum* ATCC-202195 under its intended conditions of use. Therefore, the intended use of *L. plantarum* ATCC-202195 is GRAS by scientific procedures.

6.7. Affirmative Statement Concerning Data and Information

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with Danisco's conclusion of GRAS status under the conditions of intended use.

Part 7: References

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CONCLUSION OF THE GRAS PANEL: GRAS DETERMINATION FOR THE USE OF *LACTIPLANTIBACILLUS PLANTARUM* ATCC-202195 IN INFANT FORMULA, TODDLER FOODS, AND CONVENTIONAL FOODS

Prepared for:

Danisco USA, Inc.

(A Wholly Owned Subsidiary of International Flavors and Fragrances, Inc.)

October 2022

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We, the members of the GRAS panel, have individually and collectively critically evaluated the publicly available information on *Lactiplantibacillus plantarum* ATCC-202195 summarized in a monograph prepared by Danisco USA, Inc., and JHEIMBACH LLC, as well as other material deemed appropriate or necessary. Our evaluation included review of the identity, phenotypic, and genotypic properties of the microorganism, including production methods, the potential exposure resulting from the intended use of *L. plantarum* ATCC-202195, and published research bearing on the safety of the species and the strain. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- The notified microorganism is *Lactiplantibacillus plantarum* strain ATCC-202195, formerly denominated as *"Lactobacillus plantarum,"* and so referred to in supporting documents and scientific studies published prior to April 15, 2020, sold under the brand name *L. plantarum* (HOWARU Lp-202195™). It is a Gram-positive, obligate hetero-fermentative, lactic acid bacterium.

- *L. plantarum* ATCC-202195 was isolated from infant human feces and deposited in the American Type Culture Collection (ATCC) in January, 1999. The whole-genome average nucleotide identity (ANI) indicates that there is 99% similarity between *L. plantarum* ATCC-202195 and the reference strain *L. plantarum* WCFS1 and *L. plantarum* Lp-115, a generally recognized as safe (GRAS) acknowledged strain (GRN 722) notified by DuPont and filed as GRAS in 2017 for use in conventional foods at a serving level of 10^{10} cfu and estimated daily exposure up to 10^{11} cfu.

- Intended uses of the strain are non-exempt infant and toddler formulas based on intact or partially hydrolyzed milk or soy protein, extensively hydrolyzed exempt formula, conventional foods including foods for infants and young children, juice and drinks for infants and young children, milk products including flavored milk beverages, meal replacement and powdered drink mixes, milk product analogs including soy, soy products, processed fruits and fruit juices, confectionary snacks, and baked goods.

- Addition to infant formula will not exceed 10^8 cfu of *L. plantarum* ATCC-202195/g powdered formula, resulting in a daily intake that will not exceed $1.1x10^{10}$ cfu of the microorganism per day. Assuming an approximate average body weight at birth of 3.55 kg and at 6 months of 7.6 kg (WHO), the maximum EDI is $2.8x10^9$ cfu/kg bw/day for newborns and $1.3x10^9$ cfu/kg bw/day for older infants.

- Addition of *L. plantarum* ATCC-202195 to conventional foods, including those intended for consumption by children as young as 2 years of age, will not exceed $2x10^{10}$ cfu/serving. Toddlers at age 2 are reported to consume about 6 servings of food and 2 servings of milk; if half of these servings each contain $2x10^{10}$ cfu *L. plantarum* ATCC-202195, the daily intake will be $8x10^{10}$ cfu, equivalent to $5.7x10^{9}$ cfu/kg bw/day. Older children and adults may consume up to 20 servings of foods and beverages. If half of these contain the microorganism at $2x10^{10}$ cfu/serving, the maximum daily intake would be $<2x10^{11}$ cfu, equivalent to $2.9\ x10^{9}$ cfu/kg bw/day.

- *L. plantarum* ATCC-202195 produces both D(-)- and L(+)-lactic acid isomers. Of the total lactic acid produced, about 60% is D(-)-lactate and 40% is L(+)-lactate. The strain is not intended to be used by individuals with certain medical conditions and conditions carrying an increased risk of developing D-lactate acidosis such as marked carbohydrate malabsorption, short bowel syndrome, gastrointestinal bypass surgery, or patients with increased risk of developing small intestinal bacterial overgrowth, including those with gastrointestinal dysmotility, or long-term use of proton pump inhibitor or opioid medication.

- Using the sequenced genome, screening for the presence of antibiotic resistance genes and mobile elements was completed. No transposons, transposases, insertion sequences, phages, or plasmids of health concern were found. No virulence or toxin genes were identified based on a genome survey using the VFDB database and RAST. While a few genes were identified in *L. plantarum* ATCC-202195 as potentially associated with virulence (toxin-antitoxin system, bacteriocins, hemolysin), further analysis demonstrated that these elements were not associated with virulence and as such it was concluded that these genes do not present a risk for human health.

