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Division of Biotechnology and GRAS Notice Review
Center for Food Safety & Applied Nutrition (HFS-255)
U.S. Food & Drug Administration
5100 Campus Drive
College Park, MD 20740

28 June 2022

Dear FDA Administrator,

In accordance with 21 CFR Part 170, Subpart E, we are hereby submitting this GRAS Notice on behalf of BiomEdit, LLC who has concluded that the use of a mixture of *Limosilactobacillus reuteri* strains 3360 and 3362 are generally recognized as safe based upon scientific procedures in accordance with 21 CFR 170.30(b), and therefore exempt from premarket approval for their use as an ingredient in select foods.

Sincerely,

James La Marta, Ph.D.

BIOMEDIT

THE SAFETY AND THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF THE 1:1 CFU BASIS MIXTURE OF *Limosilactobacillus reuteri* STRAINS 3630 & 3632 AS AN INGREDIENT IN THE INTENDED CONVENTIONAL CATEGORIES OF HUMAN FOOD

Submitted by the Notifier:
BiomEdit, LLC
Indianapolis, IN

Assembled by:
James La Marta, Ph.D., CFS
Splitrock Regulatory Solutions, LLC

BIOMEDIT

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Abbreviations:

AOAC – American Organization of Analytic Chemists
ATCC – American Type Culture Collection
BAM – Bacteriological Analytical Manual
BLAST – Basic Local Alignment Search Tool
CFR – Code of Federal Regulations
CFUI – Colony Forming Unit
CMMEF – Compendium of Methods for the Microbiological Examination of Foods
CRL – Charles River Labs
DSLD – Dietary Supplement Label Database
DSM – Deutsche Sammlung von Mikroorganismen (German Culture Collection)
EFSA – European Food Safety Authority
FDA – Food & Drug Administration
GRAS – Generally Recognized As Safe
IFN-g – Interferon Gamma
Ige – Immunoglobulin ‘e’
ISO – International Standards Organization
Kc/GRO - Keratinocyte chemoattractant (KC)/human growth-regulated oncogene (GRO)
MIC – Microbial Inhibition Concentration
MRS – de Man, Rogosa and Sharpe
NDIN – New Dietary Ingredient Notice
NEC – necrotizing enterocolitis
NLT – Not Less Than
NMT – Not More Than
PCR – Polymerase Chain Reaction
ppb- parts per billion
ppm – parts per million
QC – Quality Control
RH – Relative Humidity
rRNA – Ribosomal Ribonucleic Acid
SOP – Standard Operating Procedure
SQF – Safe Quality Food
TRPO - Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR
UBCG – Up-to-date Bacterial Core Gene
USC – United States Code
USDA – United States Department of Agriculture
USPM – United States Pharmacopeia Method
VF – Virulence Factor
VFDB – Virulence Factor Database
WGA – Whole Genome Analysis
WGS – Whole Genome Sequence

Executive Summary

Elanco Animal Health isolated two strains of *Limosilactobacillus reuteri* (previously described as *Lactobacillus reuteri* and referred to herein as *L. reuteri* [Zheng et al, 2020]) from chickens. BiomEdit, LLC, a new company spun off from Elanco Animal Health Inc. in April 2022, has concluded that the addition of a mixture of the *L. reuteri* strain 3630 and *L. reuteri* strain 3632 in a ratio of 1:1 on a colony-forming unit (CFU) basis (referred to herein as "*L. reuteri* mixture") is generally recognized as safe for use as a live microbial ingredient in the intended conventional human food categories at up to 1 x 10^{10} CFU/day. The purpose of the ingredient is to increase the concentration of members of the *Lactobacillaceae* in the digestive tract in the same manner as other members of the taxonomic family that have been notified to the FDA.

The strains are grown independent of each other using a food grade nutrient media following standard large scale fermentation practices and cGMP applicable to producing live microbials for use in human foods. Each organism is harvested and dried and then the strains are combined to provide equal concentrations on a CFU basis. Batch data confirm that the strains can be grown consistently to meet the established specifications.

To conclude that the *L. reuteri* mixture is a safe ingredient for the intended use, the sponsor unambiguously identified the two novel *L. reuteri* isolates, ATCC PTA-126787 (3630) and ATCC PTA-126788 (3632), and then evaluated the safety of the *L. reuteri* mixture by conducting *in silico* and *in vitro* analyses on each of the strains and an *in vivo* toxicity study using the *L. reuteri* mixture and reviewing all literature pertaining to the safety of this microbial species. Using the information from whole genome sequencing and *in silico* analysis, it was found that the strains do not produce known toxicants nor antibiotics. Antimicrobial susceptibility testing revealed that the strains are susceptible to common antibiotics. The strains do not produce virulence factors or toxins, biogenic amines, nor antibiotics to typically tested pathogens (Gangaiah, D, et al, 2022). A 28-day study with Sprague-Dawley rats did not reveal any hematologic nor histopathologic differences that were statistically significant between the *L. reuteri* mixture treatment groups and control. The no-observed-adverse-effect-level (NOAEL) was concluded to be the high dose of the *L. reuteri* mixture in this study, 1.6 x 10^{10} CFU/kg bw/day for strain 3630 plus 5.7 x 10^{10} CFU/kg bw/day for strain 3632.

The extensive review of the published safety literature included three GRAS notices to FDA CFSAN and more than 30 papers on human clinical trials regarding the consumption of different strains of *L. reuteri* at doses of up to 1 x 10^{11} CFU/person/day and there were no adverse effects due to consumption. The published literature and the results of the toxicological study support the safe use of the *L. reuteri* mixture as a live microbial ingredient in conventional human food categories.

1 Introduction and claim of exemption from premarket approval requirements

1.1 **Accordance with 21 CFR 170.225(c)(1)**

This Notice is being submitted in compliance with 21 CFR 170 subpart E.

1.2 Name and address of Sponsor

BiomEdit, LLC (hereafter "BiomEdit")
10100 Lantern Rd, Suite 150
Fishers, IN 46037

BiomEdit, LLC was formed in April 2022 as a microbiome-focused spin-off from Elanco Animal Health, Inc. ("Elanco"). Much of the data and information supporting this GRAS conclusion were developed by Elanco; however, they were transferred to BiomEdit with its formation. This explains the references to Elanco in this dossier and supporting documents.

1.3 Individuals responsible for the dossier:

Emily Bulian Helmes, MBA
Vice President Regulatory
BiomEdit, LLC
10100 Lantern Rd, Suite 150
Fishers, IN 46037
Tel: 703-424-1855

James La Marta, Ph.D., CFS
Splitrock Regulatory Solutions, LLC
107 Decker Road
Boonton, NJ 07005
Tel: 973-335-1670

1.4 Name and Address of Manufacturer of Pilot Batches

BioSource Cultures & Flavors
S66 W14328 Janesville Road
Muskego, WI 53150-0777

1.5 Name of the GRAS Substance

The GRAS substance is a mixture of *Limosilactobacillus reuteri* strains 3630 and 3632 in a ratio of 1:1 on a CFU basis and will be sold as a proprietary *L. reuteri* blend (referred to herein as "*L. reuteri* mixture"). Historically, this species was included in and known as the

Lactobacillus genus (Zheng et al, 2020), hence it is also referred to herein as either *L. reuteri* or *Lactobacillus reuteri*.

1.6 Intended conditions of use of the GRAS substance

The two strains of *Limosilactobacillus reuteri*, 3630 and 3632, in a ratio of 1:1 on a CFU basis, are to be used as a live microbial ingredient in conventional human food categories. The ingredient could be added to products that support the continued viability of the microorganisms, such as, but not limited to beverages, dairy products, nutritional powders and bars. The product is not intended to be used in infant formulae nor in meat and poultry products regulated by the USDA. The estimated maximum exposure for the 90th percentile is 2 x 10^{10} CFU/day of the combined strains based upon the maximum consumption of 10 servings of food.

1.7 Basis for GRAS conclusion

Pursuant to 21 C.F.R. § 170.30(a)(1), a panel of independent experts (the GRAS Panel) was assembled to review the accumulated data regarding the safety of *Limosilactobacillus reuteri* strains 3630 and 3632 and to evaluate the data using scientific procedures and decide if they agree with the BiomEdit conclusion that the equal CFU count mixture of *Limosilactobacillus reuteri* strains 3630 and 3632 is safe for use as an ingredient in the specified human food products. The GRAS Panel was further requested to conclude whether other qualified experts reviewing the same data as had been reviewed by the GRAS Panel would also conclude that the equal CFU count mixture of *Limosilactobacillus reuteri* strains 3630 and 3632 would be Generally Recognized As Safe (GRAS) for use as an ingredient in the specified human food products.

1.8 GRAS Exemption from Pre-market Approval Claim

Pursuant to the GRAS rule [81 Fed. Reg. 159, 54960 (17 August 2016)] and 21 C.F.R. § 170.225 (c) (6), BiomEdit, LLC has concluded that the equal CFU count mixture of *Limosilactobacillus reuteri* strains 3630 and 3632 is GRAS through scientific procedures, in accordance with 21 CFR 170.30 (b) and therefore is exempt from the requirement for premarket approval.

1.9 Availability of information for FDA review

Pursuant to 21 C.F.R. § 170.225 (c) (7),

A) The data and information that are the basis for BiomEdit, LLC's GRAS conclusion is available for the FDA's review and copying upon request during normal business hours at:

BiomEdit, LLC
10100 Lantern Rd, Suite
150 Fishers, IN 46037

A copy of all the information is also maintained by:

Splitrock Regulatory Solutions LLC
107 Decker Rd
Boonton, NJ 07005
973-335-1670

B) Upon FDA's request, we will provide FDA with a complete copy of the data and information either in an electronic format that is accessible for their evaluation or on paper.

1.10 Freedom of Information Act Exemption

Pursuant to 21 C.F.R. § 170.225 (c) (8), all the data or information presented in Sections 2 through 7 of this GRAS assessment do not contain any trade secret, commercial or financial information that is exempt from disclosure under the Freedom of Information Act, (5 U.S.C. § 552).

1.11 Certification

In compliance with 21 C.F.R. § 170.225 (c) (9), we hereby state that to the best of our knowledge, this GRAS dossier is a complete, representative, and balanced submission that includes favorable as well as unfavorable information, known to BiomEdit, LLC and pertinent to the evaluation of the safety and GRAS status of the use of the equal CFU count mixture of *Limosilactobacillus reuteri* strains 3630 and 3632 as an ingredient in human food.

James La Marta, Ph.D., CFS
Splitrock Regulatory Solutions, LLC

Date: 27 June 2022

Emily Bulian Helmes

Digitally signed by Emily Bulian Helmes
Date: 2022.06.30 10:57:31 -04'00'

Emily Bulian Helmes
Vice President Regulatory
BiomEdit, LLC

Date: 27 June 2022

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2 Identity of the GRAS substance

2.1 Common or usual name of the notified substance

The common or usual name of the substance is a 1:1 CFU basis mixture of *L. reuteri* strains 3630 and 3632, shortened to '*L. reuteri* mixture' in the dossier. The taxonomic identification was confirmed by genomic analysis using 16S rRNA sequence analysis with biochemical profiling by use of the API 50 CHL test, see Annex 1. *Limosilactobacillus reuteri* was previously known as *Lactobacillus reuteri*, and this taxonomic reclassification was established in April 2020 (Zheng et al., 2020).

2.2 Characterization of *L. reuteri* strains 3630 and 3632

The *L. reuteri* 3630 and 3632 strains were isolated from a single chicken cecum (sourced from University of Hannover, Germany) in 2017 in Cuxhaven, Germany, by Elanco Animal Health scientists and have been deposited in the American Type Culture Collection as ATCC PTA-126787 and ATCC PTA-126788, respectively. See Annex 2 for the strain deposit receipts. These isolates have been studied in several animal research trials and were found to have unique properties which led to further development in a microbial ingredient for use in human food. These strains have not been genetically engineered.

2.2.1 Taxonomy

Table 1 Taxonomy of the bacterial strains

Lineage	***L. reuteri* 3630**	***L. reuteri* 3632**
Kingdom	Bacteria	Bacteria
Phylum	Firmicutes	Firmicutes
Class	Bacilli	Bacilli
Order	Lactobacillales	Lactobacillales
Family	Lactobacillaceae	Lactobacillaceae
Genus	*Limosilactobacillus*	*Limosilactobacillus*
Species	*reuteri*	*reuteri*
Strain	3630	3632

2.3 Phenotypic identification of the bacterial strains

2.3.1 Morphology

- Opaque, circular colonies with slight whitish center (Figure 1)
- Whitish colonies for *L. reuteri* 3630; dull orange pigmentation for *L. reuteri* 3632
- Non-spore forming

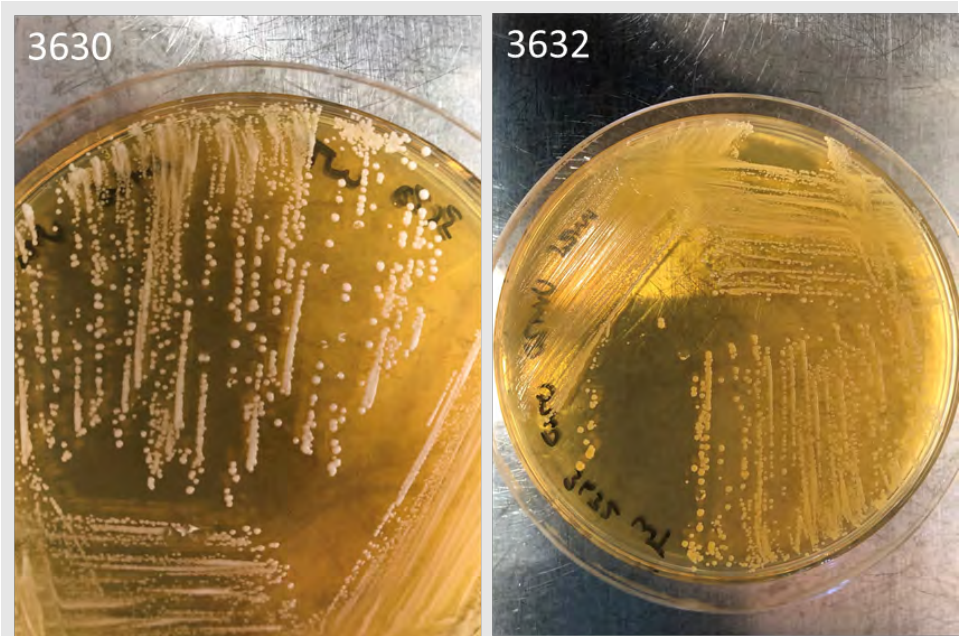


Figure 1 Colony morphology of *L. reuteri* 3630 and 3632 on MRS agar.

2.3.2 Microscopic observations

Both strains are Gram-positive, non-motile rods, most frequently occurring as doublets, but singlets and long chains are often present as well (Figure 2).

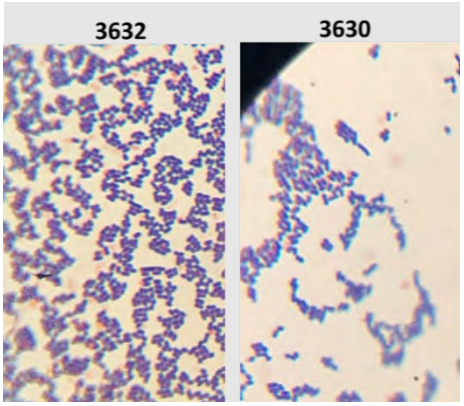


Figure 2 Microscopic observation of Gram-stained 3632 and 3630.

2.3.3 Biochemical properties of the *L. reuteri* mixture

The biochemical, growth and enzyme production properties of *L. reuteri* 3630 and 3632 strains are summarized in Table 2 and Table 3.

Table 2 **Biochemical and growth properties**

Property	**Observations of *L. reuteri* 3630 and 3632**
Fermentation metabolism	Obligately homofermentative, primarily produces lactic acid as the end product
Gram stain	+
Beta-galactosidase	+
Hydrogen peroxide	+
Optimal growth temperature	37-39 C
Oxygen requirement	Microaerophilic or anaerobic
Auto aggregation	3632, +; 3630, -

Enzyme production profiles of *L. reuteri* 3632 and 3630 were determined using the API Zym kit (BioMerieux USA), which assays for 19 different enzymes as shown in Table 3.

Table 3 Enzyme production profiles of *L. reuteri* 3630 and 3632 using API ZYM kit.

No.	Enzyme Assayed For	*L. reuteri* 3630	*L. reuteri* 3632
1	Control		
2	Alkaline phosphatase	-	-
3	Esterase (C 4)	+	+
4	Esterase Lipase (C 8)	-	-
5	Lipase (C 14)	-	-
6	Leucine arylamidase	+	+
7	Valine arylamidase	+	+
8	Cystine arylamidase	-	-
9	Trypsin	-	-
10	α-chymotrypsin	-	-
11	Acid phosphatase	-	-
12	Naphthol-AS BI-phosphohydrolase	+	+
13	α-galactosidase	+	+
14	β-galactosidase	+	+
15	β-glucuronidase	-	-
16	α-glucosidase	-	-
17	β-glucosidase	-	-
18	N-acetyl-β-glucosaminidase	-	-
19	α-mannosidase	-	-
20	α-fucosidase	-	-

+, positive reaction; -, negative reaction

2.3.4 Genomic Analysis

2.3.4.1 16S rRNA Sequence Analysis

Genotypic identification of *L. reuteri* strains 3632 and 3630 was conducted by 16S rRNA sequence analysis, which is commonly used for taxonomical classification of bacteria and has become a standard practice to identify new bacterial isolates. DNA was isolated from each of the two *L. reuteri* strains, and the 16S rRNA sequences were obtained by Sanger sequencing; BLAST analysis of the 16S rRNA sequences showed that the sequences of 3632 and 3630 have closest relationship to *L. reuteri* as shown in Figure 3A & 3B.

Figure 3 PCR (A) and 16S rRNA sequence identification (B) of *L. reuteri* 3632 and 3630. The human *L. reuteri* strain ATCC 23272 and *L. acidophilus* strain ATCC 4356 were used as controls for phylogenetic analyses.

2.3.4.2 Whole-genome sequencing (WGS)

The strains were sequenced by PacBio sequencing. *L. reuteri* strain 3632 contained 7 contigs yielding a total estimated genome size of 2.4 Mb, and *L. reuteri* strain 3630 contained 5 contigs yielding an estimated genome size of 2.4 Mb. Phylogenetic relationships of the genomes were explored with UBCG v3.0 using default settings. This software tool employs a set of 92 single-copy core genes commonly present in all bacterial genomes. These genes then were aligned and concatenated within UBCG using default parameters. The estimation of robustness of the nodes is done through the gene support index (GSI), defined as the number of individual gene trees, out of the total genes used, that present the same node. A maximum-likelihood phylogenetic tree was inferred using FastTree v.2.1.10 with the GTR+CAT model. As shown in Figure 4, the *L. reuteri* 3632 and *L. reuteri* 3630 strains showed closest relationship to the genus and species, *L. reuteri*. In addition, Figure 4 reveals that *L. reuteri* 3630 and 3632 are close to one another in the phylogenetic tree; the average nucleotide identity analysis revealed these two strains have 99.95% similarity.

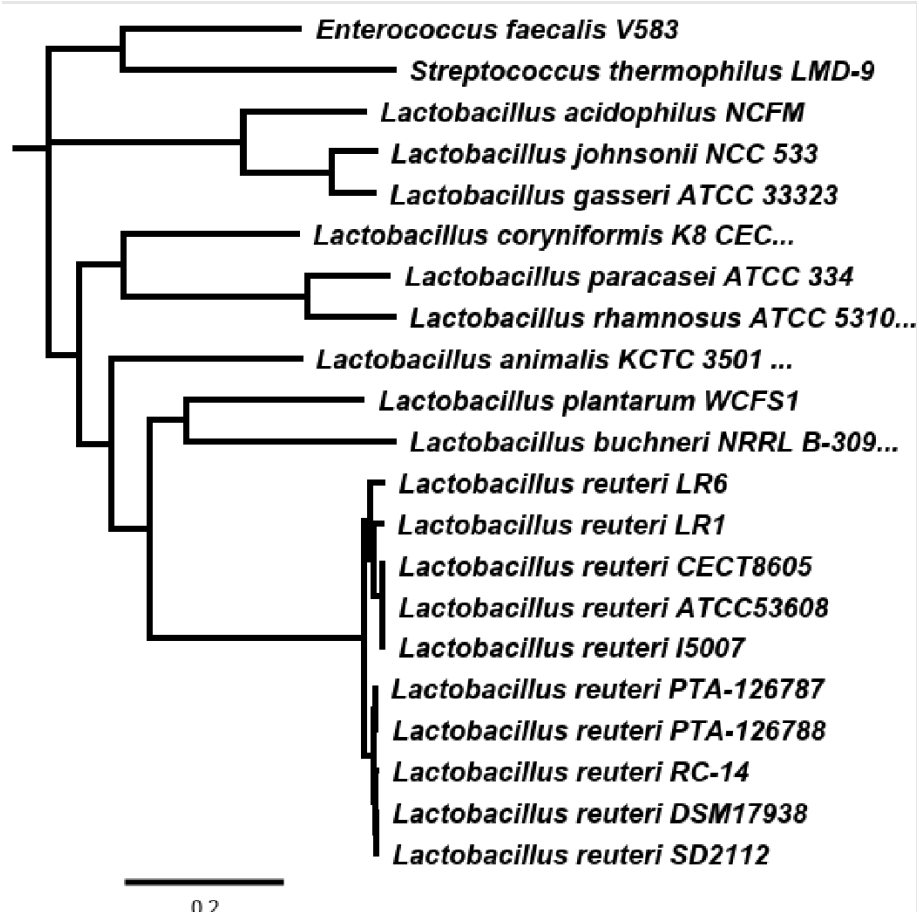


Figure 4 Phylogenetic relationship of *L. reuteri* 3630 (*L. reuteri* PTA-126787) and *L. reuteri* 3632 (*L. reuteri* PTA-126788) to other known *L. reuteri* strains using 92 core genes.