- The antibiogram for this strain resulted in MIC values below or equal to the microbial break points defined by EFSA for *Lactobacillus plantarum/pentosus* with the exception of kanamycin, resistance to which is due to the presence of nucleotidyltransferase (*ant9-*la). This gene is genomically based and not located within 5000 bp upstream or downstream of any mobile elements (transposase, insertion sequences, etc.) and is therefore not considered a risk for transfer of antibiotic resistance genes.

- *L. plantarum* ATCC-202195 was demonstrated to promote alpha-hemolysis in culture conditions, which was determined to be most likely mediated through secretion of hydrogen peroxide. The strain has a protein, catalase KatE (EC 1.11.1.6), which converts $2\ H_2O_2 = O_2 + 2\ H_2O$. This protein limits but does not totally prevent the secretion of hydrogen peroxide This does not pose a health risk.

- The seed lot is fully characterized as *L. plantarum* ATCC-202195 to verify its identity prior to production. Ingredients utilized in the fermentation media and the cryoprotectant are approved food-grade substances that do not contain protein derived from milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts, soybeans, or sesame seed. Furthermore, these ingredients are not derived from gluten-containing grains nor grown on media derived from such grains. Equipment that may have come in contact with potential allergens is managed by preventive controls. Cleaning is verified through visual examination and testing. Neither *Lactiplantibacillus* nor *L. plantarum* are known to be

food allergens and there have been no reported allergenic responses in the *L. plantarum* clinical studies.

- The strain is produced using industry-standard fermentation techniques under a HACCP system. Each lot is tested using whole-genome sequencing to establish the identity of each master-seed batch to the genus, species, and strain level. The microbiological quality of each lot is determined by testing for microbiological contamination, including enterobacteriaceae, *Cronobacter sakazakii, Salmonella* spp., *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus*, sulfite-reducing bacteria, coliforms, yeast, and mold, as well as heavy metals.

- *L. plantarum* strains are widely regarded as safe. They received "Qualified Presumption of Safety" listing in 2007 and have remained QPS since. They are listed as "Natural Health Products" in Canada. In the U.S., several strains of *L. plantarum* have been accepted for use in dietary supplements and numerous strains have been notified as GRAS with no questions from FDA, including strain 299v, Lp-115, ECGC 13110402, DSM 33452, CECT 7527, CECT 7528, and CECT 7529.

- An unpublished study of subchronic oral toxicity of *L. plantarum* ATCC-202195 provides corroborating evidence of safety with a NOAEL of 8.71×10^{11} cfu/kg bw/day in male and female Sprague-Dawley rats. Published acute, subacute, and subchronic oral toxicity studies in numerous other *L. plantarum* strains have also demonstrated safety:

- Two published human trials demonstrated the safety of ingestion of 10^9 cfu of *L. plantarum* ATCC-202195/day by healthy term neonates. The second study included 4,556 infants delivered by C-section and regarded as being at elevated risk of sepsis. The 10 deaths among the treated infants were not attributable to the intervention and represented a 40% decrease in mortality from the control group. Numerous human trials of other strains of *L. plantarum* provided further evidence of safety

- Based on the decision tree published by Pariza et al. (2015), the strain is "deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption."

Conclusion

We, the undersigned GRAS panel members, are qualified by scientific education and experience to evaluate the safety of ingredients, including microorganisms, intended to be added to foods. We have individually and collectively critically evaluated the materials summarized above and conclude that Danisco USA, Inc.'s, *Lactiplantibacillus plantarum* ATCC-202195, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting food grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures for use as a microbial ingredient in exempt and non-exempt infant and toddler formulas at levels not to exceed 10^8 cfu/g powdered formula, and in conventional foods at levels not to exceed $2x10^{10}$ cfu/serving.

It is our professional opinion that other qualified experts would also concur in this conclusion.

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany
Berthold Koletzko, M.D., Ph.D.

Signature: _____ Date: 4 October 2022

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin
Michael Pariza, Ph.D.