2.3.5 Phenotypic Properties

2.3.5.1 Absence of Toxins and other Virulence Factors

Both *L. reuteri* 3630 and 3632 strains were confirmed to be free of known virulence factors and/or toxins by BLAST analysis of the whole genome sequence (WGS) of each of the two *L. reuteri* strains against the virulence factor database (VFDB; see Annex 3), which is an integrated comprehensive online resource database for curating information about bacterial virulence factors and/or toxins (Liu, B et al., 2019). As of May 17, 2022, the VFDB contained 32543 non-redundant VF-related genes of which 3603 were with curation.

2.3.5.2 Absence of hemolysin production

Both *L. reuteri* strains were evaluated for hemolytic activity using blood agar plates with 5% sheep's blood, incubated for 48 hours at 37 °C. No hemolysis zones around the colonies were identified confirming a lack of hemolytic activity, see Annex 4.

2.3.5.3 Absence of prophage and extra-chromosomal elements

L. reuteri 3630 and 3632 genomes were analyzed for potential sequences encoding prophages using the software program PhiSpy v4.2.6, which combines similarity- and composition-based strategies (Akhter et al., 2012). Several genes encoding known prophage proteins were identified in both *L. reuteri* genomes; however, these genes did not appear to encode for any known antimicrobial resistance proteins, virulence factors or toxins.

The *L. reuteri* genomes were also scanned for extrachromosomal elements such as plasmids by BLAST analysis of PacBio contigs. *L. reuteri* 3632 and 3630 each contained three potential native plasmids; however, these plasmids do not appear to encode for any known antimicrobial resistance proteins, virulence factors or toxins.

2.3.5.4 Assessment of Biogenic Amine Production

Many lactic acid bacteria are known to produce biogenic amines such as histamine, tyramine, putrescine and cadaverine. These biogenic amines are normally produced by amino acid decarboxylation of histidine, tyrosine, ornithine and lysine. Toxicity from these amines is generally rare, and most toxicity cases are associated with histamine and to some extent tyramine.

In silico analyses were performed on *L. reuteri* 3632 and 3630 genomes to identify homologs of histidine decarboxylase, tyrosine decarboxylase, lysine decarboxylase, ornithine decarboxylase, agmatine deiminase, agmatine: putrescine antiporter and multicopper oxidase, which are all involved in the production of biogenic amines. *L. reuteri* 3632 and 3630 do not appear to contain any of these homologs in their genomes as shown in Table 4. The lab report is in Annex 5.

Table 4 Bioinformatics analysis of *L. reuteri* 3632 and 3630 genomes for genes involved in biogenic amine production.

Gene	Description	Source	Length (nt)	GenBank Accession #	Protein (aa)	*L. reuteri* 3632	*L. reuteri* 3630
hdcA	Histidine decarboxylase (histamine production)	*Lactobacillus buchneri*	951	AJ749838.1	317	Absent	Absent
tyrDC	Tyrosine decarboxylase (tyramine production)	*Lactobacillus curvatus*	1866	MF537630.1	622	Absent	Absent
ldc	Lysine decarboxylase (cadaverine production)	*Lactobacillus saerimneri* 30A	2181	ANAG01000014.1	727	Absent	Absent
odc	Ornithine decarboxylase	*Lactobacillus acidophilus*	2091	AY542890.1	697	Absent	Absent
aguA	Agmatine deiminase (N-carbamoyl putrescine production)	*Lactobacillus brevis*	1095	AF446085.5	365	Absent	Absent
aguD	Agmatine:putrescine antiporter (N-carbamoyl puterscine production)	*Lactobacillus brevis*	1389	AF446085.5	463	Absent	Absent
sufI	Multicopper oxidase	*Lactobacillus paracasei* strain CB9CT	1530	KU962939.1	510	Absent	Absent

L. reuteri 3630 and 3632 were also tested for their ability to produce biogenic amines as described previously (Branton et al., 2011). Consistent with the bioinformatics results, neither of the subject *L. reuteri* strains were able to produce the major biogenic amines histamine, tyramine, putrescine or cadaverine. The positive control *L. reuteri* ATCC 23272, which contains a gene encoding histidine decarboxylase, produced a positive reaction in the area of bacterial growth on the decarboxylase base media supplemented with L-histidine. Control plates lacking these amino acids showed no positive reaction for any of the strains tested.

2.3.5.5 Absence of antimicrobial resistance

The Pariza et al. (2015) decision tree and the European Food Safety Authority (EFSA Panel on Additives and Products or Substances used in Animal Feed (EFSA, FEEDAP 2012) recommend that microbial strains used in food applications must not harbor acquired antimicrobial resistance genes to clinically relevant antimicrobials. Whole genome analysis found the presence of the *tetW*, the ribosomal protection protein for resistance to

tetracycline. The gene is located in the chromosome in both strains. No elements indicative of horizontal transfer (plasmids, phages, transposons, or conjugation elements) were identified in the 15-kb flanking regions on both sides of *tetW.* See report in Annex 6.

Susceptibility testing of *L. reuteri* 3630 and 3632 strains for clinically relevant antimicrobials indicates that these strains do not express acquired antimicrobial resistance factors to known antibiotics of clinical significance. See report in Annex 7. Minimum inhibitory concentrations were analyzed against relevant antibiotics according to EFSA guidelines (EFSA Panel on Additives and Products or Substances used in Animal Feed (EFSA, FEEDAP 2012), including Ampicillin, Gentamicin, Kanamycin, Streptomycin, Erythromycin, Clindamycin, Tetracycline and Chloramphenicol. *L. reuteri* 3632 and 3630 strains were determined to be sensitive to all relevant tested antibiotics according to EFSA guidelines (EFSA, 2018), with minimum inhibitory concentrations (MIC) values at or below the reported species characteristic cut-off values (Table 5).

Table 5 Susceptibility of *L. reuteri* 3632 and 3630 to EFSA Critically Important Antibiotics (CIAs)

	***L. reuteri* 3632**	***L. reuteri* 3630**	**EFSA microbiological cut off values for *L. reuteri* mg/L**
Clindamycin	<0.06	<0.06	4
Chloramphenicol	2	2	4
Erythromycin	0.12	0.12	1
Gentamicin	1	1	8
Kanamycin	16	16	64
Streptomycin	8	8	64
Tetracycline	32/64	32/64	32
Ampicillin	1	1	2

All MIC values are at or below the reported species characteristics (cutoff values), except for tetracycline. For tetracycline, the MIC values for the strains are equal to or 2-fold above the EFSA microbiological cut off value, in both of the two biological replicates. However, a 2-fold variation in MIC is considered acceptable due to the technical variation of the phenotypic method as also recognized by EFSA (EFSA, 2018).

2.3.5.6 Absence of antibiotic production

Supernatant from three lots of both *L. reuteri* strains were used to saturate paper discs. ATCC strains of *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Bacillus*

circulans, Streptococcus pyogenes and *Serratia marcescens* were inoculated into molten trypticase agar and were overlayed onto solidified TSA agar and allowed to harden. The paper disks were placed onto the agar, allowed to incubate at 2- 8°C overnight and then incubated overnight at 36±2°C. The plates were observed for zones of inhibition. Sterile water was used as a control.

No zones of inhibition were observed on plates treated with supernatant from either strain indicating that the strains do not produce antibiotics to the six organisms tested. The lab reports are in Annex 8.

2.4 Specifications for each of the *L. reuteri* strains used to produce the *L. reuteri* mixture

Each production batch of the *L. reuteri* strains will be analyzed for compliance with the following specifications, as in the Technical Data Sheets for these two strains (Annex 15):

Table 6 Specifications for *L. reuteri* strains 3630 and 3632

Parameter	Specification	Method
Identification	*Limosilactobacillus reuteri*	PCR ID*
Total Viable Cell Count of *L. reuteri* 3630	NLT 4 x 10^9 CFU/g	TRPO**
Total Viable Cell Count of *L. reuteri* 3632	NLT 4 x 10^9 CFU/g	TRPO
Microbiological Testing		
Non-Lactics	< 5000 CFU/g	ISO 13559-2002
E. coli	Absent / 10g	AOAC 966.24
Enterobacteriaceae	<10 MPN/g	AOAC 966.24
Staphylococcus (coagulase +)	Negative / 25 g	AOAC 975.55
Listeria	Negative / 25 g	FDA BAM Ch. 10
Salmonella	Negative /25 g	AOAC 2004.03
Enterococcus	< 100 CFU/g	CMMEF, 4th Ed
Molds and Yeast	< 100 CFU/g	USP 2021
Heavy Metals		
Arsenic	NMT 0.3 ppm	AOAC 2015.01
Cadmium	NMT 0.2 ppm	AOAC 2015.01

Parameter	Specification	Method
Mercury	NMT 0.05 ppm	AOAC 2015.01
Lead	NMT 0.2 ppm	AOAC 2015.01

* Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR
** Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

2.5 Specifications for the *L. reuteri* mixture

Each production batch of the *L. reuteri* mixture will be analyzed for compliance with the following specifications and the methods are validated for use in microbial ingredients and the methods are valid for the listed parameters:

Table 7 Specifications for *L. reuteri* Mixture

Parameter	Specification	Method
Appearance	Free-flowing powder	Visual
Color	Off-white to dull orange	Visual
Identification	*Limosilactobacillus reuteri*	PCR ID or Riboprinter
Total Viable Cell Count of *L. reuteri*	≥ 2.6 x 10^9 CFU/g	TRPO
Microbiological Testing		
Non-Lactics	< 5000 CFU/g	ISO 13559-2002
E. coli	Negative / 10 g	AOAC 966.24
Enterobacteriaceae	< 10 MPN/g	AOAC 966.24
Staphylococcus (coagulase +)	Negative /25 g	AOAC 975.55
Listeria	Negative / 25 g	FDA BAM Ch. 10
Salmonella	Negative / 25 g	AOAC 2004.03
Enterococcus	< 100 CFU/g	CMMEF, 4th Ed
Molds and Yeast	< 100 CFU/g	USPM 2021
Heavy Metals		
Arsenic	NMT 0.3 ppm	AOAC 2011.19 and 993.14 (modified)
Cadmium	NMT 0.2 ppm	AOAC 2011.19 and 993.14 (modified)

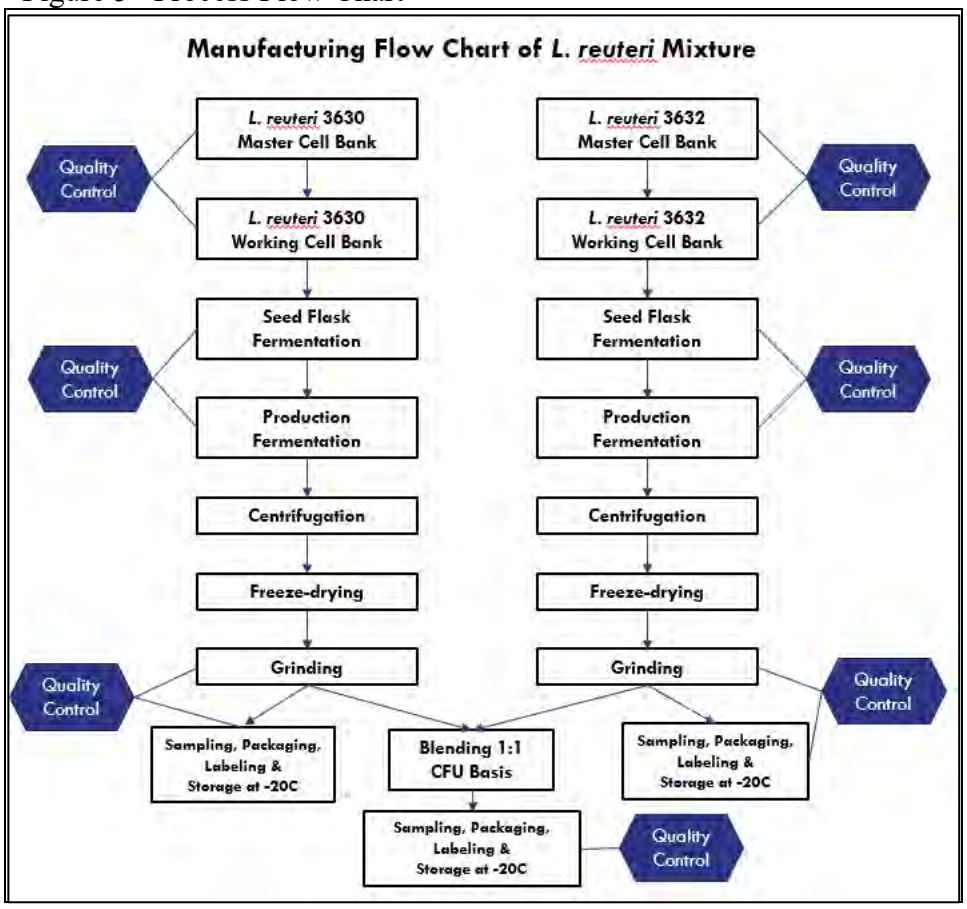
Parameter	Specification	Method
Mercury	NMT 0.05 ppm	AOAC 2011.19 and 993.14 (modified)
Lead	NMT 0.2 ppm	AOAC 2011.19 and 993.14 (modified)

2.6 Manufacture

Commercial manufacturing of *L. reuteri* strains 3630 and 3632 and the *L. reuteri* mixture follows a process similar to that used for Lactobacilli strains as described in a recent publication (Fenster et al, 2019), and requires supplying the strains with their specific nutritional requirements for growth and performance and maintaining process control and consistency. The main production steps include media preparation with ultra-high temperature sterilization; fermentation; centrifugation and/or filtration; cryoprotectant addition; pelleting in liquid nitrogen; and freeze drying.

Ensuring the microbial ingredient meets the established product quality requirements is the responsibility of the quality control (QC) team, involved during production planning and throughout manufacturing. Key QC activities consist of but are not limited to overseeing the development and periodic review of the food safety plan, qualifying raw material suppliers, assigning raw material requirements, confirming that process control points are within documented alert limits, confirming batches meet prior-established product specifications, and releasing product batches for packaging and distribution to customers.

Figure 5 Process Flow Chart



2.6.1 Methodology to ensure the stability of the production strains

Standard operating procedures (SOPs) are followed to produce the master cell banks of each of the *L. reuteri* strains 3630 and 3632, including documentation of verification of the manufacturing batch records, review of Certificates of Analysis listing testing results and acceptance requirements, post-bank testing protocol, post-bank testing reports, cell line microbial strain history report, post-bank viability testing, and raw material records. Quality review of these documents is required at each step. The master cell banks are stored at -80 °C. The working cell banks, which will be used as production seed vials, are produced from the master cell banks in accordance with specific SOPs, and with review by the Quality Department. These vials are also stored at -80 °C until ready for use.

2.6.2 Media Preparation

The raw materials (e.g., nutrients, vitamins, minerals) are received by the production plant and processed in accordance with the plant quality SOPs, which includes confirming identity and specifications. The raw materials are then weighed per the culture media recipe and sterilized. All ingredients used are of appropriate food grade and are suitable for use in this process (Annex 9).

2.6.3 Fermentation Process

The Lab fermentation chain is performed in multiple stages. First, a small laboratory scale flask with sterile media is inoculated with a cryo-vial and is incubated in a temperature-controlled incubator until the culture reaches stationary phase. The broth culture is then used to inoculate an approximately 20 L vessel, which is then allowed to grow at the optimal growth conditions to stationary stage. It is then cooled and stored refrigerated until ready for production. The vessel is then used to inoculate a production scale inoculation tank. The production scale tanks are filled with a sterilized nutrient broth of raw materials and water. This tank is pH controlled with ammonia. Once the bacteria growth begins to slow, the broth is used to inoculate the large fermentation vessels. The bacteria grow in these vessels under aerobic conditions with the ammonia used to control pH and keep the bacteria in the growth phase.

2.6.4 Cell Harvesting

Once the bacteria reach the maximum biomass (measured by the level of pH adjustment and in-process sampling), the cells are ready to be harvested. The fermentation vessel is cooled, and the contents are pumped through a centrifuge to separate the cells (culture concentrate) from the nutrient broth (supernatant). The culture concentrate is collected in a vessel, where cryoprotectant is added. The cryoprotection solution is a sterilized mix of sugars, carbohydrates and organic salts and vitamins to protect the cells during the freezing step.

2.6.5 Freeze Drying Process

After mixing with the cryoprotectant, the culture concentrate is pelletized into frozen spheres with liquid nitrogen. These are stored frozen until they can be loaded into a freeze dryer and dried under vacuum. This removes the water from the culture concentrate, which can then be milled into a powder.

2.6.6 Production of *L. reuteri* mixture

The commercial formulation sold as *L. reuteri* mixture is produced by mixing the two individually freeze-dried strains in a proportion to produce a final product with a 1:1 CFU ratio of the strains. The exact proportion of the two freeze-dried powders is dictated by the concentration of the organism in each batch on a weight basis and is controlled by the quality control laboratory at the production facility.

2.6.7 Packaging Process

The powder is then passed through a metal detector before being filled into poly/foil bags and sealed.

2.6.8 Good Manufacturing Practice

All production steps are performed under an approved Food Safety Plan in accordance with current Good Manufacturing Practices and Hazard Analysis and Risk-based Preventive Controls for Human Food per 21 CFR Part 117. The contract manufacturing site where the *L. reuteri* strains have been produced is certified by SGS as meeting the requirements of the SQF Food Safety Code for Manufacturing, Edition 8.1 for the manufacture of microbial products. The site certification is in Annex 10.

2.7 Batch Data

2.7.1 *L. reuteri* strains batch data

Five pre-commercial batches of each *L. reuteri* strain were produced and analyzed for conformance to the specifications. The results of the analyses are in Table 8 and Table 9 below and the analytic reports are in Annexes 11 and 12.

Table 8 Batch Data strain 3630

Parameter	Specification	Lot A	Lot C	Lot E	Lot 201123LRE 3630	Lot 210713LRE 14
Identification	Riboprinter >0.85 Similarity Index	Pass	Pass	Pass	Pass (0.9)	Pass (0.97)
Total Viable Cell Count of *L. reuteri* 3630, CFU/g	≥ 4 x 10^9	5.9x 10^9	6.8 x 10^9	6.2 x 10^9	2.7 x 10^{11}	2.6 x 10^{11}
Non-Lactics, CFU/g	< 5000	<10	<10	30	<100	2100
E. coli	Absent /10 g	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae CFU/g	<10	Absent	Absent	Absent	<10	<10
CP Staphylococcus	Negative in 10 g	Absent[1]	Absent	Absent	Absent	Absent

Parameter	Specification	Lot A	Lot C	Lot E	Lot 201123LRE3630	Lot 210713LRE14
Listeria	Not detected in 25 g	Not tested	Not tested	Not tested	Not detected	Not detected
Salmonella	Negative in 25g	Absent	Absent	Absent	Absent	Absent
Enterococcus, CFU/g	< 100	Absent	Absent	Absent	<10	<10
Molds and Yeast, CFU/g	< 100	<10	<10	<10	<10	<10
Arsenic, ppm	≤ 0.3	<0.01	<0.01	<0.01	0.194	0.06
Cadmium, ppm	≤ 0.2	<0.005	<0.005	<0.005	0.115	0.037
Mercury, ppm	≤ 0.05	<0.005	<0.005	<0.005	<0.005	<0.005
Lead, ppm	≤ 0.2	<0.005	<0.005	<0.005	0.046	0.15
Lots A, C and E: E. coli tested in 0.3g; Staphylococcus (coagulase+) tested in 40 g; Salmonella tested in 40 g						

Table 9 Batch Data strain 3632

Parameter	Specification	Lot A	Lot C	Lot E	Lot 201123LRE 3632	Lot 2107 12LRE3632
Identification	Riboprinter >0.85 Similarity Index	Pass	Pass	Pass	Pass (0.89)	Pass (0.97)
Total Viable Cell Count of *L. reuteri* 3630, CFU/g	≥ 2 x 10^9	4.8x 10^9	3.4 x 10^9	3.3x 10^9	4.3 x 10^{11}	1.8 x 10^{11}
Non-Lactics, CFU/g	< 5000	<10	<10	<10	300	<100
E. coli	Negative in 10 g	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae (MPN), CFU/g	< 10	Absent	Absent	Absent	< 10	<10
CP Staphylococcus	Negative by test in 10 g	Absent	Absent	Absent	Absent	Absent
Salmonella	Negative in 25 g	Absent	Absent	Absent	Absent	Absent
Listeria	Not detected in 25 g	Not tested	Not tested	Not tested	Not detected	Not detected
Enterococcus, CFU/g	< 100	Absent	Absent	Absent	<10	<10
Molds and Yeast, CFU/g	< 100	<10	<10	<10	<10	<10
Arsenic, ppm	≤ 0.3	<0.01	<0.01	<0.01	0.282	0.110
Cadmium, ppm	≤ 0.2	<0.005	<0.005	<0.005	0.195	0.052
Mercury, ppm	≤ 0.05	<0.005	<0.005	<0.005		<0.005
Lead, ppm	≤ 0.2	<0.005	<0.005	<0.005	0.103	0.190
Lots A, C and E: E. coli tested in 0.3g; Staphylococcus (coagulase+) tested in 40 g; Salmonella tested in 40 g						

2.8 Product stability

The *L. reuteri* mixture has a minimum shelf life of 9 months when stored at 5º C and ambient humidity in sealed containers. The shelf life at 25 ºC and 30 ºC – both at 60% relative humidity (RH) – is at least 6 months, and at 37º C and 65% RH the shelf life is 1 month. The product will be stored and shipped at a refrigerated condition (5-8 °C) with instructions to keep the product refrigerated until ready to use.