Signature: _____ Date: _____

Steve Taylor, Ph.D.
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Munich, Germany

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin

Signature: [redacted] Date: 5 October 2022

Steve Taylor, Ph.D.
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Signature: [redacted] Date: [redacted]

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- Addition of *L. plantarum* ATCC-202195 to conventional foods, including those intended for consumption by children as young as 2 years of age, will not exceed $2x10^{10}$ cfu/serving. Toddlers at age 2 are reported to consume about 6 servings of food and 2 servings of milk; if half of these servings each contain $2x10^{10}$ cfu *L. plantarum* ATCC-202195, the daily intake will be $8x10^{10}$ cfu, equivalent to $5.7x10^{9}$ cfu/kg bw/day. Older children and adults may consume up to 20 servings of foods and beverages. If half of these contain the microorganism at $2x10^{10}$ cfu/serving, the maximum daily intake would be $<2x10^{11}$ cfu, equivalent to $2.9 x10^{9}$ cfu/kg bw/day.

- *L. plantarum* ATCC-202195 produces both D(-)- and L(+)-lactic acid isomers. Of the total lactic acid produced, about 60% is D(-)-lactate and 40% is L(+)-lactate. The strain is not intended to be used by individuals with certain medical conditions and conditions carrying an increased risk of developing D-lactate acidosis such as marked carbohydrate malabsorption, short bowel syndrome, gastrointestinal bypass surgery, or patients with increased risk of developing small intestinal bacterial overgrowth, including those with gastrointestinal dysmotility, or long-term use of proton pump inhibitor or opioid medication.

- Using the sequenced genome, screening for the presence of antibiotic resistance genes and mobile elements was completed. No transposons, transposases, insertion sequences, phages, or plasmids of health concern were found. No virulence or toxin genes were identified based on a genome survey using the VFDB database and RAST. While a few genes were identified in *L. plantarum* ATCC-202195 as potentially associated with virulence (toxin-antitoxin system, bacteriocins, hemolysin), further analysis demonstrated that these elements were not associated with virulence and as such it was concluded that these genes do not present a risk for human health.

- The antibiogram for this strain resulted in MIC values below or equal to the microbial break points defined by EFSA for *Lactobacillus plantarum/pentosus* with the exception of kanamycin, resistance to which is due to the presence of nucleotidyltransferase (*ant9-*la). This gene is genomically based and not located within 5000 bp upstream or downstream of any mobile elements (transposase, insertion sequences, etc.) and is therefore not considered a risk for transfer of antibiotic resistance genes.

- *L. plantarum* ATCC-202195 was demonstrated to promote alpha-hemolysis in culture conditions, which was determined to be most likely mediated through secretion of hydrogen peroxide. The strain has a protein, catalase KatE (EC 1.11.1.6), which converts $2 H_2O_2 = O_2 + 2 H_2O$. This protein limits but does not totally prevent the secretion of hydrogen peroxide This does not pose a health risk.

- The seed lot is fully characterized as *L. plantarum* ATCC-202195 to verify its identity prior to production. Ingredients utilized in the fermentation media and the cryoprotectant are approved food-grade substances that do not contain protein derived from milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts, soybeans, or sesame seed. Furthermore, these ingredients are not derived from gluten-containing grains nor grown on media derived from such grains. Equipment that may have come in contact with potential allergens is managed by preventive controls. Cleaning is verified through visual examination and testing. Neither *Lactiplantibacillus* nor *L. plantarum* are known to be

food allergens and there have been no reported allergenic responses in the *L. plantarum* clinical studies.

- The strain is produced using industry-standard fermentation techniques under a HACCP system. Each lot is tested using whole-genome sequencing to establish the identity of each master-seed batch to the genus, species, and strain level. The microbiological quality of each lot is determined by testing for microbiological contamination, including enterobacteriaceae, *Cronobacter sakazakii, Salmonella* spp., *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus*, sulfite-reducing bacteria, coliforms, yeast, and mold, as well as heavy metals.

- *L. plantarum* strains are widely regarded as safe. They received "Qualified Presumption of Safety" listing in 2007 and have remained QPS since. They are listed as "Natural Health Products" in Canada. In the U.S., several strains of *L. plantarum* have been accepted for use in dietary supplements and numerous strains have been notified as GRAS with no questions from FDA, including strain 299v, Lp-115, ECGC 13110402, DSM 33452, CECT 7527, CECT 7528, and CECT 7529.

- An unpublished study of subchronic oral toxicity of *L. plantarum* ATCC-202195 provides corroborating evidence of safety with a NOAEL of 8.71×10^{11} cfu/kg bw/day in male and female Sprague-Dawley rats. Published acute, subacute, and subchronic oral toxicity studies in numerous other *L. plantarum* strains have also demonstrated safety:

- Two published human trials demonstrated the safety of ingestion of 10^9 cfu of *L. plantarum* ATCC-202195/day by healthy term neonates. The second study included 4,556 infants delivered by C-section and regarded as being at elevated risk of sepsis. The 10 deaths among the treated infants were not attributable to the intervention and represented a 40% decrease in mortality from the control group. Numerous human trials of other strains of *L. plantarum* provided further evidence of safety

- Based on the decision tree published by Pariza et al. (2015), the strain is "deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption."

Conclusion

We, the undersigned GRAS panel members, are qualified by scientific education and experience to evaluate the safety of ingredients, including microorganisms, intended to be added to foods. We have individually and collectively critically evaluated the materials summarized above and conclude that Danisco USA, Inc.'s, *Lactiplantibacillus plantarum* ATCC-202195, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting food grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures for use as a microbial ingredient in exempt and non-exempt infant and toddler formulas at levels not to exceed 10^8 cfu/g powdered formula, and in conventional foods at levels not to exceed $2x10^{10}$ cfu/serving.

It is our professional opinion that other qualified experts would also concur in this conclusion.

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany
Berthold Koletzko, M.D., Ph.D.

Signature: _____ Date: 4 October 2022

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin
Michael Pariza, Ph.D.

Signature: _____ Date: _____

Steve Taylor, Ph.D.
Professor Emeritus
University of Nebraska
Lincoln, Nebraska

Signature: _____ Date: _____

Conclusion

We, the undersigned GRAS panel members, are qualified by scientific education and experience to evaluate the safety of ingredients, including microorganisms, intended to be added to foods. We have individually and collectively critically evaluated the materials summarized above and conclude that Danisco USA, Inc.'s, *Lactiplantibacillus plantarum* ATCC-202195, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting food grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures for use as a microbial ingredient in exempt and non-exempt infant and toddler formulas at levels not to exceed 10^8 cfu/g powdered formula, and in conventional foods at levels not to exceed $2x10^{10}$ cfu/serving.

It is our professional opinion that other qualified experts would also concur in this conclusion.

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin

Signature: [redacted] Date: 5 October 2022

Steve Taylor, Ph.D.
Professor Emeritus
University of Nebraska
Lincoln, Nebraska

Signature: _____ Date: _____

Conclusion

We, the undersigned GRAS panel members, are qualified by scientific education and experience to evaluate the safety of ingredients, including microorganisms, intended to be added to foods. We have individually and collectively critically evaluated the materials summarized above and conclude that Danisco USA, Inc.'s, *Lactiplantibacillus plantarum* ATCC-202195, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting food grade specifications, is Generally Recognized as Safe (GRAS) based on scientific procedures for use as a microbial ingredient in exempt and non-exempt infant and toddler formulas at levels not to exceed 10^8 cfu/g powdered formula, and in conventional foods at levels not to exceed $2x10^{10}$ cfu/serving.

It is our professional opinion that other qualified experts would also concur in this conclusion.

Berthold V. Koletzko, Dr med, Dr med habil (M.D., Ph.D.)
Professor of Pediatrics
University of Munich
Munich, Germany

Signature: _____ Date: _____

Michael W. Pariza, Ph.D.
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin

Signature: _____ Date: _____

Steve Taylor, Ph.D.
Professor Emeritus
University of Nebraska
Lincoln, Nebraska

Signature: [redacted] Date: OCTOBER 5, 2022

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001127	Oct 13, 2022
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Jayne Davies	Director of Global Regulatory Affairs

Organization *(if applicable)*: Danisco USA, subsidiary of International Flavors & Fragrances, Inc.

Mailing Address *(number and street)*: DuPont Experimental Station - E353 200 Powder Mill Road

City	State or Province	Zip Code/Postal Code	Country
Wilmington	Delaware	19803	United States of America

Telephone Number	Fax Number	E-Mail Address
610-864-7219		jayne.c.davies@iff.com

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

Name of Contact Person	Position or Title
James Heimbach	President

Organization *(if applicable)*: JHeimbach LLC

Mailing Address *(number and street)*: 923 Water Street #66

City	State or Province	Zip Code/Postal Code	Country
Port Royal	Virginia	22535	United States of America

Telephone Number	Fax Number	E-Mail Address
8047425543		JH@JHEIMBACH.COM

SECTION C GENERAL ADMINISTRATIVE INFORMATION

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

Lactiplantibacillus plantarum strain ATCC-202195

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

☒ Electronic Submission Gateway ☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D INTENDED USE

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

Intended uses are non-exempt infant and toddler formulas based on milk (intact or partially hydrolyzed) or soy protein, extensively hydrolyzed exempt formula, conventional foods including foods for infants and young children, juice and drinks for infants and young children, milk products including flavored milk beverages, meal replacement and powdered drink mixes, milk product analogs including soy, soy products, processed fruits and fruit juices, confectionary snacks and baked goods. Addition to infant formula will not exceed 10E8 cfu/g powdered formula and addition to conventional foods will not exceed 2x10E10 cfu/serving.