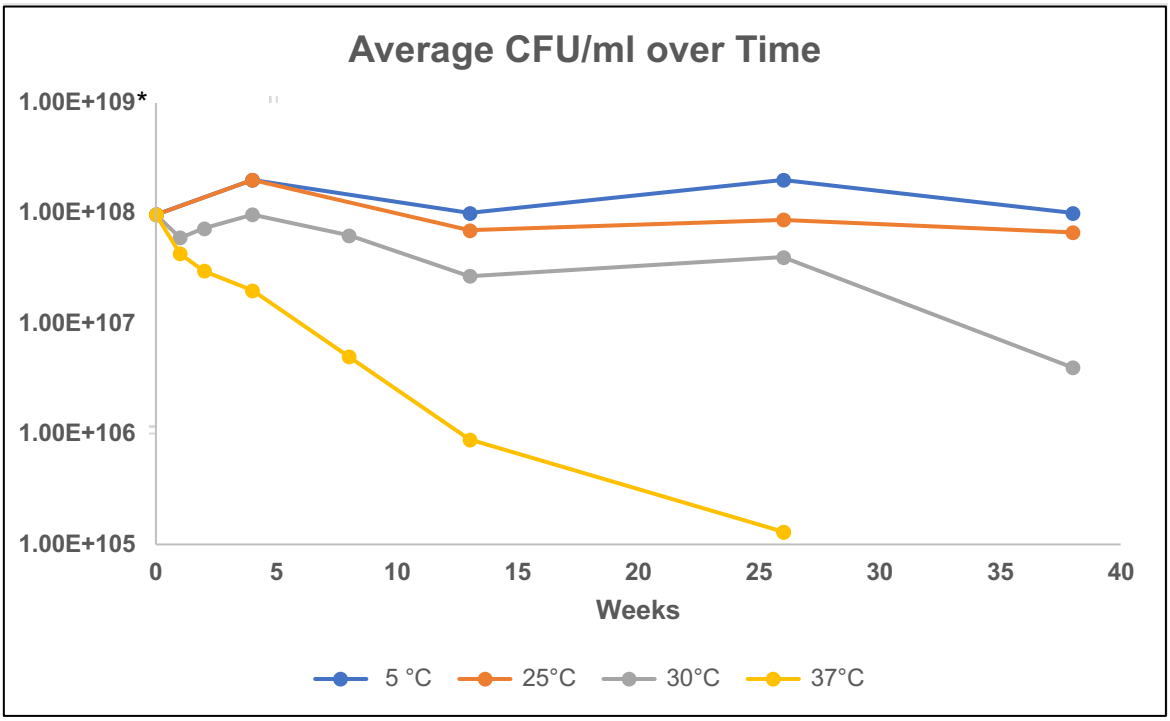
2.8.1 Storage study

Standard storage conditions for the *L. reuteri* mixture are 5-8 °C and 60% RH in sealed containers. A nine-month storage study was performed at 5 °C, 25 °C and 60% RH as well as at 30 °C and 65% RH, 37 °C and 65% RH and appearance, moisture, total viable count were evaluated initially and then at different time intervals (see Table 10 and Table 11). Due to the anticipated loss in viability at higher temperatures, more frequent inspections were performed. Three lots of the strain mixture were evaluated and three enumeration plates were run for each lot at each time point with the averages of the samples provided in Table 10 and Table 11 and shown in Figure 6. Total viable count and appearance were evaluated at each time point. Moisture was determined initially and at 3-month intervals, see Table 11. The samples were packaged in 20 mL flint borosilicate glass serum vials with a 20 mm neck finish with a 20mm 3 leg lyo stopper and a 20mm open top aluminum seal. Commercially, the packaging will be a hermetically sealed multi-layer pouch providing the same level of moisture infiltration inhibition.

The data indicate that the mixture of *L. reuteri* stains is stable for 9 months when stored in sealed containers at 5 °C.

Table 10 Stability of *L. reuteri* mixture at different temperatures

Parameter	Initial	1 Week	2 Weeks	1 Month	2 Months	3 Months	6 Months	9 Months
Appearance	Yellow-white	Yellow-white	Yellow-white	Yellow-white	Yellow-white	Yellow-white	Yellow-white	Yellow-white
CFU/ml @ 5 °C	9.7×10^7	-------	---------	2×10^8	----------	1×10^8	2×10^8	1×10^8
CFU/ml @25°C	9.7×10^7	---------	----------	2×10^8	----------	$7x\ 10^7$	8.7×10^7	$6.7x\ 10^7$
CFU/ml @30°C	9.7×10^7	6×10^7	7.3×10^7	9.7×10^7	6.3×10^7	2.7×10^7	4.0×10^7	0.4×10^7
CFU/ml @37°C	9.7×10^7	4.3×10^7	$3.0x\ 10^7$	2.0×10^7	0.5×10^7	8.9×10^5	1.3×10^5	0



*109 = 10^9

Figure 6 Stability of *L. reuteri* mixture

Table 11 Average moisture content of *L. reuteri* mixture over time

% Moisture	Initial	3 months	6 Months	9 Months
5 °C	3.2	3.0	2.9	2.4
25 °C	3.2	3.1	2.8	2.3
30°C	3.2	2.8	2.8	2.0
37 °C	3.2	3.1	3.3	2.7

3 Dietary exposure

3.1 Dietary Exposure to the *L. reuteri* mixture

The *L. reuteri* mixture is intended to be used as a food ingredient in conventional foods (see Table 12) that are compatible with the addition of live microbial cultures including dairy products, beverages, nutritional powders, bars, confections and other foods, with the exception of both infant formula and USDA-regulated foods and in foods where standards of identity preclude such use. Intended levels of use are expected to be at least 5×10^8 CFU and up to 1×10^9 CFU of the *L. reuteri* mixture per serving of food. The total estimated consumer exposure for high users of these foods is no more than 1×10^{10} CFU/person/day. These levels are consistent with common and usual practice in the US and as described in previous GRAS Notices 254, 410 and 440. The function of *L. reuteri* mixture is to serve as an ingredient that will increase the concentration of members of these specific Lactobacillaceae (*L. reuteri* 3630 and 3632) in the digestive tract in the same manner as other members of the taxonomic family that have been notified to the FDA. The *L. reuteri* mixture will serve as a source of these live microorganisms to be consumed as a component of the diet by the general population and is expected to replace other commercial sources of *L. reuteri* in these food groups and not to increase the total exposure to *L. reuteri*.

Table 12 Proposed Uses of *L. reuteri* Mixture

Food Type	*L. reuteri* mixture use level (CFU/serving)	Serving Size*	Serving Size (CFU/100g food)
Cream cheese	5.00E+08	30 g	1.67E+09
Natural cheese	5.00E+08	30 g	1.67E+09
Processed cheese and spreads	5.00E+08	30 g	1.67E+09
Sour cream	5.00E+08	30 g	1.67E+09
Energy & Meal replacement Bars	5.00E+08	40 g	1.25E+09
Granola Bars	5.00E+08	40 g	1.25E+09
Frozen Novelties	1.00E+09	120 g	8.33E+08
Frozen yogurt	1.00E+09	120 g	8.33E+08
Ice Cream	1.00E+09	120 g	8.33E+08
Yogurt	1.00E+09	170 g	5.88E+08
Fermented milks	1.00E+09	240 mL	4.17E+08
Meal replacement Beverages	1.00E+09	240 mL	4.17E+08
Milk Drinks, Flavored Milks	1.00E+09	240 mL	4.17E+08
Milk-based meal replacement beverages	1.00E+09	240 mL	4.17E+08
Refrigerated fruit & vegetable beverages	1.00E+09	240 mL	4.17E+08
Soy-based beverages	1.00E+09	240 mL	4.17E+08
Yogurt drinks	1.00E+09	240 mL	4.17E+08
*Serving size taken from 21 CFR §101.12			

In a most conservative scenario, an individual could consume 10 servings of foods containing the *L. reuteri* mixture, which would result in an exposure between 5×10^9 and 1×10^{10} CFU/person/day. It is anticipated that even the estimated maximum exposure (2 x the most conservative assumption) would not result in exposure to more than 2×10^{10} CFU/person/day. This estimate for maximum exposure level is based on published data (Millen, AE et al, 2006).

4 Self-Limiting Levels of Use

There is no known self-limiting level of use for the *L. reuteri* mixture (*L. reuteri* strain 3630 and 3632) other than current Good Manufacturing Practices. Current industry practice indicates that the *L. reuteri* microbial ingredients provide from 1 x 10^9 CFU to 1 x 10^{10} CFU of these microorganisms/ serving of food (GRN 254 and 440).

5 Common Use in Foods Prior to 1958

BiomEdit, LLC is unaware of any documented use of *L. reuteri* mixture (*L. reuteri* strains 3630 and 3632) having been used in any human food application before 1958. Production of *L. reuteri* mixture for use as a live ingredient in conventional foods did not commence on a pre-industrial scale until 2019.

6 Narrative of the Basis for the GRAS Conclusion

L. reuteri is a naturally occurring microorganism in mammalian -- including human -- and avian gastrointestinal tracts as well as in the human urogenital tract and in breast milk. As a result, it has been intensively studied for its strain-specific beneficial properties in human health, and comparative genomic studies (Spinler et al., 2014).

According to the International Probiotics Association (IPA), *Lactobacillus reuteri* is considered a "grandfathered/exempted" live microbial ingredient on the basis that it has been used in foods since before October 15,1996 (IPA, 2016).

6.1 History of Safe Use of *L. reuteri*

L. reuteri is considered one of the few naturally occurring *Lactobacillus* species in the human GI tract (Mu et al., 2018). It has been found to occur naturally in human milk and in foods including salami, milk, several cheese varieties, sourdough and fermented molasses (Klantschitsch et al., 1996). *L. reuteri* strain ATCC 55730 (also known as *L. reuteri* ATCC SD2112) was the first *L. reuteri* strain used in human food in Sweden in 1991, specifically in fermented and non-fermented milk products. The fermented milk product included *L. reuteri* together with *L. acidophilus* and *Bifidobacterium infantis*. Market expansion of *L. reuteri* in the early to mid-1990s occurred in Switzerland, Japan, US, and Finland for use in functional foods, including yogurts and fruit juices, with food products formulated to deliver 5 x 10^5 to 1 x 10^6 CFU of *L. reuteri* per ml or g. When used in juices or ultra-pasteurized dairy products, *L. reuteri* was added using special aluminum-sealed packaging, so that the organisms could be added immediately before consumption (Casas and Dobrogosz, 2000; Szajewska et al., 2014).

A study with immune compromised individuals who consumed 1 x 10^{10} CFU/person/ day of *L. reuteri* SD2112 did not identify any clinically significant safety or tolerance problems, indicating that *L reuteri* is not pathogenic (Wolf et a., 1998)

Between 2008 and 2013, *L. reuteri* has been the subject of four Generally Recognized as Safe (GRAS) Notices for the use of *L. reuteri* DSM 17938 in human food and in infant formula, and for *L. reuteri* 30242 in human food, at doses ranging from 3.3 x 10^8 to 1 x 10^{10} CFU/serving. In each case the Agency has replied with no objection letters to the notifiers. These GRAS Notices are summarized in Table 13and are incorporated herein as reference.

Derived from *L. reuteri* ATCC 55730, with plasmid-borne antibiotic resistance traits removed, *L. reuteri* DSM 17938 is now commercially produced and distributed in more than 50 countries around the world. As of 2014, more than 3 billion doses (1 dose = 10^8 CFU) of this closely related *L. reuteri* strain (see Table 16 for genomic similarity data) had been sold (Klantschitsch et al., 1996; Oberhelman et al., 2014; Szajewska et al., 2014).

Table 13 GRAS Notices for *Lactobacillus reuteri*

Substance	GRN # / Closure Date	Intended Use	Use Rate
***Lactobacillus reuteri* strain DSM 17938**	GRN254 18 Nov 2008	Ingredient in processed cheeses, yogurt, ice cream, fruit juices, fruit drinks, processed vegetables, processed vegetable drinks, beverage bases, energy bars, energy drinks, and chewing gum and drinking straws at a level up to 10^8 CFU per serving, and may be as high as 10^9 CFU at time of manufacture	Up to 10^9 CFU per serving/per straw; maximum anticipated exposure < 10^{10} CFU/person/day
***Lactobacillus reuteri* strain DSM 17938**	GRN410 26 Mar 2012	As an ingredient in powdered whey-based term infant formula	10^6 CFU/g to 10^8 CFU/g; estimated average consumption of 10^8 CFU/day by each term infant.
***Lactobacillus reuteri* strain NCIMB 30242**	GRN440 12 Feb 2013	As an ingredient in beverages and beverage bases, breakfast cereals, cheeses, dairy product analogs, fats and oils, frozen dairy desserts, grain products and pastas, milk products, processed fruits and fruit juices, and sugar substitutes	$3.3x10^8$ to $1x10^{10}$ CFU/serving; estimated maximum use (90th percentile) of $3.5x10^{10}$ CFU/person/day

6.1.1 Use as a dietary supplement

Lactobacillus reuteri is commonly used as a live microbial ingredient in dietary supplements in the U.S. The Dietary Supplement Label Database (DSLD) identified 87 products with various strains of *L. reuteri* (DSLD, 2021). The strains specifically identified in the DSLD search included: SD-5865, LRE02, HA-188, SD-1357, UALRE-16, E1E, HDS-0188, NCIMB 30242, and RC-14. While many product labels do not specifically claim the amount of *L. reuteri* strain they contain in CFUs (when inclusion is as part of a proprietary blend of microorganisms), a cursory review of product labels found some that did identify an *L. reuteri* dose with a range of 5 to 7 $x10^9$ CFU/serving (1 serving recommended per day), and that *L. reuteri* was part of live microbial product blends that provided up to 1.50 $x10^{11}$ total CFU/serving, 6.12 $x10^{10}$ of which are from 12 strains of lactobacilli (1 serving recommended per day). See Annex 13 for DSLD report.

A search of FDA's New Dietary Ingredient Notification (NDIN) list Identified three successful notices: NDIN 78, NDIN 460 and NDIN 1182 and one that was declined due to insufficient information. See Table 14 for a summary of the notices. These NDINs are considered

supplemental information and corroborative of the safety of *L. reuteri* strains for use in human food.

Table 14 New Dietary Ingredient Notices for *L. reuteri*

Substance	NDIN # / Date of FDA's Response	Recommended Daily Dose
Lactobacillus reuteri **(strain ATCC 55730)**	NDIN 78, Sept. 18, 2000	One chewable tablet daily containing up to 10^9 CFU/tablet
RepHresh Pro-B1 *Lactobacillus reuteri* R - 14™ and *Lactobacillus rhamnosus* GR-1™	NDIN 488, Mar. 10, 2008	Encapsulated form providing $5x10^9$ CFU/day
Lactobacillus rhamnosus, strain DSM33426 & *Lactobacillus reuteri,* strain DSM33106	NDIN 1155, Aug. 28, 2020 FDA concluded there was insufficient safety data for *L. reuteri* strain DSM33016	*L. rhamnosus* strain DSM33426 and *L. reuteri* strain DSM33016 in equal proportions (2.5×10^9 to 5.5×10^9 CFU each)
Lactobacillus reuteri 12246-CU DS 80913	NDIN 1182, December 2020	Supplements at up to 2×10^{10} CFU/day

6.1.2 Acceptance of *L. reuteri in Canada and the EU*

A summary of the regulatory reviews conducted on *L. reuteri* in Canada and the EU is provided below.

Table 15 Canadian and European Safety Evaluations of the *L. reuteri* species and strains for human food use

Date	Authority or Entity	Evaluation	Dose	*L. reuteri strain, if applicable*	Reference
2007, 2020	European Food Safety Authority (EFSA) BIOHAZ Panel	Qualified Presumption of Safety (QPS) List	N/A	*L. reuteri species*	EFSA Journal, 2007; EFSA Journal, 2020
25-Mar-19	Health Canada	Probiotics Monograph	N/A	*L. reuteri species as whole cell and by strain designation*	Health Canada, 2019
2018	International Dairy Federation (IDF)	Inventory of Microbial Food Cultures with Safety Demonstration in Fermented Food Products	N/A	*L. reuteri species*	IDF, 2018

6.1.3 Similarity with other commercial *L. reuteri* strains

Both *L. reuteri* strains 3630 and 3632 have greater than 98% average nucleotide identity to commercially used *L. reuteri* strains SD2112 and RC-14 (Table 16). In addition, phylogenetic analyses based on gene content as well as on core genomes showed that the subject poultry *L. reuteri* isolates cluster with human *L. reuteri* strain SD2112 (poultry/human lineage VI), suggesting that human *L. reuteri* strain SD2112 potentially may have originated from poultry.

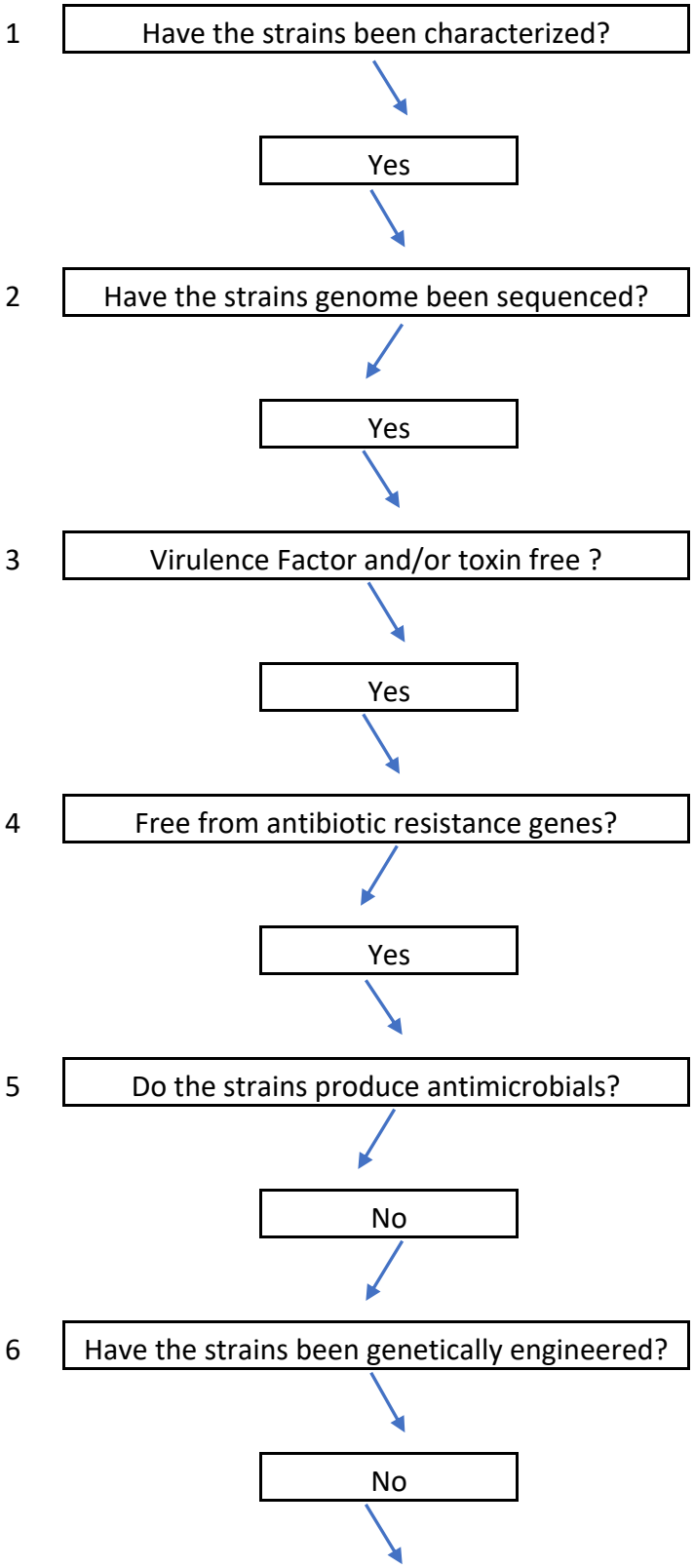
Table 16 Average Nucleotide Identity (ANI) of *L. reuteri* 3632 and 3630 with commercial microbial strains with a history of safe use in human food.