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E PARTS 2 7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

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

☐ Yes ☐ No

SECTION F SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Danisco USA

(name of notifier)

has concluded that the intended use*(s)* of Lactiplantibacillus plantarum strain ATCC-202195

(name of notified substance)

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

2. Danisco USA *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

Office of JHeimbach LLC at 923 Water Street, Port Royal VA 22535

(address of notifier or other location)

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	James T. Heimbach, President, JHeimbach LLC	10/06/2022

SECTION G LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	COSM_Form3667_DaniscoUSAsubsidiaryofInternationalFlavors FragrancesInc_10-06-2022.pdf	Administrative
	GRASNoticeforLp202195.pdf	Administrative
	SteveTaylorSignature.pdf	Administrative
	BertKoletzkoSignature.pdf	Administrative
	MikeParizaSignature.pdf	Administrative
	L.plantarumCoverLetter20221006.pdf	Administrative

From: James Heimbach
To: Anderson, Ellen; jh@jheimbach.com
Subject: [EXTERNAL] RE: GRAS notice for Lactiplantibacillus plantarum ATCC-202195
Date: Friday, May 5, 2023 9:05:32 AM
Attachments: image001.png

Dear Ellen—

Please note our responses below in red ink.

Regards,
Jim

From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Thursday, May 4, 2023 12:46 PM
To: jh@jheimbach.com
Subject: GRAS notice for Lactiplantibacillus plantarum ATCC-202195

Hello Jim,

I hope your health has improved with the arrival of spring and you are feeling much better!

We are processing the GRAS notice for *Lactiplantibacillus plantarum* ATCC-202195 that you submitted on behalf of Danisco USA. We would like to get some clarification on the intended uses. We note that on page 26 the notice states, "Intended uses are non-exempt infant and toddler formulas based on milk (intact or partially hydrolyzed) or soy protein, extensively hydrolyzed exempt formula, conventional foods including foods for infants and young children, juice and drinks for infants and young children, milk products including flavored milk beverages, meal replacement and powdered drink mixes, milk product analogs including soy, soy products, processed fruits and fruit juices, confectionary snacks and baked goods." There is also a table listing the intended uses on page 26.

We have the following questions:

1. The text states that flavored milk beverages are an intended food use; however, this use is not listed in the table under the "Food Use" column. Please confirm that the table on page 26 accurately lists the intended uses and clarify whether flavored milk beverages should be included as a food use. Flavored milk beverages are included, but simply included with "Milk Products" rather than listed separately.
2. For non-exempt infant formula, please clarify:
    a. That the formula is intended for term infants Yes

b. If partially hydrolyzed soy protein formula is included in the intended use No.

3. For exempt infant formula, please clarify:
   a. That the formula is intended for term infants Yes
   b. The protein base that is extensively hydrolyzed (e.g., cow milk, soy). Yes and no: extensively hydrolyzed cow's milk protein formulas are included, but not soy protein bases.

Thank you in advance for addressing these questions.

Sincerely,
Ellen

Ellen Anderson (she/her/hers)
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

JHeimbach LLC

August 3, 2023

Ellen Anderson
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition
U.S. Food & Drug Administration

Dear Ms. Anderson,

Below please find our responses to the questions from the FDA regarding GRAS Notice GRN 001127 sent on July 24, 2023.

1. Question: On page 15, the notice states, "In view of the potential risk of development of D-lactate acidosis and encephalopathy in infants and children with gastrointestinal conditions, products containing *L. plantarum* ATCC-202195 should be labelled with a statement indicating that the product should not be taken by patients with marked carbohydrate malabsorption such as patients with short bowel syndrome or gastrointestinal bypass surgery, and patients with increased risk of developing small intestinal bacterial overgrowth such as gastrointestinal dysmotility, or long term use of proton pump inhibitor or opioid medication. Cautions regarding use of *L. plantarum* ATCC-202195 in immune-compromised individuals and others under medical supervision are similar to those for other administered bacteria and many food ingredients and do not compromise the GRAS status of the intended use of the strain." We note that you responded to our pre-filing questions about the intended use of *L. plantarum* ATCC-202195 in an email dated May 5, 2023. In light of the statements made on page 15 of the notice, please confirm that *L. plantarum* ATCC-202195 is intended for use in the following types of infant formula for term infants:

non-exempt cow milk- or soy-based infant formula for term infants
non-exempt partially hydrolyzed cow milk-based infant formula for term infants
exempt extensively hydrolyzed cow milk-based infant formula for term infants.