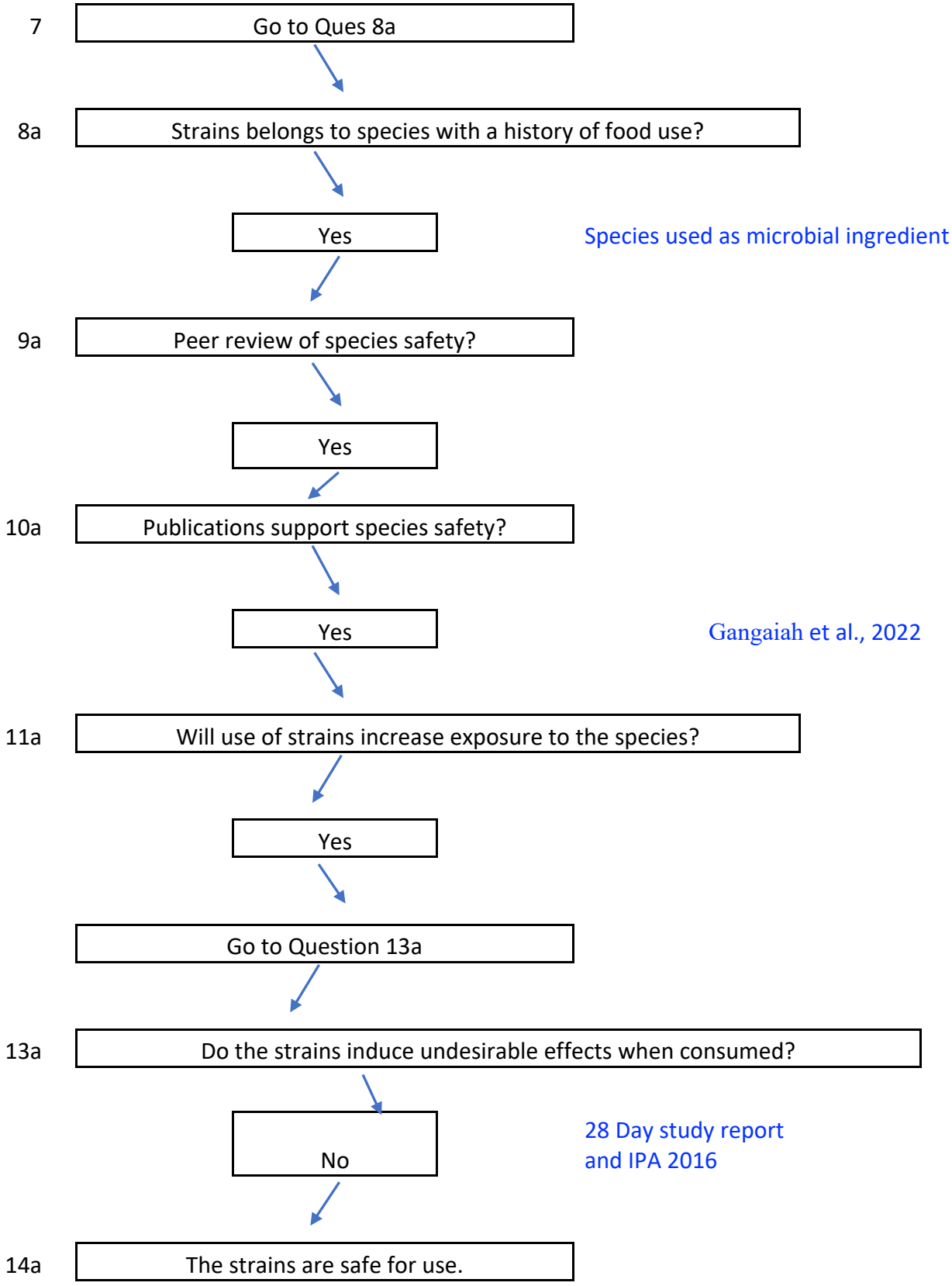
Query genome	Reference genome	%ANI	Orthologous matches	Sequence fragments
LR3630	CF48-3A	98.1372	560	797
LR3630	RC-14	98.0939	537	797
LR3630	RC-18	98.0939	537	797
LR3630	SD2112	98.0825	590	797
LR3630	DSM17938	98.0805	591	797
LR3630	DSM20016	95.4979	523	797
LR3630	ATCC53608	95.1714	532	797
LR3632	CF48-3A	98.2214	553	825
LR3632	SD2112	98.1492	581	825
LR3632	DSM17938	98.1422	582	825
LR3632	RC-14	98.0633	532	825
LR3632	RC-18	98.0633	532	825
LR3632	DSM20016	95.6098	516	825
LR3632	ATCC53608	95.0288	545	825

6.2 Decision tree for bacterial cultures used as food

A decision tree for determining the suitability of a microorganism for consumption by humans was published by Pariza et al. in 2015. The application of the specific criteria in this decision tree in the current evaluation indicates that the *L. reuteri* mixture is safe for use in the manufacture of food and dietary supplements for human consumption.

Question:


1
Have the strains been characterized?
Yes
2
Have the strains genome been sequenced?
Yes
3
Virulence Factor and/or toxin free ?
Yes
4
Free from antibiotic resistance genes?
Yes
5
Do the strains produce antimicrobials?
No
6
Have the strains been genetically engineered?
No


7
Go to Ques 8a
8a
Strains belongs to species with a history of food use?
Yes
Species used as microbial ingredient
9a
Peer review of species safety?
Yes
10a
Publications support species safety?
Yes
Gangaiah et al., 2022
11a
Will use of strains increase exposure to the species?
Yes
Go to Question 13a
13a
Do the strains induce undesirable effects when consumed?
No
28 Day study report
and IPA 2016
14a
The strains are safe for use.

6.3 Toxicological studies

Because *L. reuteri* has a history of safe use in food and therefore a high presumption of safety, no OECD or FDA guideline toxicology studies with the strains contained in *L. reuteri* mixture are available in the published literature.

6.3.1 Studies with Other *L. reuteri* strains with animals

An acute toxicity study with *L. reuteri* NCIMB 30253 fed at 2 x 10^{10} CFU / day to male Sprague-Dawley rats (Sulemankhil et al., 2012), a calculated CFU per kg body weight of 8 x 10^{10} using the reported upper weight of 250 g/rat, found that:

> *"Following a 28-day repeated oral dose of L. reuteri NCIMB 30253 (2 Å~ 1010 CFU) in normal SD rats, no statistically significant differences in body weight (P = 0.835) and food consumption (P = 0.458) were seen in animals treated with L. reuteri NCIMB 30253 versus vehicle control. Analysis of biochemical markers of safety showed no significant differences (P > 0.05) between treated and vehicle control treated animals for endpoints other than bicarbonate, in which a significant difference (P = 0.029) in mean bicarbonate levels was found between L. reuteri NCIMB 30253 treated (24.69 mmol/L) and vehicle control treated (26.12 mmol/L) animals. Results from hematologic analysis did not reveal significant differences (P > 0.05) between L. reuteri NCIMB 30253 treated, and vehicle control treated animals."*

Newborn rats fed cow's milk with and without *E.coli* lipopolysaccharide and gastrointestinal inflammatory response was evaluated when the milk was augmented with one of four *L. reuteri* strains, DSM17938, PTA 4659, PTA 5289, and PTA 6475. There was no toxic effect of *L. reuteri* on intestinal cells or mucosa. (Liu et al., 2010).

Two *L. reuteri* strains were evaluated for their ability to minimize necrotizing enterocolitis (NEC) in newborn, Sprague-Dawley rats.

> *Both L. reuteri strains DSM 17938 and ATCC PTA 4659 supported intestinal health* (Liu et al., 2012).

L. reuteri strain DSM 17938 fed to newborn mice found that:

> *feeding LR (10^7 CFY/day) did not affect clinical phenotype or inflammatory biomarkers in plasma and stool, but LR increased the proportion of Foxp3_regulatory T cells (Tregs) in the intestine. LR also increased bacterial diversity and the relative abundance of p_Firmicutes, f_Lachnospiraceae, f_Ruminococcaceae, and genera Clostridium and Candidatus arthromitus, while decreasing the relative abundance of p_Bacteriodetes, f_Bacteroidaceae, f_Verrucomicrobiaceae, and genera Bacteroides, Ruminococcus, Akkermansia, and Sutterella.* In short, LR is linked to proliferation of beneficial gut microbiota (Liu et al., 2019).

In a similar study with *L. reuteri* strain DSM 17938 fed to female 12-week old mice induced to develop experimental autoimmune encephalomyelitis (EAE), the research team discovered that:

> *The loss of diversity of gut microbiota induced by EAE was largely restored by feeding L. reuteri.* (He et al., 2019).

A concern with the consumption of bacterial cultures has been the possibility of the organism to cross the intestinal epithelium and translocate to another organ. Zegarra-Ruiz and co-workers studied 6-week-old SPF B6 and TLR7.1 TgB6 mice, (models for Lupus) fed 2 x 10^8 CFU as a single dose by oral gavage, to ascertain if *L. reuteri* could alter the autoimmune system (Zegarra-Ruiz et al., 2019). The animals were euthanized after 16 weeks and were found to have both splenomegaly and hepatomegaly. This study indicates that an underlying pathology must exist for translocation to occur and that *L. reuteri* alone was sufficient to exacerbate systemic autoimmunity under both specific pathogen-free and germ-free conditions.

L. reuteri 23272 was fed to mice in drinking water at 5 x 10^7 CFU/mL to determine if the microorganism attenuated the impact of being orally infected with *Citrobacter rodentium* when exposed to physical stress for 6 consecutive nights, (Makos et al., 2013). Although *L. reuteri* fed mice exhibited the same histopathology as non-treated mice, there was a reversal of the 5-fold decrease in β-defensin 3 gene expression found in mice not receiving the *L. reuteri* treatment. *L. reuteri* reduced the stressor-enhanced susceptibility to infectious colitis and pathogen translocation from the colon to the spleen.

Administration of *Lactobacillus reuteri* CRL 1098 (10^4 cells/d) to mice for 7 days before inducing hypercholesterolemia (by feeding mice with a fat-enriched diet for the subsequent 7 days) was evaluated by Taranto et al., (Taranto et al., 1999). *L. reuteri* was effective in preventing hypercholesterolemia in mice fed a high fat diet compared to the mice not receiving *L. reuteri,* but was still higher than control mice, producing a 17% increase in the ratio of high-density lipoprotein to low-density lipoprotein. Total cholesterol and triglycerides decreased by 22 and 33%, respectively, compared to the group that was not fed the lactobacilli.

In healthy breastfed mice, oral administration of 10^7 CFU/day of *Lactobacillus reuteri* DSM 17938 promoted intestinal immune tolerance and was linked to proliferation of beneficial gut microbiota; *P. firmicutes, F. lachnospiraceae, F. ruminococcaceae*, and genera Clostridium and *Candidatus arthromitus*. *L. reuteri* upregulated plasma metabolites that are involved in the urea cycle, the TCA cycle, methionine methylation, and the polyamine pathway. They also found that *L. reuteri* administration increased levels of tryptophan metabolites and the purine nucleoside adenosine that are known to enhance tolerance to inflammatory stimuli, (Liu et al., 2019).

6.3.2 Human studies with *L. reuteri* strains

L. reuteri strain ATCC SD2112, also referred to as ATCC 55730, is the parent strain of commercially used *L. reuteri* strain DSM 17938 (GRN000254). Two plasmids containing antimicrobial resistance genes have been cured from SD2112, resulting in DSM 17938. SD2112 and its derivative DSM 17938 as well as RC-14 have been used as dietary supplements in many countries in the form of capsules/tablets. A large number of studies have established the safety of SD2112 and its derivative DSM 17938 as well as RC-14 in humans as described in Table 17 and Table 18 below. Several of the below studies were conducted to the explore potential health benefits conferred by ingestion of the different *L. reuteri* strains. It is not the intent of the sponsor to promote the use of *L. reuteri* strains 3630 and 3632 (*L. reuteri* mixture) to prevent, mitigate, treat or cure a disease in humans.

Table 17 Published studies of *L. reuteri* DSM 17938 and its parent ATCC 55730 (SD2112) in humans

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Reizzo et al., 2019	To investigate: (1) the effects of long-term administration of *Lactobacillus reuteri* DSM 17938 on the serum levels of serotonin (5-HT) and brain-derived neurotrophic factor (BDNF); (2) the possible link between 5-HT, BDNF, and specific constipation-related symptoms; (3) whether genetic variability at the 5-HTT gene-linked polymorphic region (5-HTTLPR) and BDNF Val66Met loci could be associated with serum 5-HT and BDNF variations.	Randomized, double blind, placebo controlled trial	56 functionally constipated patients	4 x 10^8 CFUs/day of *L. reuteri* DSM 17938 orally	105 days	**The authors reported no adverse events** from long-term administration of *Lactobacillus reuteri* DSM 17938.
Savino et al., 2010	To understand the underlying mechanism of probiotic in breastfed subjects.	Randomized, double blind, placebo controlled trial	46 infants younger than 60 days, of whom 25 subjects had colic and 21 were healthy controls	1 x 10^8 CFUs/day of *L. reuteri* DSM 17938 orally	21 days	The authors concluded that, "**no adverse events were reported by parents during the study in either group of infants**" and that L. reuteri DSM 17 938 at a dose of 10^8 colony-forming units per day in early breastfed infants was well tolerated and safe.
Savino et al., 2018	To evaluate crying time, retinoid-related orphan receptor-γ	Randomized, double blind,	60 infants, 32 with colic and 28	1 x 10^8 CFUs/day of *L.*	30 days	The authors concluded that **"no adverse events were reported by parents during the study in either group of infants"**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
	(RORγ) and forkhead box P3 (FOXP3) messenger RNA levels (transcription factors that can modulate T cell responses to gut microbes), and to investigate gut microbiota and fecal calprotectin in infants treated with *Lactobacillus reuteri* for infantile colic.	placebo controlled trial	healthy control infants	*reuteri* DSM 17938 orally		
Oberhelman et al., 2014	Phase I safety study to assess the safety and tolerability of *Lactobacillus reuteri* DSM 17938 in healthy adult volunteers.	Randomized to one of two treatment groups in a 2:1 ratio (treatment to placebo)	45 Healthy adults 18-65	1 x 10^8 CFUs/day of *L. reuteri* DSM 17938 orally for 5 days	36 days (1st 5 days as treatment days)	**No adverse effects were reported.**
Stensson et al., 2013	To evaluate the effect on oral health, at age 9 years, of daily oral supplementation with the probiotic *Lactobacillus reuteri*, strain ATCC 55730, to mothers during the last month of gestation and to children through the first year of life.	Randomized, double-blind, placebo controlled, trial	232 mothers; 113 children, 60 in the probiotic and 53 in the placebo group	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	28 days for mothers and 365 days for the infant	**No adverse effects were reported**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Abrahamsson et al., 2013	To evaluate whether perinatal and infant supplementation with *L. reuteri* reduced the prevalence of respiratory allergic disease in school age and to explore whether this supplementation was associated with any long-term side effects.	Randomized, double-blind, placebo controlled, trial	232 families	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally for 30 days (last month of gestation) and the first year of infancy	30 days of gestation; 365 days of infancy	The authors concluded that **"administration of *L. reuteri* during the last weeks of gestation and in infancy was not associated with any long-term side effects. No severe adverse events were reported".**
Iniesta et al., 2012	To investigate the effects of an orally administered probiotic on the oral microbiota.	Randomized, double blind, placebo controlled trial	40 gingivitis subjects	2 x 10^8 CFUs/day of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 orally	56 days	The authors concluded that **"no relevant adverse effects were reported".**
Francavilla et al., 2012	To test the efficacy and safety of Lactobacillus reuteri DSM 17938 derived from *L. reuteri* ATCC 55730 in children with acute diarrhea. Primary outcomes were the rate of unresolved diarrhea after 3 days of treatment and duration of diarrhea.	Randomized, double-blind, placebo controlled, trial	69 patients with acute diarrhea with clinical sign of dehydration, 35 in *L. reuteri* and 34 in placebo group.	4 x 10^8 CFUs/day of *L. reuteri* DSM 17938, twice daily, orally	7 days	The authors concluded that **"there no adverse events were recorded"**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Takemura, 2017	To assess long-term efficacy and safety of lozenges containing Lactobacillus reuteri (Prodentis™) on gingivitis	Randomized, double-blind, placebo controlled trial	62 participants	1 x 10^8 CFUs/day of *L. reuteri* DSM 17938 and 1 x 10^8 CFUs/day of *L. reuteri* ATCC PTA 5289, once or twice daily, orally	168 days	**There were no serious adverse events reported.** Other adverse events (nausea, allergic sinusitis, cold, and sore throat) were not attributable to the treatment. No statistical analysis of the results was published.
Cimperman et al., 2011	To evaluate Lactobacillus reuteri for the prevention of antibiotic-associated diarrhea (AAD) in hospitalized adults.	Randomized, double-blind, placebo controlled, trial	31 patients with antibiotic-associated diarrhea (AAD)	2 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	28 days	The authors concluded that **"*L. reuteri* was safe and well tolerated"** among hospitalized adults".
Abrahamsson et al., 2009	To identify factors affecting the prevalence of Lactobacillus reuteri in maternal feces and breast milk and infant feces after oral supplementation with L reuteri and to assess the influence on microbial ecology, particularly Clostridium difficile and Bifidobacterium colonization.	Randomized, double-blind, placebo controlled, trial	232 mothers and their infants	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	28 days of pregnancy for mothers and first 365 days after birth for infants	**No adverse events were reported**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Indrio et al., 2008	To investigate the effect of dietary supplementation with a probiotic on feeding tolerance and gastrointestinal motility in healthy formula-fed preterm infants.	Randomized, double-blind, placebo controlled, trial	30 preterm newborns	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	30 days	The authors concluded that "**no adverse events reported related to trial**" in formula-fed preterm newborns.
Sung et al., 2014	To determine whether the probiotic *Lactobacillus reuteri* DSM 17938 reduces crying or fussing in a broad community-based sample of breastfed infants and formula fed infants with colic aged less than 3 months.	Randomized, double-blind, placebo controlled, trial	167 Breastfed infants or formula fed infants aged less than 3 months meeting Wessel's criteria for crying or fussing: 85 randomized to receive probiotic and 82 to receive placebo	1 x 10^8 CFUs/day of *L. reuteri* DSM 17938 orally	30 days	The authors concluded that **"no study related adverse events occurred**" in breastfed and formula fed infants.
Nation et al, 2017	Impact of *L. reuteri* colonization on gut microbiota, inflammation, and crying time in infant colic	Randomized, double-blind, placebo controlled, trial	65 infants (31 probiotic, 34 placebo)	1 x 10^8 CFUs/day of *L. reuteri* DSM 17938 orally	28 days	No adverse events were reported in this paper.
Francavilla et al., 2008	To test whether Lactobacillus reuteri ATCC 55730 reduces H. pylori intragastric load in vivo,	Randomized, double-blind, placebo controlled,	40 H. pylori positive subjects	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	28 days	The authors concluded that **"no adverse events were reported"**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
[In GRN440]	decreases dyspeptic symptoms, and affects eradication rates after conventional treatment.	trial				
Abrahamsson et al., 2007 [In GRN254]	To prevent eczema and sensitization in infants with a family history of allergic disease by oral supplementation with the probiotic Lactobacillus reuteri.	Randomized, double-blind, placebo controlled, trial	232 families with allergic diseases	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	28 days of pregnancy for mothers and first 365 days after birth for infants	The authors concluded that "**no adverse events were reported**".
Lionetti et al., 2006 [IN GRN254]	To determine whether adding the Lactobacillus reuteri to an anti-H. pylori regimen could help to prevent or minimize the gastrointestinal side-effects burden in children.	Randomized, double-blind, placebo controlled, trial	40 H. pylori positive children	1 x 10^8 CFUs/day of *L. reuteri* ATCC 55730 orally	10 days	The authors concluded that "**no adverse events were reported**".
Niv et al., 2005 [In GRN254]	To assess the short- and long-term effects of Lactobacillus reuteri administration on clinical symptoms of IBS.	Randomized, double-blind, placebo controlled, trial	54 subjects with IBS	2 x 10^8 CFUs/day of *L. reuteri* ATCC 55730, orally	180 days	**There were no significant differences between the groups in terms of the rate of adverse events.**

Table 18. Published studies of *L. reuteri* RC14 in humans.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Yang et al., 2020	To evaluate the effects of oral administration of GR-1 and RC-14 on the vaginal microbiota, as well as dampen the vaginal concentration of pro-inflammatory cytokines and chemokines	Randomized, double blind, placebo controlled, trial	86 asymptomatic pregnant women who had an intermediate bacterial vaginosis Nugent score at 13 weeks	5 x 10^9 CFUs/day of L. rhamnosus GR-1 and *L. reuteri* RC-14, once daily, orally	84 days	The authors concluded that **this study showed no adverse issues resulting from 12-week use of *Lactobacillus* strains GR-1 and RC-14 during pregnancy in women at low risk for premature birth.**
Yefet et al., 2020	To study vaginal colonization of orally administered Lactobacillus Reuteri RC-14 and Rhamnosus GR-1 in pregnant women at high risk for preterm labor	Randomized controlled crossover study	40 pregnant women at high risk for preterm birth with normal vaginal flora	5 x 10^9 CFUs/day of L. rhamnosus and *L. reuteri*, once daily, orally	60 days	The authors concluded that "**no adverse events related to the study treatment were reported**".
Ho et al., 2016	To examine the effect of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 taken orally before bedtime on Group B Streptococcus (GBS)-positive pregnant women with respect to becoming GBS negative.	Randomized, double blind, placebo controlled, trial	110 pregnant women at 35-37 weeks of gestation who were diagnosed by GBS culture as being GBS positive for both vaginal and rectal GBS colonization were randomly assigned	2 x 10^9 CFUs/day of L. rhamnosus GR-1 and *L. reuteri* RC-14, once daily, orally	21 days	The authors concluded that "**there were no adverse treatment effects in terms of nausea, vomiting, diarrhea, abdominal pain, skin rash, or systemic infections after taking the capsules in any of the participants during the trial**".

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Vujic et al., 2013	To assess the efficacy of orally administered capsules containing the probiotics Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 (Lactogyn, JGL, Rijeka, Croatia) compared to placebo in otherwise healthy women diagnosed with bacterial vaginosis.	Randomized, double blind, placebo controlled trial	544 subjects, women older than 18 years old, diagnosed with vaginal infection	1 x 10^9 CFUs/day of L. rhamnosus GR-1 and *L. reuteri* RC-14, once daily, orally	42 days	**No adverse effects nor side effects were observed. -**
De los Angeles-Pineda et al., 2011	To examine the effect of probiotics as adjunctive therapy for the treatment of rheumatoid arthritis (RA). A sample size of 30 subjects was calculated to determine a moderate effect.	Randomized, double blind, placebo controlled trial	30 patients with rheumatoid arthritis, 14 patients for placebo and 15 patients for probiotics	2 x 10^9 CFUs of L. rhamnosus and *L. reuteri*, twice daily, orally	90 days	The authors concluded that "**there were no adverse effects from the probiotics reported by the subjects**".
Hummelen et al., 2011	To evaluate the impact of 25 weeks use of probiotics, a randomized, double blind, controlled study was undertaken on 65 women who were naïve to anti-retroviral treatment.	Randomized, double blind, placebo controlled trial	65 HIV positive patients, 33 assigned placebo and 32 assigned probiotics	2 x 10^9 CFUs of L. rhamnosus and *L. reuteri*, twice daily, orally	175 days	**No adverse events were associated with the long-term use of probiotics.**

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Krauss- Silva et al., 2009	To evaluate the effectiveness of the early administration of selected probiotics to pregnant women with asymptomatic bacterial vaginosis/ intermediate degree infection to reduce the occurrence of spontaneous preterm delivery and related neonatal mortality and morbidity.	Randomized, double blind, placebo controlled trial	65 HIV infected women with an aberrant microbiota	2×10^9 CFUs of L. rhamnosus and *L. reuteri*, twice daily, orally	175 days	**No differences in adverse events between probiotic treated and placebo groups**.
Cianci et al., 2008	To investigate the efficacy of the use of Lactobacillus rhamnosus GR-1 and of Lactobacillus reuteri RC-14 administrated orally in the treatment and prevention of vaginoses and bacterial vaginitis relapses.	Randomized controlled trial	50 women in good health, aged between 18 and 48 years, with assessed diagnosis of bacterial vaginosis and vaginitis	No info on the dose available	15 days	**No withdrawals due to adverse effects**. .
Petricevic et al., 2008	To evaluate the influence of the orally administered probiotic strains Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the quality of the vaginal flora in	Randomized, double blind, placebo controlled trial	72 postmenopausal women with Nugent scores between 4 and 6 in initial vaginal swab, were randomized into two groups	2.5×10^9 CFUs of L. rhamnosus and *L. reuteri*, once daily, orally	14 days	**No withdrawals due to adverse effects**.

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
	postmenopausal women.					
Martinez et al., 2009 [In GRN440]	To evaluate the beneficial effect of Lactobacilli to women being treated with antibiotics for an infectious condition	Randomized, double blind, placebo controlled trial	64 Brazilian women diagnosed with bacterial vaginosis	2 x 10^9 CFUs of L. rhamnosus and *L. reuteri*, once daily, orally	28 days	**No withdrawals due to adverse effects**.
Anukam et al., 2006 [IN GRN254]	To evaluate the synergistic effect of oral probiotic L. rhamnosus GR-1 and *L. reuteri* RC-14	Randomized, double blind, placebo controlled trial	125 premenopausal women	2 x 10^9 CFUs of L. rhamnosus and *L. reuteri*, once daily, orally	30 days	**No withdrawals due to adverse effects.**
Wolf et al., 1995 [IN GRN254]	To evaluate the safety and efficacy of the probiotic Lactobacillus reuteri in healthy adult male subjects	Two group, double blinded, parallel design	30 healthy male subjects	1 x 10^{11} CFU/day of Lactobacillus reuteri	28 days	The authors concluded that supplemental ***L. reuteri*** **may be fed at 1 x 10^{11} CFU/day without any clinically significant safety or tolerance problems**.
Wolf et al., 1998 [IN GRN254]	To evaluate the safety and tolerance of Lactobacillus reuteri supplementation to a population infected with HIV	Double masked, parallel design study	39 HIV infected subjects	1 x 10^{10} CFUs/day of *Lactobacillus reuteri*	21 days	The authors concluded that ***L. reuteri*** **may be fed to HIV-positive individuals at 1 x 10^{10} colony forming units/day without any clinically significant safety or tolerance problems.**

Table 19 Published studies with other *L. reuteri* strains

Reference	Objective	Study design	Subjects	*L. reuteri* dose	Duration	Safety related results
Li et al. 2022	Evaluate safety & Efficacy of *L. reuteri* CCFM1040 to treat allergic rhinitis and asthma	Random, placebo controlled	77 individuals aged 16 to 60 yrs. with rhinitis and asthma	1 x 10^9 CFU/day of *L. reuteri*	8 weeks	No adverse effects reported. Statistically relevant change in microbiota profile at the end of study in treatment group
Lee et al. 2021	Safety assessment of L. reuteri IDCC3701 by phonotypic and genomic analysis	Whole genome sequencing, MIC, hemolysis, biogenic amines, rat oral toxicity	12 female rats, 3 at four treatments	13.8 x 10^{10} CFU/Kg BW	14 days	Whole genome sequence showed Non-transferable, intrinsic resistance to gentamicin & kanamycin. Hemolysis and D-lactate negative, no biogenic amine production. No evidence of acute toxicity in rodents.
Jones et al. 2012	Safety & tolerance of *L. reuteri* NCIMB30242	Double-blind, placebo-controlled randomized study	131 individuals, 56 male & 75 female adults	2.9 x 10^9 CFU/capsule, twice/day	13 weeks	No clinically significant differences in blood chemistry/hematology were observed. The adverse events and safety parameters were found to be similar in treatment and placebo groups.

6.3.3 28-day rodent study with a mixture of the *L. reuteri* strains 3630 and 3632

The mixture of the two *L. reuteri* strains 3630 and 3632 was administered by gavage to 80 CRL Sprague-Dawley CD® IGS rats, evenly divided between the sexes and divided into 4 groups, control and treatments 1 through 3 for 28 days. All animals survived until termination of the study except for one male in Group 2, which upon full necropsy was found to have suffered a torn esophagus. Inflammation of the trachea, pharynx and into mediastinal tissues, consistent with a trauma-induced tear were also noted.

Clinical pathology of samples from the 79 animals that survived until termination of the study did not find any treatment related abnormalities. Macroscopic evaluation of the animals in the other groups did not find any abnormalities that could be associated with the treatment. Microscopic evaluation of an extensive tissue collection also did not find any treatment related abnormalities. The pathologist concluded that the NOAEL was the geometric mean of the high dose, 1.6×10^{10} CFU/kg bw/day of strain 3630 plus 5.7×10^{10} CFU/kg bw/day of strain 3632 for both male and female Sprague-Dawley rats.

The final report is in Annex 14.

6.3.4 Translocation study

Blood and tissue from the 28-day study were separated during necropsy, packed on dry-ice and sent to a third-party lab for determination if the *L. reuteri* strains translocated into the rodents' tissues. The lab report is in Appendix L of the 28-day study report, Annex 14.

The laboratory found that in no instances were test substance treated group translocated bacterial colony counts significantly different than the counts seen in the same sex vehicle control group. The male high-dose group results are nominally higher than the vehicle control group. However, no adverse clinical signs or findings regarding clinical pathology and urinalysis and no morphological changes indicate any correlation to treatment. Therefore, these higher numbers are considered to be without toxicological significance.

The test strains *L. reuteri* (genotypes 1) did not translocate in a greater abundance than the endogenous *L. reuteri* (genotype 2).

6.4 Potential Anti-Nutritional Factors

6.4.1 Allergens

The fermentation media does not contain any ingredients nor derivatives of the current list of allergenic compounds requiring labeling (FDA, 2021), fish, crustaceans, wheat, peanuts, tree nuts, milk, soy or sesame, see Annex 9.

6.4.2 Heavy Metals

Heavy metals are monitored as a component of the manufacturing process and specifications have been established, see sections 2.4 of this dossier. Batch data in section 2.6.1 indicate that the level of heavy metals are below acceptable concentrations for food ingredients.

6.4.3 Hemolysin production

As noted in section 2.3.5.2 of this dossier, an evaluation of the ability of the two strains to produce hemolysin were negative, see Annex 4 for the lab report.

6.4.4 Biogenic amine production

In silico analyses were performed on *L. reuteri* 3632 and 3630 genomes to identify homologs of histidine decarboxylase, tyrosine decarboxylase, lysine decarboxylase, ornithine decarboxylase, agmatine deiminase, agmatine: putrescine antiporter and multicopper oxidase, which are all involved in the production of biogenic amines. *L. reuteri* 3632 and 3630 do not appear to contain any of these homologs in their genomes, see section 2.3.5.5 of this dossier for additional details. Consistent with the bioinformatics results, neither of the subject *L. reuteri* strains were able to produce the major biogenic amines, histamine, tyramine, putrescine or cadaverine.

6.5 Environmental Safety

L. reuteri mixture is a GRAS substance and per 21 CFR 25.32, foods, food additives and color additives, including GRAS substances, are categorically excluded from the requirement to provide an environmental impact statement or an environmental assessment.

6.6 Summary

No published animal studies have revealed any potential toxicological issues with *L. reuteri*, except for one study in mice which showed the potential for translocation of *L. reuteri* and other bacteria to the liver and spleen at doses ≥10^8 CFU/day, (Zegarra-Ruiz et al., 2019). Most of the studies reviewed in section 6.3.1.2 of this dossier indicate a beneficial effect from the addition of *L. reuteri* to the animal's gut microbiota. A 28-day study by the sponsor did not reveal any adverse effects due to the consumption of the *L. reuteri* strain mixture nor translocation of the organisms to other organs.

The data support that the *L. reuteri* strains 3630 and 3632 are non-pathogenic and non-toxigenic and that the *L. reuteri* mixture is non-allergenic.

In healthy human male adults, up to 1x10^{11} CFU/day *L. reuteri* (1.4 x 10^9 CFU/kg bw/day based on an estimated 70 kg weight for the male subjects did not illicit any clinically significant safety or tolerance problems, (Wolf et al., 1995). In pregnant women, up to 1.2 x 10^9 CFU *L. reuteri*/day has been administered for 12 weeks without causing adverse effects. *L. reuteri* has been fed to healthy infants at up to 1.0 x 10^9 CFU *L.* reuteri/day for 28 days without adverse effects, (Abrahamsson et al., 2009).

All the clinical studies reviewed in section 6.3.3 of this dossier did not report adverse effects from the consumption of strains of *L. reuteri*. The NDINs for strains of *L. reuteri* used as a live microbial ingredient corroborate the safety of the genus and species.

The estimated intake of *L. reuteri* mixture in adults of no more than 1 x 10^{10} CFU/day (1.4 x 10^8 CFU/kg bw/day for a 70 kg adult), is 521 times less than the dose of *L. reuteri* mixture that was shown to have no adverse effects in a 28-day toxicity study in rats, 7.3 x 10^{10} CFU/Kg bw/day, see section 6.3.4 of this dossier and Annex 14. When compared to prior GRAS Notices for *L. reuteri* strains, the estimated intake of the subject strains of 1.4 x 10^8 CFU/kg bw/day is about the same as the maximum anticipated intake of *L. reuteri* strain DSM 17938 (GRAS 00254), and one third of the maximum estimated intake of *L. reuteri* NCIMB 30242 (GRAS 00440).

7 Supporting data and information

7.1 Literature Search

Splitrock Regulatory Solutions LLC and BiomEdit performed searches of the publicly available literature looking for both favorable and unfavorable information regarding the safety of the *L. reuteri* mixture and *L. reuteri* strains in general. The most recent search on 7 June 2022 used the search terms ‘reuteri, adverse and safety’ for the following databases: Google Scholar, ScienceDirect, PubMed, ClinicalTrials.gov, Regulations.gov (for NDI filings), Science.gov and the EPA document databases. The information discovered has been discussed in section 6 of this dossier. The pertinent results of all the searches are included in the citations within the dossier and in the bibliography, section 7.3.

7.2 Annexes

Annex 1	API 50 CHL Identification
Annex 2	Strain Receipts
Annex 3	Virulence Factor Report
Annex 4	Hemolytic Activity Report
Annex 5	Biogenic Amine Production Report
Annex 6	Antibiotic Resistance Genes Report
Annex 7	Antimicrobial Susceptibility Report
Annex 8	Antibiotic Production Reports
Annex 9	Fermentation Medium
Annex 10	Manufacturer's GMP Certificate
Annex 11	CoA Strain 3630
Annex 12	CoA Strain 3632
Annex 13	Dietary Supplements Label Database Report
Annex 14	28-day Study-Final Report

7.3 References

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7.4 GRAS Panel Summary

No published animal studies have revealed any potential toxicological issues with L. reuteri, except for one study in mice which showed the potential for translocation of L. reuteri and other bacteria to the liver and spleen at doses ≧108 CFU/day, (Zegarra-Ruiz et al., 2019). Most of the studies reviewed in the dossier indicate a beneficial effect from the addition of L. reuteri to the animal's gut microbiota. A 28-day study by the sponsor did not reveal any adverse effects due to the consumption of the L. reuteri strain mixture nor translocation of the organisms to other organs.

In healthy human male adults, up to 1x1011 CFU/day L. reuteri (1.4 x 109 CFU/kg bw/day based on an estimated 70 kg weight for the male subjects did not illicit any clinically significant safety or tolerance problems, (Wolf et al., 1995). In pregnant women, up to 1.2 x 109 CFU L. reuteri/day has been administered for 12 weeks without causing adverse effects. L. reuteri has been fed to healthy infants at up to 1.0 x 109 CFU L. reuteri/day for 28 days without adverse effects, (Abrahamsson et al., 2009).

The estimated intake of L. reuteri culture in adults of no more than 1 x 1010 CFU/day (1.4 x 108 CFU/kg bw/day for a 70 kg adult), is 521 times less than the dose of L. reuteri culture that was shown to have no adverse effects in a 28-day limited study in rats, 7.3 x 1010 CFU/Kg bw/day. When compared to prior GRAS Notices for L. reuteri strains, the estimated intake of the subject strains of 1.4 x 108 CFU/kg bw/day is about the same as the maximum anticipated intake of L. reuteri strain DSM 17938 (GRAS 00254), and one third of the maximum estimated intake of L. reuteri NCIMB 30242 (GRAS 00440).

BIOMEDIT

7.5 GRAS Panel Conclusion Statement

CONCLUSION

We, the undersigned independent qualified members of the GRAS Panel, have individually and collectively, critically evaluated the data and information summarized above, as well as other data and information that we deemed pertinent to the safety of the intended conditions of use of *Limosilactobacillus reuteri* strains 3630 and 3632 in human food. We unanimously conclude that the proposed uses of the strains produced in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate established specifications as presented in the supporting dossier *["The Safety and Generally Recognized as Safe (GRAS) Status of the proposed uses of Limosilactobacillus reuteri strains 3630 and 3632 in Human Food"]* is safe for consumption at use levels of up to 1 x 10^{10} CFU/day.

We, the members of the GRAS Panel, further unanimously conclude that the intended uses of *Limosilactobacillus reuteri* strains 3630 and 3632, produced in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate established specifications as presented in the supporting dossier is Generally Recognized as Safe (GRAS) based on scientific procedures, under the intended conditions of use in human food.

It is our professional opinion that other qualified experts critically evaluating the same information would concur with this conclusion.

[redacted] November 17, 2021

Michael w. Pariza Ph.D.
(Panel Member)
Emeritus Professor, Univ. of Wisconsin-Madison

Date

[redacted] November 18, 2021

John Specchio, Ph.D., CFS
(Panel Member)
Professor, Montclair University

Date

[redacted] October 25, 2021

Stanley M. Tarka, Jr., Ph.D., FATS.
The Tarka Group, Inc.(Chair), The Pennsylvania State University College of Medicine

Date

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
001112	Jul 4, 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):* 2022-02-03

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
Emily Bulian-Helmes	V.P. Regulatory

Organization *(if applicable)*: BiomEdit, LLC

Mailing Address *(number and street)*: 10100 Lantern Rd. Suite 150

City	State or Province	Zip Code/Postal Code	Country
Fishers	Indiana	46037	United States of America

Telephone Number	Fax Number	E-Mail Address
703-424-1855		emily@biomedit.com

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

Name of Contact Person	Position or Title
James La Marta	Principal Consultant

Organization *(if applicable)*: Splitrock Regulatory Solutions LLC

Mailing Address *(number and street)*: 107 Decker Road

City	State or Province	Zip Code/Postal Code	Country
Boonton	Indiana	07005	United States of America

Telephone Number	Fax Number	E-Mail Address
973-335-1670		james-SRS@optimum.net

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Limosilactobacillus reuteri

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.

The two strains of Limosilactobacillus reuteri, 3630 and 3632, in a ratio of 1:1 on a CFU basis, are to be used as a live microbial ingredient in conventional human food categories. The ingredient could be added to products that support the continued viability of the microorganisms, such as, but not limited to beverages, dairy products, nutritional powders and bars. The product is not intended to be used in infant formulae nor in meat and poultry products regulated by the USDA. The estimated maximum exposure for the 90th percentile

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 BiomEdit, LLC

(name of notifier)

has concluded that the intended use*(s)* of Limosilactobacillus reuteri strains 3630 and 3632

(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. James La Marta 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.

(name of notifier)

107 Decker Road, Boonton, NJ 07005

(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 La Marta, Principal Consultant	06/30/2022

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
	GRAS_Notice_Cover_Letter.pdf	Administrative
	Part_1-BiomEdit_GRASDossier.pdf	Administrative
	GRASDossier_BiomEdit.pdf	Administrative
	Annex_1-API50CHLidentification.pdf	GRAS Notice
	Annex_3_VirulenceFactorReport.pdf	GRAS Notice
	Annex_4_HemolyticActivityReport.pdf	GRAS Notice
	Annex_5_BiogenicAmineProductionReport.pdf	GRAS Notice
	Annex_6_AntibioticResistanceGenesReport.pdf	GRAS Notice

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Annex_7_AntimicrobialSusceptibilityReport.pdf	GRAS Notice
	Annex_8-Antibiotic_Production_Reports.pdf	GRAS Notice
	Annex_9-Fermentation_Medium.pdf	GRAS Notice
	Annex_10_GMP_Certificate-Biosource.pdf	GRAS Notice
	Annex_11_CoA_Strain_3630.pdf	GRAS Notice
	Annex_12_CoA_Strain_3632.pdf	GRAS Notice
	Annex_13-DSLD_Report.pdf	GRAS Notice
	Annex_2-ATTC-Strain_Receipts.pdf	GRAS Notice
	Annex_14a_28-DayStudy_rpt.pdf	GRAS Notice

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Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Annex_14b_28-DayStudy_rpt.pdf	GRAS Notice
	Annex_14c_28-DayStudy_rpt.pdf	GRAS Notice
	Annex_14d_28-DayStudy_rpt.pdf	GRAS Notice

Splitrock Regulatory Solutions, LLC

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Dr. Deng
Food & Drug Administration
Division of Food Ingredients
Center for Food Safety & Applied Nutrition

23 June 2023

Dear Dr. Deng,

On 12 June 2023, the FDA sent a letter to Splitrock Regulatory Solutions, LLC requesting additional information regarding the Generally Recognized As Safe Notice that was submitted on behalf of BiomEdit, LLC. in July 2022, now identified as GRN 001112. Provided below are our answers to those questions with the FDA question in blue text and our replies in black text.

1. In Tables 8 and 9 (pp. 24-25 and 26, respectively), the notifier provides the results from five batch analyses for each of the two *L. reuteri* strains.

a) Please confirm that the five batches tested are non-consecutive.

BiomEdit, LLC is confirming that the five batches are non-consecutive.

b) We note that some of the results for heavy metals are reported as "<" [a value]. Please provide the limit of quantitation (LOQ) or the limit of detection (LOD) of the analytical method used to analyze the batches for heavy metals.

The Limit of Quantification (LOQ) of the analytical method used by the third-party laboratory to analyze the batches for each of the heavy metals is as follows: Arsenic, 10 ppb, and 5 ppb for Cadmium, Lead, and Mercury.

c) Please explain why the result for mercury is missing for Lot 201123LRE3632 (Table 9).

We apologize for the missing value in Table 9. It was accidently deleted when generating the Adobe Acrobat pdf copy. Here is Table 9 with all the values.

Parameter	Specification	Lot A	Lot 210712LRE3632	Lot C	Lot 201123LRE3632	Lot E
Identification	*Limosilactobacillus reuteri*	Pass	Pass	Pass	Pass	Pass
Total Viable Cell Count of *L. reuteri* 3630, CFU/g	NLT 2 x 10^9	4.8x 10^9	1.8 x 10^{11}	3.4 x 10^9	4.3 x 10^{11}	3.3x 10^9
Non-Lactics, CFU/g	< 5000	<10	<100	<10	300	<10
E. coli	Negative in 10 g	Absent	Absent	Absent	Absent	Absent
Enterobacteriaceae (MPN/g)	< 10	Absent	<10	Absent	< 10	Absent
Staphylococcus (coagulase +)	Negative by test in 40 g	Absent	Absent	Absent	Absent	Absent
Salmonella	Negative in 40 g	Absent	Absent	Absent	Absent	Absent
Enterococcus, CFU/g	< 100	Absent	<10	Absent	<10	Absent
Molds and Yeast, CFU/g	< 100	<10	<10	<10	<10	<10
Arsenic	NMT 0.3 ppm	<10 ppb	110 ppb	<10 ppb	282 ppb	<10 ppb
Cadmium	NMT 0.2 ppm	<5 ppb	52 ppb	<5 ppb	195 ppb	<5 ppb
Mercury	NMT 0.05 ppm	<5 ppb	<5 ppb	<5 ppb	6.4 ppb	<5 ppb
Lead	NMT 0.2 ppm	<5 ppb	190 ppb	<5 ppb	103 ppb	<5 ppb

2. Regarding the results provided in Tables 8 and 9 (pp. 24-25 and 26, respectively), we note that there is a noticeable difference (up to approximately 40 times) among the batches with regard to the heavy metal levels (except for mercury). The provided results demonstrate that in three out of five batches the levels of heavy metals are consistently below 0.005 mg/kg or 0.01 mg/kg. In addition, the heavy metal levels in those three batches are at least 30-40 times lower than the corresponding specification limits (0.2–0.3 mg/kg), indicating that achieving levels of heavy metals significantly lower than the specification limits is feasible. However, the results from the two remaining batches show that some heavy metals are present in the final ingredient at levels higher than 0.1 mg/kg and up to 0.28 mg/kg. We note that the provided results show levels of heavy metals and a variation in heavy metal levels that we would not expect for an ingredient produced by controlled fermentation under good manufacturing practices.

a) Please discuss the variation in the results from the batch analyses and comment on the possible source of significantly higher heavy metals levels in the two batches of the final ingredient.