Answer: Danisco confirms that *L. plantarum* ATCC-202195 is intended for use only in the following types of infant formula:

non-exempt cow milk- or soy-based formula for term infants
non-exempt partially hydrolyzed cow milk-based formula for term infants
exempt extensively hydrolyzed cow milk-based formula for term infants

923 Water Street #66, Port Royal, Virginia 22535, USA
tel. (+1) 804-742-5548 cell (+1) 202-320-3063 jh@jheimbach.com

2. Question: The notifier uses the term “toddler formula” to describe a formula intended for use at the age of 9 months and older. While FDA does not define toddler formula, an infant is defined as “a person not more than 12 months of age.” Therefore, please be aware that a formula intended for 9 months and older would be regulated as an infant formula.

Answer: Danisco would like to change the statement of the intended use of “toddler formula” as describing a formula intended for use at the age of 12 months and older (rather than 9 months and older).

3.Question: In Part 3 of the notice (page 26), the intended uses of L. plantarum ATCC-202195 include “conventional foods including foods for infants and young children.” Please confirm that the intended uses of *L. plantarum* ATCC-202195 do not include foods where standards of identity preclude its use.

Answer: **Danisco confirms that the intended uses of *L. plantarum* ATCC-202195 do not include foods where standards of identify preclude its use.**

4. Question: Part 3 of the notice (page 26) includes a table of the minimum and maximum use levels for each of the intended food categories. The serving sizes for the conventional food categories, other than infant formula and formula for young children, are listed in this table as 250 g. We note that a serving size of 250 g does not match the reference amounts customarily consumed per eating occasion listed in 21 CFR 101.12 for the food categories listed. For example, under CFR 101.12(b) Table 1, the reference amount for juices for infants and young children (aged 1 to 3 years) is 120 mL (4 fl oz). Please discuss the rationale for the serving size of 250 g or clarify the serving sizes and resulting use levels.

Answer: Danisco would like to submit the revised table below with minimum and maximum uses for each of the intended food categories. The target fortification remains a maximum of $2x10^{10}$ per serving with a minimum of $1x10^{9}$ per serving. The serving sizes are the reference amounts as listed under Tables 1 and 2 in 21 CFR 101.12 (b).

Food Category	Food Use	Max	Min
Non-exempt formula for infants and young children-milk, soy, partially hydrolyzed Extensively Hydrolyzed exempt infant formula	Infant formula (0-12 months), including extensively hydrolyzed exempt formula	10^{8} cfu/g provides 11B ($1.1x10^{10}$) cfu/d	10^{6} cfu/g to provide 1.1 $x10^{8}$ cfu/d
Formula for infants and young children	Toddler formula (12 mo and above)	10^{8} cfu/g provides 11B ($1.1x10^{10}$) cfu/d	10^{6} cfu/g to provide 1.1 $x10^{8}$ cfu/d
Foods for infants	Cereal and grain products, dry ready-to-eat cereals, puffs/melts, fruit and vegetable purees	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Foods for young children	Cereal and grain products, dry ready-to-eat cereals	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Juice and drinks for infants and young children	Juice/drinks/Dry-blended beverages	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Milk products	Yogurt, spoonable and drinkable, smoothies	$2x10^{10}$/serving	1 x 10^{9} cfu/ serving
Milk product analogs including soy	Smoothies, high-protein beverages, yogurts (non-dairy)	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Soy products		$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Powdered meal replace-ment or nutritional beverages		$2x10^{10}$/ serving	1 x 10^{9} cfu/serving
Processed fruits and fruit juices	Fruit juices and nectars (including fruit-based beverages)	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Confectionary snacks	Candies	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving
Baked Goods	Cereal and Nutrition Bars	$2x10^{10}$/ serving	1 x 10^{9} cfu/ serving

Reference: 21 CFR 101.12 (b)Tables 1 and 2 eCFR :: 21 CFR 101.12 -- Reference amounts customarily consumed per eating occasion.

5. Question: The specifications on page 23 of the notice include limits for lead (<0.5 mg/kg), cadmium (<0.2 mg/kg), and tin (<5 mg/kg), and the results of the analyses of three batches of *L. plantarum* ATCC-202195 for these heavy metals on page 24 are well below the specified limits. We note that specifications help to ensure that the ingredient is being manufactured in accordance with good manufacturing practices.
In addition, FDA's recent "Closer to Zero" initiative focuses on reducing dietary exposure to heavy metals from food. Please discuss the potential source of tin in *L. plantarum* ATCC-202195. Further, we request that specifications for heavy metals be as low as possible and consistent with the methods used and the results obtained from the batch analyses.

Answer: We do not have any source of tin that could be introduced into our process. Tin has been included in the current specification as part of our surveillance commitment to the pediatric nutrition industry.

IFF has been proactive in the evaluation and testing of dietary ingredients for potential contaminants. Our approach includes following the FDA "Closer to Zero" initiative focusing on reducing dietary exposure to heavy metals from food. The established testing limits are set at very low levels (ppb) which allows us to monitor our strains and proactively mitigate any risk well before it would ever reach the targeted limits (ppm). Probiotics are small inclusion ingredients in infant formula and other infant products. This low inclusion has been utilized to calculate the targeted specification listed in this submission.

6. Question: The estimated maximum dietary exposures for *L. plantarum* ATCC-202195 from the intended use in infant formula presented in the notice (page 27) are based on the estimated mean consumption of infant formula and average infant body weights at birth and 6 months. Please discuss the estimated dietary exposures to *L. plantarum* ATCC-202195 for the upper percentile (e.g., 90th percentile) consumers of infant formula.

Answer: Food consumption data reported in the 2015-2016 National Health and Nutrition Examination Survey (NHANES) dataset compiled by the U.S. Department of Health and Human Services, National Center for Health Statistics, and the Nutrition Coordinating Center (as cited in GRN 952) can be used to estimate the dietary exposure of *L. plantarum* 202195 from infant formula (as consumed, ready-to-drink or reconstituted formula prepared from powder) at the 90tile for 0-6 mo. and 6-12 mo. can be calculated.
See Table 9 in GRN 952. https://www.fda.gov/media/148117/download

The 90tile intake for 3-5.9 mo. can be used as representative of infants 0-6 mo. Formula volume is 1239 ml/d and provides 1.67 x10^{10} cfu/d (assuming addition level of 10^8 cfu/g). The 90tile intake for 9-11.9 mo. can be used as representative for infants 6-12 mo. Formula volume is 1097 ml/d and provides 1.48x10^{10} cfu/d (assuming addition level of 10^8 cfu/g). These exposures are well within the levels shown to be safe.

7. Question: We note that some of the methods used for microbial specifications are not specific to the notified substance. For example, the method used to detect total viable count, ISO 7889/IDF 117, is intended for use in yogurt and the method used to detect yeast and mold, USP 61, is intended for dietary supplements. Please clarify that all methods used for specifications are validated for the intended use in the notified substance and are fit for purpose.

Answer: All methods that are utilized for the notified substance, *L. plantarum* 202195, have either been qualified and/or validated for the intended use in the notification and are fit for the purpose.

President

cc. Jayne Davies, IFF

From: James Heimbach
To: Anderson, Ellen
Cc: "Jim Heimbach"; Jayne Davies
Subject: RE: [EXTERNAL] Responses to Questions on GRN 1127
Date: Wednesday, September 13, 2023 12:55:10 PM
Attachments: image001.png
image002.png

Dear Ellen—

You concluded your last email to me by requesting that we "please consider reducing the specification for lead and cadmium to a level comparable to arsenic."

The level for arsenic is <0.1 mg/kg. We agree to reduce the specification for cadmium to a comparable level, ≤0.1 mg/kg, and to reduce the specification for lead to a level half that, i.e., ≤0.05 mg/kg.

We are confident that this complies with your request.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street #66
Port Royal VA 22535
USA
Tel: (+1) 804-742-5543
Cell: (+1) 202-320-3063
Email: jh@jheimbach.com

From: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Sent: Wednesday, August 30, 2023 3:52 PM
To: James Heimbach <jheimbach@va.metrocast.net>
Subject: RE: [EXTERNAL] Responses to Questions on GRN 1127

Hello Jim,

Thank you again for your response to our questions received on August 3. A copy of your response is attached for your reference.

We have one follow-up to our previous request (Question #5) regarding the heavy metal specifications for *Lactiplantibacillus plantarum* ATCC-202195, in which we noted that the specifications for lead (<0.5 mg/kg) and cadmium (<0.2 mg/kg) are significantly higher than the observed results from the analyses of three batches of *L.*

plantarum ATCC-202195. You stated that "The established testing limits are set at very low levels (ppb) which allows us to monitor our strains and proactively mitigate any risk well before it would ever reach the targeted limits (ppm)." We note that the specification for arsenic (<0.1 mg/kg) is lower than lead and cadmium; however, batch results demonstrated that arsenic was detectable and found at higher levels (24.3 to 63.3 µg/kg) compared to lead (<10 to 33 µg/kg) and cadmium (<10 to <20 µg/kg). Based on these observations, please consider reducing the specification for lead and cadmium to a level comparable to arsenic.

Please let me know if you have any questions regarding this follow-up.

Sincerely,
Ellen

From: Anderson, Ellen
Sent: Friday, August 4, 2023 8:21 AM
To: James Heimbach <jheimbach@va.metrocast.net>
Subject: RE: [EXTERNAL] Responses to Questions on GRN 1127

Good morning Jim,

Thank you for the responses. We will review the information and I will contact you if we need further clarification.