Batches A, C and E were produced at pilot scale in BiomEdit's R&D facility whereas the other two lots were produced by the toll manufacturer identified in Annex 10. The two facilities are located in different parts of the country. Heavy metal concentrations are strongly affected by water quality at the source. The Pilot Plant uses highly purified Reverse Osmosis water whereas the toll manufacturer uses potable water that meets the EPA drinking water standards and food production standards as supported by the facility's SFQ certification that was provided in Annex 10.

b) We encourage you to review your specifications for arsenic, cadmium, and lead, and keeping in line with FDA's Closer to Zero initiative that focuses on reducing dietary exposure to heavy metals, provide specification limits that are reflective of the batch analyses and are as low as possible.

BiomEdit, LLC supports the FDA Closer to Zero initiative to reduce dietary exposure to contaminants to as low as possible while continuing to focus on delivering nutritious foods. Heavy metal specification values were established based upon development batches which included production scale to ensure that they are representative of the realities of full-scale manufacturing capabilities. At this time, industry is guided by 21 CFR 172 under which FDA regulates the maximum permitted limits of some heavy metals in certain food additives permitted for direct addition to food for human consumption. Another resource is the Codex Alimentarius, with limits for heavy metals for direct food additives for human foods and for a variety of human food products. A third dataset that informs industry is the compilation of heavy metal specifications in other live microbial food ingredients that were the subject of recent FDA no questions letters. Table 2b below shows these heavy metal limits together with the specifications in the BiomEdit, LLC, GRAS Notice 1112.

Table 2b – Specific References for Heavy Metal Limits in Food Additives and GRAS Substances

	21 CFR 172.25	**Codex**	**BiomEdit**	**GRN 871**	**GRN 988**	**GRN 1013**	**GRN 1062**	**GRN 1022**
All amounts in ppm	Certain food additives	Certain direct food additives	*L. reuteri*	*L. acidophilus*	*L. fermentum*	*L. rhamnousus*	*S. cerevisiae*	*S. salivarius*
Arsenic	0.1 - 3	0.1 - 0.35	0.3	1	1	not provided	0.5	0.1
Cadmium	0.05 - 0.13	0.05 - 0.4	0.2	0.3	1	not provided	0.03	0.1
Lead	0.1 - 10	0.05 - 1	0.2	1	1	<= 0.05	1	0.3
Mercury	0.5 - 1	0.001 - 0.1	0.05	0.05	1	not provided	not provided	0.1

The specification values for the *L. reuteri* strains of 0.3 ppm for arsenic, 0.2 ppm for cadmium, 0.2 ppm for lead, and 0.05 ppm for mercury are within the limits for most other food additives and recent GRAS Notices for other similar microbials. Because the manufacturing scale batches had heavy metal levels greater than the pilot batches, those values are more indicative of probable future levels and do not allow for confident reduction in the specifications at this time. If future commercial batches reveal a consistent trend of lower levels, the specifications will be adjusted downward following appropriate quality control procedures.

3. In Table 7 (p. 21), the notifier provides the specifications for the *L. reuteri* combination. Please provide the results from the analyses of a minimum of three (preferably five) non-consecutive batches of the *L. reuteri* combination.

Provided below are analytical data from three lots.

Table 7.1 Analytical Data from Three Representative Non-Consecutive Lots of L. reuteri Mixture

Parameter	Specification	Lot# 184	Lot# 625	Lot# 223
Appearance	Free-flowing powder	Pass	Pass	Pass
Color	Off-white to dull orange	Pass	Pass	Pass
Identification	*Limosilactobacillus reuteri*	Pass	Pass	Pass
Total Viable Cell Count of *L. reuteri* (CFU/g)	$\geq$ 2.6 x 10^9	5 x 10^9	4.5 x 10^9	3.78 x 10^9
Microbiological Testing				
Non-Lactics (CFU/g)	< 5000	100	100	900
E. coli	Negative / 10g	Negative	Negative	Negative
Enterobacteriaceae (MPN/g)	< 10	< 10	< 10	< 10
Staphylococcus (coagulase +)	Negative/25 g	Negative	Negative	Negative
Listeria	Negative/25 g	Negative	Negative	Negative
Salmonella	Negative/ 25 g	Negative	Negative	Negative
Enterococcus (CFU/g)	< 100	< 10	< 10	< 10
Molds and Yeast (CFU/g)	< 100	< 10	< 10	< 10
Heavy Metals				
Arsenic (ppm)	NMT 0.3	0.22	0.22	0.08
Cadmium (ppm)	NMT 0.2	0.14	0.14	0.05
Mercury (ppm)	NMT 0.05	< 0.005	< 0.005	< 0.005
Lead (ppm)	NMT 0.2	0.075	0.075	0.17

4. Please provide a statement that all processing aids and cryoprotectants used in the manufacture of *L. reuteri* ATCC PTA-126787 and ATCC-PTA 126788 are used in accordance with applicable U.S. regulations, are GRAS for their respective uses or are the subject of an effective food contact notification.

BiomEdit is confirming that the cryoprotectants are approved food additives or GRAS substances for use in the production of live microbials for human consumption.

5. In Table 10 (p. 27), the notifier provides data from the stability studies on the L. reuteri combination. We note that the initial concentration of the tested samples was 9.7 x 10^7 CFU/mL while the lower specification limit for the total viable cell count in the ingredient was stated as ≥ 2.6 x 10^9 CFU/g (Table 7, p. 21). Please clarify why the stability studies were performed on samples that do not meet the specification limit for the total viable cell count.

The stability studies were started when it was believed that the concentration of the mixture of *L. reuteri* strains would be ≥ 9 x 10^7 CFU/g. It was later decided for marketing purposes that the concentration should be increased to ≥ 2.6 x 10^9 CFU/g. The concentration does not affect the stability. As the data indicates, the equal mixture of the two strains is stable at 5 °C and 25 °C and loses viability at 30°C and above. There is no reason to believe that the same would not hold true for a mixture that has a higher concentration at time zero.

6. On p. 8, the notifier states that the subject of GRN 00112 is a combination of two strains of *L. reuteri* in a 1:1 ratio on a CFU basis. Please confirm that the strains are intended to be used only in a combination and not individually. If you intend to use each strain individually, please clarify whether the intended uses of the individual strains will be substitutional for the strains used in combination.

BiomEdit, LLC is hereby confirming that it is the mixture of the two strains and not the individual strains that will be used as a food ingredient.

7. On p. 29, the notifier states that the ingredient is intended to be used in dairy products, beverages, nutritional powders, bars, confections and other foods. We note that the intended food uses listed in Table 12 (p. 30) are not reflective of all the intended food uses mentioned on p. 29 (e.g., Table 12 does not list confections and "other foods"). Please clarify if the intended use is only in the food categories listed in Table 12, includes additional food categories, or it is in all conventional foods. If the intended use includes food categories that are in addition to those listed in Table 12 or if it includes all conventional foods, the dietary exposure estimate should be revised to reflect all the intended uses of the of the *L. reuteri* combination.

The food groups listed in Table 12 are the intended uses, BiomEdit, LLC does not intend to sell the *L. reuteri* strains for use in 'all conventional foods'.

8. Annexes 11 and 12 are CoA documents for a September 2020 production batch of the two strains. Please clarify which lot in Table 8 and 9 the September 2020 production batch represents.

The CoA documents for the production batches for LR strain 3630 and LR strain 3632 are 201123LRE3630 (made in Nov 2020), 210713LRE14(ET3(TRE)) (made in Jul 2021) and 2011LRE3632 (Nov 2020), 210712LRE15(ET5)(TRE) (Jul 2021), respectively.

9. Please clarify that the internal methods that listed in Annexes 11 and 12 have been validated against a standardized method, such as an ISO, AOAC or FDA BAM method, for its intended use.

The Certificates of Analysis in Annexes 11 and 12 from the third-party analytical laboratory contain the appropriate compendial references at the bottom of each section. For example, in Annex 11 on page 6 after the heavy metal results, the AOAC Methods 2011.16 and 993.14 are referenced, and on page 9, the Enterobacterial count references USP chapter 62.

10. In section 2.6.1 (p. 22), the notifier describes the methodology to determine the stability of the two production strains in their master cell bank. For the administrative record, please briefly specify how the purity of the inoculum for each of the two L. reuteri strains is ensured.

Maintaining pure cultures is a critical prerequisite to successful production of Cell Banks. Preventive measures used to control microbial and cross contamination include segregation, cleaning and sanitation, facility construction to permit adequate cleaning, and wearing of protective clothing, face shields, and gloves during handling tasks. The inoculums used for generating the Master Cell Bank (MCB) are derived from well-isolated single colonies of cells produced under defined conditions, uniformly dispensed into multiple containers (vials), and stored under controlled conditions, e.g., -80 °C. The Master Cell Bank is tested and characterized in accordance with regulatory requirements for purity and quality and must meet certain company acceptance criteria for use. The Working Cell Bank is generated by the same process as the MCB except that the starting point is the well-isolated single colony of the MCB. All raw materials and formulation ingredients used in the production of the Master and Working Cell Banks must meet all applicable regulatory standards for purity and quality.

11. Please clarify that the fermentation process is continuously monitor for contaminants.

The production of the L. reuteri strains is a batch process with fermentation time of less than 30 hours. Each batch is analyzed to ensure compliance with the specifications which includes the absence of pathogens and other potential contaminants.

12. For supporting the safety conclusion, the notifier lists three GRAS notices (#254, 410, 440) related to L. reuteri strains have received no-question letter from FDA. As each GRAS notice stands on its own, for the administrative record, please provide a brief paragraph summarizing the information pertaining to safety for each of these GRAS notices.

GRAS Notice 254

On November 18, 2008, the FDA replied with a no-questions letter regarding GRAS Notice No. 254. The notice detailed the conclusion by BioGaia that *Lactobacillus reuteri* strain DSM 17938 is GRAS, through scientific procedures, for use as an ingredient in processed cheeses, yogurt, ice cream, fruit juices, fruit drinks, processed vegetables, processed vegetable drinks, beverage bases, energy bars, energy drinks, chewing gum, and in a drinking straw at a level up to 10^9 colony forming units (cfu) per serving. In terms of the identity and characteristic properties, *L. reuteri* strain DSM 17938 is a Gram-positive, non-spore-forming heterofermentative lactic acid bacterium, derived by deletion of two antibiotic resistance plasmids (pLR581 and pLR585) from an original strain *L. reuteri* ATCC 55730. Analysis of the genome sequence of *L. reuteri* strain DSM 17938 showed no presence of any gene or gene cluster known to be involved in virulence or antibiotic resistance. BioGaia noted that the parent organism, *L. reuteri* ATCC 55730, has been sold in the United States and internationally as an ingredient in a variety of dairy products including yogurts, and as a dietary supplement. BioGaia stated that the production of the *L. reuteri* strain DSM 17938 ingredient is based on standard fermentation techniques and the fermentation medium components are food-grade materials. In the safety narrative, BioGaia noted that *L. reuteri* is part of the indigenous human microflora and has been isolated from the oral cavity, breast milk, and gastrointestinal

tract of humans, and summarizes data from published studies in which rats, mice, pigs, and monkeys were fed various strains of *L. reuteri*. The GRAS notice included an overview of published studies in which human adults, children or infants consumed *L. reuteri* ATCC 55730 or other strains of *L. reuteri and* described data from an unpublished study in human adults who consumed *L. reuteri* strain DSM 17938. BioGaia concluded that the intended use of *L. reuteri* strain DSM 17938 in various human foods excluding infant formula resulting in a maximum anticipated exposure of less than 10^{10} cfu per day is GRAS.

GRAS Notice 410

FDA responded with a no questions letter to GRAS Notice 410 submitted on behalf of Nestle Nutrition, U.S. (Nestle) on March 26, 2012. Nestle informed FDA that the view of Nestle was *L. reuteri* strain DSM 17938 is GRAS, through scientific procedures, for use as an ingredient in powdered whey-based term infant formula at a minimum level of 10^6 colony forming units per gram (cfu/g), but not higher than 10^8 cfu/g of powdered formula, produced in accordance with current good manufacturing practices (cGMPs). Nestle incorporated by reference GRN 254 on the same *L. reuteri* 17938 for use in various human foods excluding infant formula. For use in infant formula, in addition to the specifications for *L. reuteri* strain DSM 17938 from GRN 254, Nestle added the specification for absence (zero colony forming units) of *Cronobacter sakazakii* (formerly classified as *Enterobacter sakazakii)* in 10 grams of freeze-dried, powdered *L. reuteri* strain DSM 17938. Nestle discussed experiments with *L. reuteri* strain DSM 17938 that assessed potential lactic acid production in reconstituted formulas held up to 6 hours at 4, 25, or 37 ^oC. Under these conditions, infant formulas prepared from powder containing 10^6 cfu *L. reuteri* strain DSM 17938 per gram were similar to controls and did not show increases in L- and D-lactic acid concentrations. Using a typical energy density of 67.6 kcal/100 mL for term infant formula, Nestle estimated consumption of infant formula to be 209 mL formula per kg bw/d at the 90^{th} percentile, corresponding to a dietary exposure of 2.7×10^7 cfu/d *L. reuteri* strain DSM 17938. Furthermore, Nestle discussed four additional clinical studies with term or pre-term infants fed *L. reuteri* strain DSM 17938 suspended in oil and given via oral drops or added to infant formula. Nestle noted these four studies, published since the completion of GRN 000254, showed no adverse events attributable to feeding *L. reuteri* strain DSM 17938 and support the safety of the intended use of this ingredient in powdered term infant formula. Finally, Nestle provided published scientific data, as well as a discussion, refuting any safety issue related to possible metabolic acidosis from use of *L. reuteri* strain DSM 17938 in term infant formula.

GRAS Notice 440

On February 12, 2013, the FDA responded to GRN 440 with a no questions letter to Micropharma Ltd. (Micropharma) who had concluded that *L. reuteri* strain NCIMB 30242 is GRAS, through scientific procedures, for use as an ingredient in beverages and beverage bases, breakfast cereals, cheeses, dairy product analogs, fats and oils, frozen dairy desserts, grain products and pastas, milk products, processed fruits and fruit juices, and sugar substitutes at levels ranging from 3.3×10^8 to 10^{10} colony forming units per serving (CFU/serving). Micropharma discussed the identity and characterization of *L. reuteri* strain NCIMB 30242. Lactobacilli are Gram-positive, non-spore-forming, rod- or coccobacillus-shaped bacteria and these microorganisms can be found in dairy products, fermented meat, sourdough, vegetables, fruits, and beverages. *L. reuteri* strains are commonly found as commensal organisms in the respiratory, gastrointestinal, and genital tracts of humans and animals. Micropharma described the production of *L. reuteri* strain NCIMB 30242 by fermentation and that the manufacturing process was conducted under cGMP using suitable food-grade raw materials that are either GRAS or permitted for use in the United States. Micropharma stated that *L. reuteri* strains have a history of safe use by the food industry as

fermentation starters in the manufacture of sourdough and other breads. The history of safe food use of *L. reuteri* strains is recognized by the European Food Safety Authority and *L. reuteri* strains meet their criteria for "Qualified Presumption of Safety" status. Micropharma discussed published *in vitro* tests (does not produce biogenic amines or antimicrobial substances and is susceptible to several antibiotics) and bioinformatic analyses (contains antibiotic resistance elements common to other lactobacilli, unlikely to be transmitted; does not contain genes encoding protein toxins or known to confer pathogenicity), to support the safety of *L. reuteri* strain NCIMB 30242. Furthermore, the notifier's search of several publicly available databases did not produce literature documenting incidences of *L. reuteri* infections. Micropharma also reported on two published human studies, in which male and female subjects consumed microencapsulated *L. reuteri* strain NCIMB 30242 at a minimum of 10^{10} CFU/serving twice a day for six weeks or two capsules per day of *L. reuteri* strain NCIMB 30242 at 2.9 X 10^9 CFU/capsule for nine weeks. Micropharma noted that *L. reuteri* strain NCIMB 30242 were well tolerated in both studies. Micropharma reported on two unpublished animal studies. In the first study, the bacteria were administered by gavage to hamsters at 10^9 CFU per day of alginate polylysine encapsulated *L. reuteri* strain NCIMB 30242 for six weeks. In the second study, 10^{10} CFU per day of *L. reuteri* strain NCIMB 30242 were administered by gavage to male rats. *L. reuteri* strain NCIMB 30242 was not detected in the blood or liver and there was no increase in plasma D-lactic acid compared to the control. Based on the totality of the information in the notice, Micropharma concluded that *L. reuteri* strain NCIMB 30242 is GRAS for the intended uses in foods.

13. In Annex 9 (fermentation medium ingredients), the notifier cited GRN 000609 as a reference for pea protein. However, the subject of GRN 000609 is rice protein. Pea protein is the subject for GRAS notices GRN 000803 and GRN 000804. Please provide correct references for the pea protein component in the fermentation medium.

We apologize for the incorrect reference. Pea protein should have had GRN 000803 and GRN 000804 as the references and not GRN 000609.

14. Please provide updated information on the literature search(es) performed to prepare the notice. This includes the date(s) (e.g., month and year) of the search(es), the resource database(s) used (e.g., PubMed), the principal search terms used, and the time period that the search spanned (e.g., 1/2022 to 5/2023).

In section 7.1 of the Notice, the date of the last search, June 2022, about one month before the Notice was submitted, and the databases and search terms that were used are identified. No date range was used in the searches in an effort to discover any relevant publications that occurred before June 2022.

15. On p. 6, the notifier states that the intended use of Limosilactobacillus reuteri ATCC PTA-126787 and ATCC-PTA 126788 (L. reuteri combination) is as a microbial ingredient in selected foods. On the same page, the notifier further states that "The purpose of the ingredient is to increase the concentration of members of the *Lactobacillaceae* in the digestive tract…". It should be pointed out that health benefits are not evaluated under the GRAS Notification Program. Therefore, the latter statement is not appropriate to describe the intended use of a GRAS substance.

BiomEdit, LLC has not made any claims of health benefits. It is logical to conclude that if you add a microorganism to a food, that the addition will increase the concentration of that organism in the digestive tract when the food is consumed.

16. We note that the term “probiotic” has been mentioned in the notice. Please note that there is no FDA regulatory definition of the term “probiotic”. The use of a substance as a “probiotic” is not acceptable with respect to use as an ingredient in conventional foods.

BiomEdit, LLC understands the FDA's position regarding the term “probiotic”. The word 'probiotic' appears in the dossier only when reviewing the literature and in the references as that word was used in some of the publications and the titles of the publications. It would not be appropriate to eliminate ‘probiotic’ when discussing those publications in the narrative as required by the GRAS Rule or when listing the publication in the bibliography.

Sincerely,

James La Marta

Splitrock Regulatory Solutions, LLC

James La Marta
Principal Consultant

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Dr. Deng
Food & Drug Administration
Division of Food Ingredients
Center for Food Safety & Applied Nutrition

11 August 2023

Dear Dr. Deng,

On 1 August 2023, the FDA sent a letter to Splitrock Regulatory Solutions, LLC requesting additional information regarding the Generally Recognized As Safe Notice that was submitted on behalf of BiomEdit, LLC, in July 2022, now identified as GRN 001112. Provided below are our answers to those questions with the FDA question in blue text and our replies in black text.

1. In the amendment dated June 23, 2023, you provided Table 2b that compared example heavy metal limits in food additives and GRAS substances to the heavy metal limits for *L. reuteri* ATCC PTA-126787 and ATCC PTA-126788. We note that the heavy metal limits provided in Table 2b for GRN 001062 were incorrect. Specifically, the limits for arsenic (< 0.5 mg/kg) and cadmium (< 0.03 mg/kg) provided in one of the amendments to GRN 001062 are for the raw materials, not for the final ingredient. In addition, the limit for lead is ≤0.005 mg/Kg, not ≤1 mg/Kg (Please see the response letter for GRN 001062 as well as the amendments).
We note that the FDA's recent Closer to Zero initiative specifically focuses on reducing dietary exposure to heavy metals in foods. We typically see levels of heavy metals (and therefore corresponding specification limits) not higher than 0.1 mg/kg in fermentation-derived ingredients produced by controlled fermentation and following current good manufacturing practices (e.g., GRNs 001074 and 001075 that were recently posted on our GRAS Notices inventory webpage). In keeping with FDA's Closer to Zero initiative, we suggest that you reconsider our recommendation regarding lowering the specification limits for lead, arsenic, and cadmium in the final ingredient.