I hope you enjoy the weekend as well!

Sincerely,
Ellen

Ellen Anderson (she/her/hers)
Regulatory Review Scientist

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1309
ellen.anderson@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: James Heimbach <jheimbach@va.metrocast.net>
Sent: Thursday, August 3, 2023 4:26 PM
To: Anderson, Ellen <Ellen.Anderson@fda.hhs.gov>
Subject: [EXTERNAL] Responses to Questions on GRN 1127

Ellen—

Here are our responses to your questions on GRN 1127. Have fun reading, and have a good weekend!

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street #66
Port Royal VA 22535
USA
Tel: (+1) 804-742-5543
Cell: (+1) 202-320-3063
Email: jh@jheimbach.com

Ellen Anderson
Regulatory Review Scientist
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

Dear Ellen:

Here are our responses to the issues you raised in your letter of November 8.

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

   Response:
   The primary search was completed in May 2020. The databases included Pubmed and Google Scholar, covering 1998 to the present, and the principal search terms were *L. plantarum* and clinical trials.

   A secondary search was based on discussion with members of the GRAS Panel and focused on food allergens and allergenicity, gastrointestinal dysmobility, and d-lactate acidosis. The search terms from the primary search were also repeated. This search was completed in October 2022, at which time the document was mailed to FDA.

2. *A brief summary of reports of bacteremia or foodborne illness involving* L. plantarum *and the relevance of these to safety of the article of commerce. This could include a discussion of the populations who mainly experience bacteremia or other evidence as to why these reports do not indicate a safety concern for healthy consumers.*

   Response:
   None of the studies identified in the literature reported cases of bacteremia or food-borne illness. There were 2 studies that included sepsis as either a primary or secondary outcome or an adverse event.

   In the Panagrahi et al. (2017) trial with the subject strain at $1x10^9$ cfu/day in combination with fructooligosaccharides, the target population was infants at an increased risk of sepsis. Sepsis was included as a primary outcome. Infants in the supplemented group had reduced incidence of sepsis. None of the blood cultures drawn from septic infants were positive for the genus *Lactobacillus* and therefore would not be positive for the species (i.e. *L. plantarum*). See study details in Table 8. Human Studies of *L. plantarum* Strain ATCC-202195 in the GRAS notice.

   The second trial, conducted by McNaught et al. (2002), studied a diseased population of elderly adults undergoing abdominal surgery who received 2.5 $x10^{10}$ cfu/day of *L.*

923 Water Street #66, Port Royal, Virginia 22535, USA
tel. (+1) 804-742-5548 cell (+1) 202-320-3063 jh@jheimbach.com

plantarum 299v . The primary outcome was reduction of sepsis. No differences were seen in any outcomes between the test and control groups, including septic complications. The authors concluded that "preoperative administration of the probiotic *L. plantarum* 299v for two weeks has no effect [either beneficial or adverse] on the human gut mucosal barrier … and the systemic inflammatory response." These findings, in a high-risk population of elderly diseased individuals, did not identify a safety concern associated with consumption of the species and hence does not indicate a safety concern for healthy consumers including infants. See study details in Table 9. Human Studies of Other Strains of *Lactiplantibacillus plantarum*.

Neither of these studies indicates a safety concern for healthy consumers, including term infants or the general population of children and adults for whom the subject strain, *L. plantarum* 202195, is intended. The species in the identified sepsis in the Panigrahi et al. (2017) trial was not *L. plantarum* and use of the strain *L plantarum* 202195 in conjunction with FOS reduced risk of sepsis.

Citations:

McNaught, C.E., Woodcock, N.P., MacFie, J., Mitchell, C.J., 2002. A prospective randomised study of the probiotic *Lactobacillus plantarum* 299V on indices of gut barrier function in elective surgical patients. Gut 51, 827–831.

Panigrahi, P., Parida, S., Nanda, N.C., Satpathy, R., Pradhan, L., Chandel, D.S., Baccaglini, L., Mohapatra, A., Mohapatra, S.S., Misra, P.R., Chaudhry, R., Chen, H.H., Johnson, J.A., Morris, J.G., Paneth, N., Gewolb, I.H. 2017. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 548, 407–412

3. *Whether findings from any publications contradict Danisco's GRAS conclusion.*

Response:

The GRAS notice included the following statement, which remains true:

6.7. Affirmative Statement Concerning Data and Information

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with Danisco's conclusion of GRAS status under the conditions of intended use.

James T. Heimbach, Ph.D., F.A.C.N.
President

cc. Jayne Davies, IFF