BiomEdit understands and supports the FDA Closer to Zero initiative. However, FDA has not established maximum limits for metals in food ingredients. Additionally, the concept of what constitutes 'zero' has changed each decade as analytic techniques have improved. What was once acceptable has now become suspect. GRN 001074 and GRN 001075 describe single,

purified molecules whereas the BiomEdit GRAS Notice describes harvested live microorganisms. The downstream processes used to isolate these two products are very different and do not allow for direct comparison of specifications for heavy metals. It is not possible to repeatedly wash, nano-filter nor treat live bacteria with activated charcoal, which is known to absorb heavy metals, without complete loss of viability from cell lysing and/or removal of the organisms. The comparisons provided in the amendment to GRN 001112 of 23 June 2023 of the BiomEdit product to other *Lactobacillaceae*, GRNs 871, 988, and 1013, are the most relevant. We apologize for misunderstanding the context of the response letter amended to GRN 001062.

Another consideration is the exposure. As noted in the GRAS Notice, the BiomEdit product has an anticipated maximum exposure of 2 x 10^{10} CFU/day and the concentration of *L. reuteri* in the finished product is a minimum of 2.6 x 10^{9} CFU/g. Therefore, the maximum amount of product consumed would be 7.7 grams/day per person. This means that the maximum exposure to heavy metals from the consumption of the BiomEdit product would be 2.3 µg for arsenic, 1.5 µg for cadmium, 1.5 µg for lead and 0.39 µg for mercury. On a body weight basis, using the typical 60 kg person, the exposure is 38.3 ng/Kg/day for arsenic, 25 ng/Kg/ day for cadmium and lead and 6.3 ng/Kg/ day for mercury. The current accepted Tolerable Daily Intakes (TDI) are 0.3 µg/Kg/ day for arsenic, 0.36 µg/Kg/ day for cadmium, 0.5 µg/Kg/ day for lead and 0.19 µg/Kg/ day for mercury, (Augustsson et al., 2021). The safety margins for the 90th percentile consumer based upon the TDI are then 7.8 for arsenic, 14.4 for cadmium, 20 for lead and 30 for mercury.

BiomEdit stands by its current data The heavy metals specifications are at the lowest levels supported by manufacturing data at the production facility. As noted in our letter of 23 June 2023, BiomEdit will lower the heavy metal specifications when sufficient manufacturing data is available to support the reduction, which is consistent with the FDA Closer to Zero initiative document and standard quality control practices.

2. In the amendment dated June 23, 2023, you did not provide a sufficient clarification regarding differences in the batch labeling (response to our Question #8).

a) Three of the batches in Table 8 of GRN 001112 are labeled as Lots A, C, and E. However, the certificates of analysis (COAs) in Annex 11 are for batches labeled as Lots 3630-A, 3630-B, and 3630-D. Please clarify which lots (A, C, and E) in Table 8 correspond to which lots (3630-A, 3630-B, and 3630-D) in Annex 11.

b) Three of the batches in Table 9 of GRN 001112 are labeled as Lots A, C, and E. However, the COAs in Annex 12 are for batches labeled as Lots 3632-A, 3632-B, 3632-C, and 3632-E. Please clarify which lots (A, C, and E) in Table 9 corresponds to which lots (3632-A, 3632-B, 3632-D, and 3632-E) in Annex 12.

We provide the following amendments to Tables 8 and 9 of the dossier.

In addition, please note BiomEdit has found typographical errors in Table 8: Lot C should be Lot B; Lot E should be Lot D; the concentration of *L. reuteri* for Lot A is 5.8 x 10^{9} CFU/g not 5.9 x 10^{9} CFU/g.

Table 8 Amended: Dossier Lot ID and Corresponding Annex 11 Sample IDs and Annex 11 Lot IDs

Lot ID	Lot A	Lot B	Lot D
Annex 11 Sample ID	9868719	9868720	9868722
Annex 11 Lot ID	3630-A	3630-B	3630-D

Table 9 Amended: Dossier Lot ID and Corresponding Annex 12 Sample IDs and Annex 12 Lot IDs

Lot ID	Lot A	Lot C	Lot E
Annex 12 Sample ID	9868714	9868716	9868718
Annex 12 Lot ID	3632-A	3632-C	3632-E

3.Concerning your response to question #15, we suggest explicitly stating that the statement of "The purpose of the ingredient is to increase the concentration of members of the *Lactobacillaceae* in the digestive tract..." is not one of the intended uses of the subject, because benefit claims are not evaluated under the GRAS Notification Program.

The purpose of the mixture of two strains of *L. reuteri*, as the FDA suggested to BiomEdit in the pre-submission meeting of 3 February 2022, is '*to be used as an ingredient in select foods*'. In Section 1.6 of the GRAS Notice, BiomEdit claims the organisms '*are to be used as a live microbial ingredient in conventional human food categories*'. In the letter of 22 June 20203, BiomEdit noted that it does not make a claim of benefit from consumption in the GRAS Notice as inferred by the FDA comment. If it pleases FDA to remove the last sentence in paragraph one of the executive summary, we do not object and a revised page 6 of the GRAS Notice is provided.

Reference:

Augustsson, A., Qvarforth, A., Engstrom, E., Paulukat, C. and Rodushkin, I.; 2021. Trace and major elements in food supplements of different origin: Implications for daily intake levels and health risks. Toxicology Reports (8): 1067-1080.

Sincerely,

James La Marta

Executive Summary

Elanco Animal Health isolated two strains of *Limosilactobacillus reuteri* (previously described as *Lactobacillus reuteri* and referred to herein as *L. reuteri* [Zheng et al, 2020]) from chickens. BiomEdit, LLC, a new company spun off from Elanco Animal Health Inc. in April 2022, has concluded that the addition of a mixture of the *L. reuteri* strain 3630 and *L. reuteri* strain 3632 in a ratio of 1:1 on a colony-forming unit (CFU) basis (referred to herein as "*L. reuteri* mixture") is generally recognized as safe for use as a live microbial ingredient in the intended conventional human food categories at up to 1 x 10^{10} CFU/day.

The strains are grown independent of each other using a food grade nutrient media following standard large scale fermentation practices and cGMP applicable to producing live microbials for use in human foods. Each organism is harvested and dried and then the strains are combined to provide equal concentrations on a CFU basis. Batch data confirm that the strains can be grown consistently to meet the established specifications.

To conclude that the *L. reuteri* mixture is a safe ingredient for the intended use, the sponsor unambiguously identified the two novel *L. reuteri* isolates, ATCC PTA-126787 (3630) and ATCC PTA-126788 (3632), and then evaluated the safety of the *L. reuteri* mixture by conducting *in silico* and *in vitro* analyses on each of the strains and an *in vivo* toxicity study using the *L. reuteri* mixture and reviewing all literature pertaining to the safety of this microbial species. Using the information from whole genome sequencing and *in silico* analysis, it was found that the strains do not produce known toxicants nor antibiotics. Antimicrobial susceptibility testing revealed that the strains are susceptible to common antibiotics. The strains do not produce virulence factors or toxins, biogenic amines, nor antibiotics to typically tested pathogens (Gangaiah, D, et al, 2022). A 28-day study with Sprague-Dawley rats did not reveal any hematologic nor histopathologic differences that were statistically significant between the *L. reuteri* mixture treatment groups and control. The no-observed-adverse-effect-level (NOAEL) was concluded to be the high dose of the *L. reuteri* mixture in this study, 1.6 x 10^{10} CFU/kg bw/day for strain 3630 plus 5.7 x 10^{10} CFU/kg bw/day for strain 3632.

The extensive review of the published safety literature included three GRAS notices to FDA CFSAN and more than 30 papers on human clinical trials regarding the consumption of different strains of *L. reuteri* at doses of up to 1 x 10^{11} CFU/person/day and there were no adverse effects due to consumption. The published literature and the results of the toxicological study support the safe use of the *L. reuteri* mixture as a live microbial ingredient in conventional human food categories.

Splitrock Regulatory Solutions, LLC

James La Marta
Principal Consultant

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1-973-335-1670
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Dr. Deng
Food & Drug Administration
Division of Food Ingredients
Center for Food Safety & Applied Nutrition

13 September 2023

Dear Dr. Deng,

After meeting with the FDA and consultation with representatives of BiomEdit LLC, we are hereby clarifying the inclusion levels of the *L. reuteri* mixture in the foods identified in GRN 001112 and amending the GRAS Notice accordingly.

The maximum use level of the *L. reuteri* mixture in each of the food groups identified in Table 12 of the GRAS Notice is 1 x 10^{10} CFU/serving, which allows for variations in composition and processing conditions within the food groups. This maximum use level would result in a total potential exposure of no more than 1 x 10^{11} CFU/ person/day if a person consumed 10 servings of food with each containing the maximum amount of the *L. reuteri* mixture.

We trust this clarification is helpful.

Sincerely,

James La Marta

Dossier Annexes

Annex 1
API 50 CHL Identification

Technical Report

Biochemical Identification of *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788 by API 50 CHL Assay

Dharanesh Gangaiah

Discovery Research – Bacteriology and Microbiome

Keywords: *Limosilactobacillus reuteri*, Biochemical identification, API 50 CHL

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology & Microbiome	04/21/2021
[Author's name] [Author's title and Department name]	Date

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Shrinivas Mane/ Principal Research scientist, Informatics	04/21/2021
[Reviewer's name] [Reviewer's title and Department name]	Date

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name] [Approver's title and Department name]	Date

1. Introduction

API 50 CHL test is a well established biochemical identification method for *both* Gram-negative and Gram-positive bacteria, including *Lactobacillus* species. The identification is based on the rationale that microorganisms belonging to specific species produce a unique carbohydrate fermentation profile.

2. Experimental methods

2.1. Bacterial growth

The *L. reuteri* strains described in this study were routinely propagated on Lactobacilli de Man Rogosa Sharpe (MRS, BD Difco) medium anaerobically at 37°C.

2.2. API 50 CHL assay

The strains were profiled for enzymatic activity and carbohydrate fermentation using API 50 CHL strips (bioMérieux), following the manufacturer's instructions. The *L. reuteri* strain DSM 17938 was used as a positive control.

3. Results and Discussion

3.1. API 50 CHL identification

When tested with API 50 CHL, the final two *L. reuteri* candidates, PTA-126787 and PTA-126788, were identified as *Limosilactobacillus fermentum* (previously *Lactobacillus fermentum*) with 92.3% identity (Table 1). The positive control *L. reuteri* DSM 17938 was also identified as *L. fermentum* with 92.3% identity (Table 1). The fermentation profile of *L. reuteri* is similar to that of *L. fermentum* and the APIwebTM software version 5.0 does not have the capability to distinguish between the 2 species.

Table 1. Carbohydrate fermentation profile of *L. reuteri* strains PTA-126787 and PTA-126788 by API 50 CHL.

Substrate	PTA-126787	PTA-126788	DSM 17938	Substrate	PTA-126787	PTA-126788	DSM 17938
Negative control	-	-	-	Esculin ferric citrate	+	+	+
Glycerol	-	-	-	Salicin	-	-	-
Erythritol	-	-	-	D-Cellobiose	-	-	-
D-Arabinose	-	-	-	D-Maltose	+	+	+
L-Arabinose	+	+	+	D-Lactose	+	+	+
D-Ribose	+	+	+	D-Melibiose	+	+	+
D-Xylose	-	-	-	D-Saccharose	+	+	+
L-Xylose	-	-	-	D-Trehalose	-	-	-
D-Adonitol	-	-	-	Inulin	-	-	-

Methyl-βD-xylopyranoside	-	-	-	D-Melezitose	-	-	-
D-Galactose	+	+	+	D-Raffinose	+	+	+
D-Glucose	+	+	+	Amidon	-	-	-
D-Fructose	-	-	-	Glycogen	-	-	-
D-Mannose	-	-	-	Xylitol	-	-	-
L-Sorbose	-	-	-	Gentibiose	-	-	-
L-Rhamnose	-	-	-	D-Turanose	-	-	-
Dulcitol	-	-	-	D-Lyxose	-	-	-
Inositol	-	-	-	D-Tagatose	-	-	-
D-Mannitol	-	-	-	D-Fucose	-	-	-
D-Sorbitol	-	-	-	L-Fucose	-	-	-
Methyl-αD-mannopyroside	-	-	-	D-Arabitol	-	-	-
Methyl-αD-glucopyranoside	-	-	-	L-Arabitol	-	-	-
N-Acetylglucosamine	-	-	-	Potassium gluconate	+	+	+
Amygdalin	-	-	-	Potassium 2-ketogluconate	-	-	-
Arbutin	-	-	-	Potassium 5-ketogluconate	-	-	-

4. References

None.

Annex 2
Strain Receipts

ATCC® Credible leads to Incredible™

Receipt in the Case of an Original Deposit (BP/4)

THIS FORM IS ISSUED BY THE ATCC® PATENT DEPOSITORY PURSUANT TO THE BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE ("BUDAPEST TREATY"), RULE 7.1 IN ACCEPTANCE OF PATENT DEPOSIT(S) BELOW.

Depositor: **Arvind Kumar, Dharanesh Gangaiah, Dwi Susanti, Alyssa Volaind, Shrinivasrao Mane, Elanco Animal Health**

Depositor Address: **2500 Innovation Way, Greenfield, IN 46140**

Date of Original Deposit: **19JUN2020**

Date of Request for Conversion: **Not applicable**

Date of Deposit Transfer: **Not applicable**

Scientific Description and/or Proposed Taxonomic Designation	Name Designation	Patent Deposit Number
Bacillus amyloliquefaciens ☐ Scientific Description Proposed ☒ Taxonomic Designation	ELA191024	PTA-126784
Bacillus amyloliquefaciens ☐ Scientific Description Proposed ☒ Taxonomic Designation	ELA191036	PTA-126785
Bacillus subtilis ☐ Scientific Description Proposed ☒ Taxonomic Designation	ELA191105	PTA-126786
Lactobacillus reuteri ☐ Scientific Description Proposed ☒ Taxonomic Designation	3630	PTA-126787
Lactobacillus reuteri ☐ Scientific Description Proposed ☒ Taxonomic Designation	3632	PTA-126788

Monica Salihi

Digitally signed by Monica Salihi
Date: 2020.09.10 17:29:20 -04'00'

Monica Salihi, Patent Depository Specialist; ATCC® Patent Depository, 10801 University Boulevard, Manassas, Virginia 20110 USA

Annex 3

Virulence Factor Report

Bioinformatic identification of virulence determinants of *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788

Shrinivas Mane

Global Computational Sciences

Keywords: Limosilactobacillus reuteri, Abricate, VFDB

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Shrinivas Mane/Principal Research scientist, Global Computational Sciences	04/21/2021
[Author's name]	Date
[Author's title and Department name]	

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology and Microbiome	04/21/2021
[Reviewer's name]	Date
[Reviewer's title and Department name]	

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name]	Date
[Approver's title and Department name]	

1. Introduction

Lactic acid bacteria added into food products may act as potential reservoirs for antimicrobial resistance genes, which is considered as a safety concern. European Food Safety Agency (EFSA) strongly recommends that probiotics intended for use in human food chain be free of potential virulence factors and toxins.

2. Experimental methods

2.1. Identification of virulence determinants

Automated screening of whole genome sequences of *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788 against the Virulence Factor Database (VFDB), a comprehensive repository of known bacterial virulence factors and other putative adverse metabolites (Liu et al. 2019) was performed using Abricate version 0.9.9 (Seemann).

3. Results and Discussion

3.1. Absence of virulence factors and toxins

Both *L. reuteri* PTA-126787 (5 contigs) and PTA-126788 (7 contigs) strains were confirmed to be free of known virulence factors and/or toxins by comparing against virulence factor database (VFDB), which is an integrated comprehensive online resource database for curating information about bacterial virulence factors and/or toxins (Liu et al. 2019).

4. References

Liu, B., D. Zheng, Q. Jin, L. Chen, and J. Yang. 2019. 'VFDB 2019: a comparative pathogenomic platform with an interactive web interface', *Nucleic Acids Res*, 47: D687-D92.

Seemann, T. 'Abricate: mass screening of contigs for antimicrobial and virulence genes.'. https://github.com/tseemann/abricate.

Annex 4

Hemolytic Activity Report

Evaluation of hemolysis by *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788

Dharanesh Gangaiah

Discovery Research – Bacteriology and Microbiome

Keywords: *Limosilactobacillus reuteri*, Hemolysis, Blood agar

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology & Microbiome	04/21/2021
[Author's name] [Author's title and Department name]	Date

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Shrinivas Mane/ Principal Research scientist, Informatics	04/21/2021
[Reviewer's name] [Reviewer's title and Department name]	Date

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name] [Approver's title and Department name]	Date

1. Introduction

European Food Safety Agency (EFSA) strongly recommends screening probiotic candidates intended to be used in food products for hemolytic activity, even if they have GRAS or QPS staus.

2. Experimental methods

2.1. Bacterial growth

The *L. reuteri* strains described in this study were routinely propagated on Lactobacilli de Man Rogosa Sharpe (MRS, BD Difco) medium anaerobically at 37°C.

2.2. Hemolysis

Hemolytic activity of *L. reuteri* strains was evaluated by streaking on blood agar plates with 5% sheep blood and incubating at 37°C for 48 hours. After 48 hours, the hemolytic activity was evaluated and classified as alpha (green zones around colonies), beta (clear zones around colonies) or gamma hemolytic (no hemolysis zones around colonies).

3. Results and Discussion

3.1. Hemolysis

Bioinformatics analyses showed that our *L. reuteri* strains do not encode for potential hemolysins or similar toxins. Consistent with this data, none of the tested *L. reuteri* strains showed alpha or beta hemolytic activity when grown on blood agar plates. The strains showed gamma hemolytic activity – no hemolysis (Fig 1).

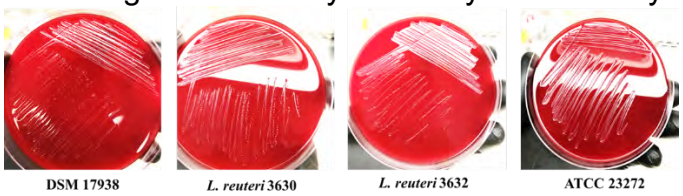

Fig 1. Hemolysis of *L. reuteri* strains on blood agar with 5% sheep blood. *L. reuteri* strains were evaluated for hemolytic activity by growing the strains on blood agar plates with 5% sheep blood. The data represent the mean ± SD from 3 independent experiments.

4. References

None

Annex 5

Biogenic Amine Production Report

Biogenic Amine Production by *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788

Dharanesh Gangaiah

Discovery Research – Bacteriology and Microbiome

Keywords: *Limosilactobacillus reuteri*, Biogenic amines, Histamine, Tyramine

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology & Microbiome	04/21/2021
[Author's name] [Author's title and Department name]	Date

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Shrinivas Mane/ Principal Research scientist, Informatics	04/21/2021
[Reviewer's name] [Reviewer's title and Department name]	Date

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name] [Approver's title and Department name]	Date

1. Introduction

Many lactic acid bacteria produce biogenic amines such as histamine, tyramine, putrescine and/or cadaverine by amino acid decarboxylation of histidine, tyrosine, ornithine and/or lysine, respectively. Toxicity from biogenic amines are rare but when reported is mostly associated with histamine and less commonly with tyramine [1-3].

2. Experimental methods

2.1. Bacterial growth

The *L. reuteri* strains described in this study were routinely propagated on Lactobacilli de Man Rogosa Sharpe (MRS, BD Difco) medium anaerobically at 37°C.

2.2. Biogenic amine production

The ability of *L. reuteri* strains to produce biogenic amines was determined as previously described [4]. Briefly, *L. reuteri* cultures were grown in MRS broth supplemented with L-tyrosine (0.1% m/v), L-histidine (0.1% m/v), L-arginine (0.1% m/v) or L-lysine (0.1% m/v) and pyridoxal-5-phosphate (0.005% m/v) under anaerobic conditions at 37°C overnight. The cultures were then plated on supplemented decarboxylase broth base as described by Bover-Cid and Holzapfel [4] and colour development was recorded after 48 hours of incubation under anaerobic conditions at 37°C.

3. Results and Discussion

3.1. Biogenic amine production

Consistent with the bioinformatics results, neither of the subject *L. reuteri* strains were able to produce the major biogenic amines histamine, tyramine, putrescine or cadaverine (Data not shown). As expected, *L. reuteri* ATCC 23272 produced a positive reaction in the area of bacterial growth on the decarboxylase base media supplemented with L-histidine. Control plates lacking these amino acids showed no positive reaction for any of the strains tested.

Genome analysis showed that our strains do not encode for any known genes encoding for histamine or tyramine production. Analysis of the strains for their ability to produce biogenic amines using decarboxylase media developed by Bover-Cid and Holzapfei [4] showed that our strains are not capable of producing histamine or tyramine. The data clearly suggest that our strains do not produce the two major biogenic amines associated with toxicity in humans - histamine, and tyramine.

Our bioinformatic search identified a CDS predicted to encode arginine deiminase in both *L. reuteri* PTA-126787 and PTA-126788. Arginine deiminase is a common enzyme

present in most lactic acid bacteria and is used to convert arginine into ornithine via citrulline and allows bacteria to adapt to non-optimal stress conditions such as acid, osmotic and temperature stresses [5]. Expectedly, a gene encoding arginine deiminase was also present in the genome of the commercially marketed *L. reuteri* strain DSM 17938 (data not shown). Our bioinformatics analysis showed that the downstream gene ornithine decarboxylase required for putrescine production is absent in the genomes of *L. reuteri* PTA-126787 and PTA-126788. Consistent with this, *in vitro* analysis of biogenic amines using decarboxylase media showed that our strains are not capable of producing putrescine using L-ornithine as a substrate. Thus, the presence of arginine deiminase may not result in production of the harmful biogenic amine putrescine.

4. References

1. Becker K, Southwick K, Reardon J, Berg R, MacCormack JN. Histamine poisoning associated with eating tuna burgers. JAMA. 2001;285(10):1327-30. Epub 2001/03/20. doi: 10.1001/jama.285.10.1327. PubMed PMID: 11255388.
2. Ohnuma S, Higa M, Hamanaka S, Matsushima K, Yamamuro W. An outbreak of allergy-like food poisoning. Intern Med. 2001;40(8):833-5. Epub 2001/08/24. doi: 10.2169/internalmedicine.40.833. PubMed PMID: 11518138.
3. Miki M, Ishikawa T, Okayama H. An outbreak of histamine poisoning after ingestion of the ground saury paste in eight patients taking isoniazid in tuberculous ward. Intern Med. 2005;44(11):1133-6. Epub 2005/12/17. doi: 10.2169/internalmedicine.44.1133. PubMed PMID: 16357449.
4. Bover-Cid S, Holzapfel WH. Improved screening procedure for biogenic amine production by lactic acid bacteria. Int J Food Microbiol. 1999;53(1):33-41. Epub 1999/12/22. doi: 10.1016/s0168-1605(99)00152-x. PubMed PMID: 10598112.
5. Vrancken G, Rimaux T, Wouters D, Leroy F, De Vuyst L. The arginine deiminase pathway of Lactobacillus fermentum IMDO 130101 responds to growth under stress conditions of both temperature and salt. Food Microbiol. 2009;26(7):720-7. Epub 2009/09/15. doi: 10.1016/j.fm.2009.07.006. PubMed PMID: 19747605.

Annex 6

Antibiotic Resistance Genes Report

Anti-Microbial Resistance Analysis of *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788

Shrinivas Mane

Global Computational Sciences

Keywords: Limosilactobacillus reuteri, Abricate, VFDB

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Shrinivas Mane/Principal Research scientist, Global Computational Sciences	04/21/2021
[Author's name]	Date
[Author's title and Department name]	

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology and Microbiome	04/21/2021
[Reviewer's name]	Date
[Reviewer's title and Department name]	

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name]	Date
[Approver's title and Department name]	

1. Introduction

Lactic acid bacteria added into food products may act as potential reservoirs for antimicrobial resistance genes, which is considered as a safety concern. European Food Safety Agency (EFSA) strongly recommends that probiotics intended for use in human food chain be free of acquired antimicrobial resistance determinants.

2. Experimental methods

2.1. Identification of antimicrobial resistance genes

Automated screening of whole genome sequences of both strains against ARG-ANNOT (1), ResFinder (2) and NCBI-AMR databases (2020-Jun-15) was performed using Abricate version 0.9.9 (3).

3. Results and Discussion

3.1. Absence of acquired antimicrobial resistance genes

The Pariza *et al*. (4) decision tree and the EFSA Panel on Additives and Products or Substances used in Animal Feed (5) recommend that microbial strains used in food applications must not harbor acquired antimicrobial resistance genes to clinically relevant antimicrobials. Search for antimicrobial resistance genes was carried out for both *L. reuteri* PTA-126787 (5 contigs) and PTA-126788 (7 contigs) strains by comparing the genomes against multiple AMR databases including NCBI-AMR, Resfinder DB and ARG-ANNOT using Abricate. The screening identified tetracycline-resistant ribosomal protection protein (*tetW*) that confers resistance to tetracycline as one potential gene of health concern (**Table**) (4).

Table 1. Predicted antimicrobial resistance genes in *L. reuteri* strains PTA-126787 and PTA-126788.

Strain	Chromosome	Location	Size (bp)	Identity (%)	Coverage (%)	Annotation
PTA-126787	IU404_1	723,894 - 725,813	1920	100	99.96	Tet(W)
PTA-126788	IVR12_1	1,090,027 - 1,091,946	1920	100	99.96	Tet(W)

Genome analysis identified the presence of *tetW* in both *L. reuteri* strains. *tetW* was found to be present on the chromosome. No elements indicative of horizontal transfer (plasmids, phages, transposons, or conjugation elements) were identified in the 15-kb flanking regions on both sides of *tetW.* No AMR genes were identified on contigs representing plasmid sequences.

4. References

1. Gupta, S. K., B. R. Padmanabhan, S. M. Diene, R. Lopez-Rojas, M. Kempf, L. Landraud, and J. M. Rolain. 2014. 'ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes', *Antimicrob Agents Chemother*, 58: 212-20.
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3. Seemann, T. 'Abricate: mass screening of contigs for antimicrobial and virulence genes.'. https://github.com/tseemann/abricate.
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5. Rychen, Guido, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Boet Glandorf, Lieve Herman, Sirpa Kärenlampi, Jaime Aguilera, Montserrat Anguita, Rosella Brozzi, and Jaume Galobart. 2018. 'Guidance on the characterisation of microorganisms used as feed additives or as production organisms', *EFSA Journal*, 16: e05206.

Annex 7

Antimicrobial Susceptibility Report

Antimicrobial Susceptibility of *Limosilactobacillus reuteri* strains PTA-126787 and PTA-126788

Dharanesh Gangaiah

Discovery Research – Bacteriology and Microbiome

Keywords: *Limosilactobacillus reuteri*, Antimicrobial susceptibility

Review and Approval Signatures

Author(s) Review: I confirm that the information in this technical report is clearly written, accurate, accurately referenced, and that all of the requirements for writing a technical report have been met.

Dharanesh Gangaiah/ Senior Research scientist, Discovery Bacteriology & Microbiome	04/21/2021
[Author's name]	Date
[Author's title and Department name]	

Technical Review: I confirm that sufficient information and detail have been reported in this technical report, that it is scientifically sound, and that appropriate conclusions have been included.

Shrinivas Mane/ Principal Research scientist, Informatics	04/21/2021
[Reviewer's name]	Date
[Reviewer's title and Department name]	

Approval: I have reviewed this technical report and confirm that the listed author(s) are appropriate, the required reviews have been completed, and the reviewers are qualified to complete their activities.

Arvind Kumar/ Senior Director, Discovery Bacteriology and Microbiome	04/21/2021
[Approver's name]	Date
[Approver's title and Department name]	

1. Introduction

Lactic acid bacteria added into food products may act as potential reservoirs for antimicrobial resistance genes, which is considered as a safety concern. European Food Safety Agency (EFSA) strongly recommends that probiotics intended for use in human food chain be free of acquired antimicrobial resistance determinants.

2. Experimental methods

2.1. Bacterial growth

The *L. reuteri* strains described in this study were routinely propagated on Lactobacilli de Man Rogosa Sharpe (MRS, BD Difco) medium anaerobically at 37°C.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using broth microdilution method, using LSB medium (Mueller Hinton broth containing 5% horse blood) following Clinical and Laboratory Standards Institute (CLSI) guidelines. Two-fold dilutions of the clinically relevant antibiotics (Clindamycin, Chloramphenicol, Erythromycin, Gentamicin, Kanamycin, Streptomycin, Tetracycline and Ampicillin, all purchased from Sigma Aldrich) were prepared in LSB medium. Approximately, 50 µL of 1 × 105 CFUs/mL of the L. reuteri cells were added into each well. “No antibiotic” and “medium” alone controls were included. *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumonia* ATCC 49619 and *Lacticaseibacillus paracasei* ATCC 334 were used as quality control organisms. The plates were incubated for 24-48 hours under microaerophilic conditions. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that showed complete inhibition of L. reuteri growth. The strains were classified as susceptible or resistant using the microbiological cut offs established by EFSA [1].

3. Results and Discussion

3.1. Antimicrobial susceptibility

Minimum inhibitory concentrations were analyzed against relevant antibiotics according to EFSA guidelines (EFSA Panel on Additives and Products or Substances used in Animal Feed) [1], including Ampicillin, Vancomycin, Gentamicin, Kanamycin, Streptomycin, Erythromycin, Clindamycin, Tetracycline and Chloramphenicol. *L. reuteri* PTA-126788 and PTA-126787 strains were determined to be sensitive to all relevant tested antibiotics according to EFSA guidelines [1], with MIC values at or below the reported species characteristic cut-off values (Table 1), except for tetracycline. For tetracycline, the MIC values for our strains were two-fold dilution above the EFSA microbiological cut off value,

in one of the two biological replicates. However, this is considered acceptable due to the technical variation of the phenotypic method as recognized previously [2].

Table 1. Susceptibility of L. reuteri PTA-126787 and PTA-126788 to EFSA Critically Important Antibiotics.

	***L. reuteri* PTA-126788**	***L. reuteri* PTA-126787**	**EFSA microbiological cut off values for *L. reuteri***
Clindamycin	≤0.06	≤0.06	4
Chloramphenicol	2	2	4
Erythromycin	0.12	0.12	1
Gentamicin	1	1	8
Kanamycin	16	16	64
Streptomycin	8	8	64
Tetracycline	32/64	32/64	32
Ampicillin	1	1	2

Genome analysis identified the presence of *tetW* in both *L. reuteri* strains. *tetW* was found to be present on the chromosome and no elements indicative of horizontal transfer (plasmids, phages, transposons, or conjugation elements) were identified in the 15-kb flanking regions on both sides of *tetW* (data not shown). Phenotypic analysis showed that the two strains are susceptible to all clinically relevant antimicrobials with MICs below the EFSA recommended microbiological cut offs, except for tetracycline. For tetracycline, both strains showed a marginal 2-fold increase in MIC than the recommended microbiological cut off and a 2-fold variation in the MIC is considered acceptable due to technical variation in the MIC assay and hence the strains can be considered phenotypically susceptible [2, 3]. Together, these data suggest that the presence of *tetW* in our *L. reuteri* poses minimal risk to human and animal health.

4. References

1. Pariza, M. W., K. O. Gillies, S. F. Kraak-Ripple, G. Leyer, and A. B. Smith. 2015. 'Determining the safety of microbial cultures for consumption by humans and animals', *Regul Toxicol Pharmacol*, 73: 164-71.
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Annex 8
Antibiotic Production Reports

DETERMINATION OF ANTIMICROBIAL PROPERTIES OF *Lactobacillus reuteri* CMG3630

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

FINAL LABORATORY REPORT

Study Sponsor:

Elanco Animal Health
2500 Innovation Way
Greenfield, IN 46140
U.S.A.

Signature Page
(Page 1 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3630

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

FINAL STUDY REPORT

This protocol has been reviewed and approved by:

[redacted] 07/12/2021

Dharanesh Mahimapura Gangaiah
Person Responsible/Study Monitor
Elanco Animal Health

Date

Signature Page
(Page 2 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3630

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

FINAL STUDY REPORT

This protocol has been reviewed and approved by:

09JUL2021

Donald J. Bade
Primary Investigator
Microbial Research Incorporated

Date

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Executive Summary

A. Objective(s)/Purpose

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3630 provided by Elanco Animal Health Inc.

B. Study Overview

Three supernatant lots from culture of the CMG3630 *Lactobacillus reuteri* strain were tested against ATCC strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus circulans, Streptococcus pyogenes* and *Serratia marcescens*. Each organism was inoculated into molten trypticase agar and then was overlayed onto solidified TSA agar and allowed to harden. A paper disk saturated with the supernatant was placed onto the agar, allowed to incubate at 2-8°C overnight and then incubated overnight at 36±2°C. The plates were observed for zones of inhibition.

C. Results

No zones of inhibition were observed for CMG3630 strain culture supernatant lots or the sterile distilled water control.

D. Conclusions

There are no substances that are inhibitory to the organisms tested in CMG3630 *Lactobacillus reuteri* strain culture supernatants from Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF or Lot# 2243324-2021-06-23-3GF when tested according to the protocol.

1.0. Personnel

Person Responsible for Study / Sponsor's Monitor	
Dharanesh Mahimapura Gangaiah, Ph.D.	Elanco Animal Health 2500 Innovation Way Greenfield, IN 46140, U.S.A. Phone: (202) 631-6459 Email: dharanesh.mahimapura_gangaiah@elancoah.com
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Investigator	
Donald J. Bade	Microbial Research Incorporated (MRI) 2290 East Prospect Road Suite #1 Fort Collins, Colorado 80525 Phone: 970.221.4695 Fax: 970.221.0916
Archives – for source data, protocol, final report	
Responsible for archiving the final report and all source data	Elanco Animal Health Bldg. B 2500 Innovation Way Greenfield, Indiana 46140

2.0. Objective

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3630 provided by Elanco Animal Health Inc.

3.0. Materials and Methods

3.1. Test Article

Three independent CMG3630 strain culture supernatant lots including Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF and Lot# 2243324-2021-06-23-3GF were provided to MRI. The lots were received frozen from ELANCO Animal Health on June 24, 2021. The tubes were thawed and stored at 2-8°C until used.

3.2. Absence of Antimicrobial Production

Each of the three independent supernatant lots was tested as requested by Elanco.

3.3. Preparation of Culture Plates

The following six organisms were tested against each supernatant:

Table 1. List of Test Organisms and Dilutions

Organism	ATCC number	MRI code	Dilution tested
Staphylococcus aureus	6538	Sta 11	1:10
Escherichia coli	11229	EC 96	1:10
Bacillus cereus	2	BC 5	1:10
Bacillus circulans	4516	Bi 1	1:10
Streptococcus pyogenes	12344	Str 59	1:20
Serratia marcescens	14041	SM 4	1:10

1. Each organism was inoculated onto a trypticase soy agar (TSA) plate. The agar plates were incubated aerobically overnight at 36±2°C.
2. From the overnight TSA culture, a trypticase soy broth (TSB) was inoculated for each culture and incubated aerobically overnight at 36±2°C. This culture was used the same day as incubation was complete.
3. On the day of testing, TSA was prepared and allowed to cool to 44-49°C in a water bath until needed.
4. Uninoculated TSA was dispensed into petri dishes and allowed to harden.
5. Aliquots of TSA (9 mL and 9.5 mL) were dispensed into separate tubes and held in a 44-49°C water bath.
6. The organism, at the dilution in Table 1, was inoculated into the molten agar (i.e., 1 mL into 9 mL TSA for all but *Streptococcus pyogenes*, or 0.5 mL into 9.5 mL TSA for *Streptococcus pyogenes*), mixed and overlayed on the hardened TSA in the petri dish.
7. The agar was allowed to solidify.

3.4. Disk Preparation

1. Paper disks that were 12.7 mm in diameter (e.g., Fisher catalog number 09-927-541) were sterilized.
2. For each lot of the supernatant, 20µL was applied to at least 6 disks. The amount of material was enough to saturate the disk, but was not excessive.
3. A single disk of each lot of each supernatant, and a control disk, was place onto each of the inoculated agar plates for each organism. Multiple disks were placed onto a single plate.

3.5. Incubation

1. The inoculated agar plates were held aerobically at 2-8°C overnight without inverting.

2. After overnight 2-8°C incubation, the agar plates were incubated overnight at 36±2°C without inversion.

3.6. Interpretation

The plates were observed for a clear zone around the disk and if present, the zone diameter was recorded to the nearest 0.1 mm. If no zone was present, the zone was recorded as ≤12.7 mm. A visually clear zone around a disk with a diameter of ≥16 mm was interpreted to have the presence of antibacterial components in the supernatant. A supernatant that showed obvious antibacterial activity against three (or more) organisms was concluded that antimicrobial agents were present. Activity against less than three organisms was interpreted as insignificant activity.

3.7. Quality Control

A sterile disk was saturated with sterile distilled water according the procedure listed above and tested against each organism to assure no activity is observed for the test system.

As a positive control, a disk containing 20 µL of a 200 µg/mL solution of enrofloxacin was tested according to the procedures listed above and tested against each organism to confirm that the system would produce a zone of inhibition.

3.8. Supernatant Sterility Testing

The sterility of the provided supernatant was verified prior to use by streaking a loopful of the supernatant onto trypticase soy agar containing 5% sheep blood (BA) and incubating overnight at 36±2°C.

4.0. Disposition of the Research Material

All research material will be disposed using standard operational procedures (SOP) of autoclaving for the Microbiology Laboratory following approval of the final report.

5.0. Results

Each of the provided supernatants was sterile prior to testing.

No zones of inhibition were observed for the CMG3630 strain culture supernatant lots or the sterile distilled water control, as indicated in the table below:

Table 2. Zone Diameters from CMG3630 Supernatant and Control

Test Article Description	Test Article Lot number	Zone Diameter for the indicated organism (mm)					
		Staphylococcus aureus	*Escherichia coli*	*Bacillus cereus*	*Bacillus circulans*	*Streptococcus pyogenes*	*Serratia marcescens*
		ATCC 6538	ATCC 11129	ATCC 2	ATCC 4516	ATCC 12344	ATCC 14041
		MRI Sta 11	MRI EC 96	MRI BC -5	MRI Bi 1	MRI Str 59	MRI SM 4
CMG3630 Supernatant	2243324-2021-06-23-1GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
	2243324-2021-06-23-2GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
	2243324-2021-06-23-3GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
Enrofloxacin	N/A	33.0	37.7	38.9	38.2	27.0	29.8
Sterile Water	N/A	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7

Following incubation, pictures were taken of each organism seeded into the agar onto which a saturated disk of each lot of CMG3630 strain culture supernatant, sterile water and enrofloxacin controls were placed according to the protocol. No zones of inhibition were observed for any of the provided supernatant lots. Zones of inhibition were observed for the enrofloxacin controls for all organisms.

6.0. Conclusion

There are no substances that are inhibitory to the organisms tested in CMG3630 *Lactobacillus reuteri* strain culture supernatants from Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF or Lot# 2243324-2021-06-23-3GF when tested according to the protocol.

7.0. Appendix 1: Protocol

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3630

Protocol

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

Study Sponsor:

ELANCO US, Inc.
2500 Innovation Way
Greenfield, IN 46140
U.S.A.

Signature Page
(Page 1 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3630

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

Protocol

This protocol has been reviewed and approved by:

[redacted] 06/23/2021

Dharanesh Mahimapura Gangaiah
Person Responsible/Study Monitor
Elanco Animal Health

Date

Signature Page
(Page 2 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3630

Elanco Study Number: MR2120-1

MRI Study Number: MR2120-1

Protocol

This protocol has been reviewed and approved by:

[redacted] 23JUN2021

Donald J. Bade
Primary Investigator
Microbial Research Incorporated

Date

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1.0. Personnel

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Sponsor Quality Assurance (QA)	
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Investigator	
Donald J. Bade	Microbial Research Incorporated (MRI) 2290 East Prospect Road Suite #1 Fort Collins, Colorado 80525 Phone: 970.221.4695 Fax: 970.221.0916
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2.0. Objective

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3630 provided by Elanco Animal Health Inc.

3.0. Materials and Methods

3.1. Test Article

Three independent CMG3630 strain culture supernatant lots including Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF and Lot# 2243324-2021-06-23-3GF. The test article will be kept under 2-8°C until it is required for the testing.

3.2. Absence of Antimicrobial Production

Each of the three independent lots of supernatant will be tested as requested by Elanco.

3.3. Preparation of Culture Plates

The following six organisms will be tested against each supernatant:

Organism	ATCC number	MRI code	Dilution tested
Staphylococcus aureus	6538	Sta 11	1:10
Escherichia coli	11229	EC 96	1:10
Bacillus cereus	2	BC 5	1:10
Bacillus circulans	4516	Bi 1	1:10
Streptococcus pyogenes	12344	Str 59	1:20
Serratia marcescens	14041	SM 4	1:10

1. Inoculate each organism onto a trypticase soy agar (TSA) plate. Incubate the agar plate overnight at 36±2°C. Hold the culture at 2-8°C for up to 7 days.
2. From the overnight TSA culture, inoculate a trypticase soy broth (TSB) of each culture. Incubate the inoculated TSB overnight at 36±2°C. Use this culture the same day as incubation is complete.

   On the day of testing, prepare >350 mL of TSA and allow to cool to 44-49°C in a water bath until needed.
3. Dispense 15 mL of uninoculated TSA into a minimum of 12 petri dishes. Allow to harden.
4. Dispense a minimum of ten 9 mL and two 9.5 mL aliquots of TSA into separate tubes and hold in a 44-49°C water bath.
5. Dilute the organism at the dilution in the above table into the molten agar (i.e., 1 mL into 9 mL TSA for all but *Streptococcus pyogenes*, or 0.5 mL into 9.5 mL TSA for *Streptococcus pyogenes*), mix and overlay the hardened agar in the petri dish.
6. Allow to solidify.

3.4. Disk Preparation

1. Sterilize paper disks that are 12.7 mm in diameter (e.g., Fisher catalog number 09-927-541).
2. Apply 20µL of the supernatant dilution to at least 6 disks. The amount of material should be enough to saturate the disk, but not in excess. Adjust the amount of material accordingly and apply to all disks.
3. Place a single disk of the supernatant on each of the 6 inoculated agar plates. Multiple disks may be placed onto a single plate.

3.5. Incubation

1. Hold the inoculated agar plates at 2-8°C overnight without inverting.
2. After overnight 2-8°C incubation, incubate the plates overnight at 36±2°C without inversion.

3.6. Interpretation

Examine the plates for a clear zone around the disk. If a zone is present, record the diameter of the zone of inhibition to the nearest 0.1 mm. A visually clear zone around a disk with a diameter of ≥16 mm indicates the presence of antibacterial components in the supernatant. If a supernatant shows obvious antibacterial activity against three (or more) organisms, it is concluded that antimicrobial agents are present. Activity against less than three organisms will be interpreted as insignificant activity.

Prepare a picture of each plate using a digital camera.

3.7. Quality Control

A sterile disk will be saturated with sterile distilled water according the procedure listed above and tested against each organism to assure no activity is observed for the test system.

As a positive control, a disk containing 20µL of a 200 µg/mL solution of enrofloxacin will be tested according to the procedures listed above and tested against each organism to confirm that the system would produce a zone of inhibition.

4.0. Disposition of the Research Material

All research material will be disposed using standard operational procedures (SOP) of autoclaving for the Microbiology Laboratory. The method of disposal will be documented in the final report.

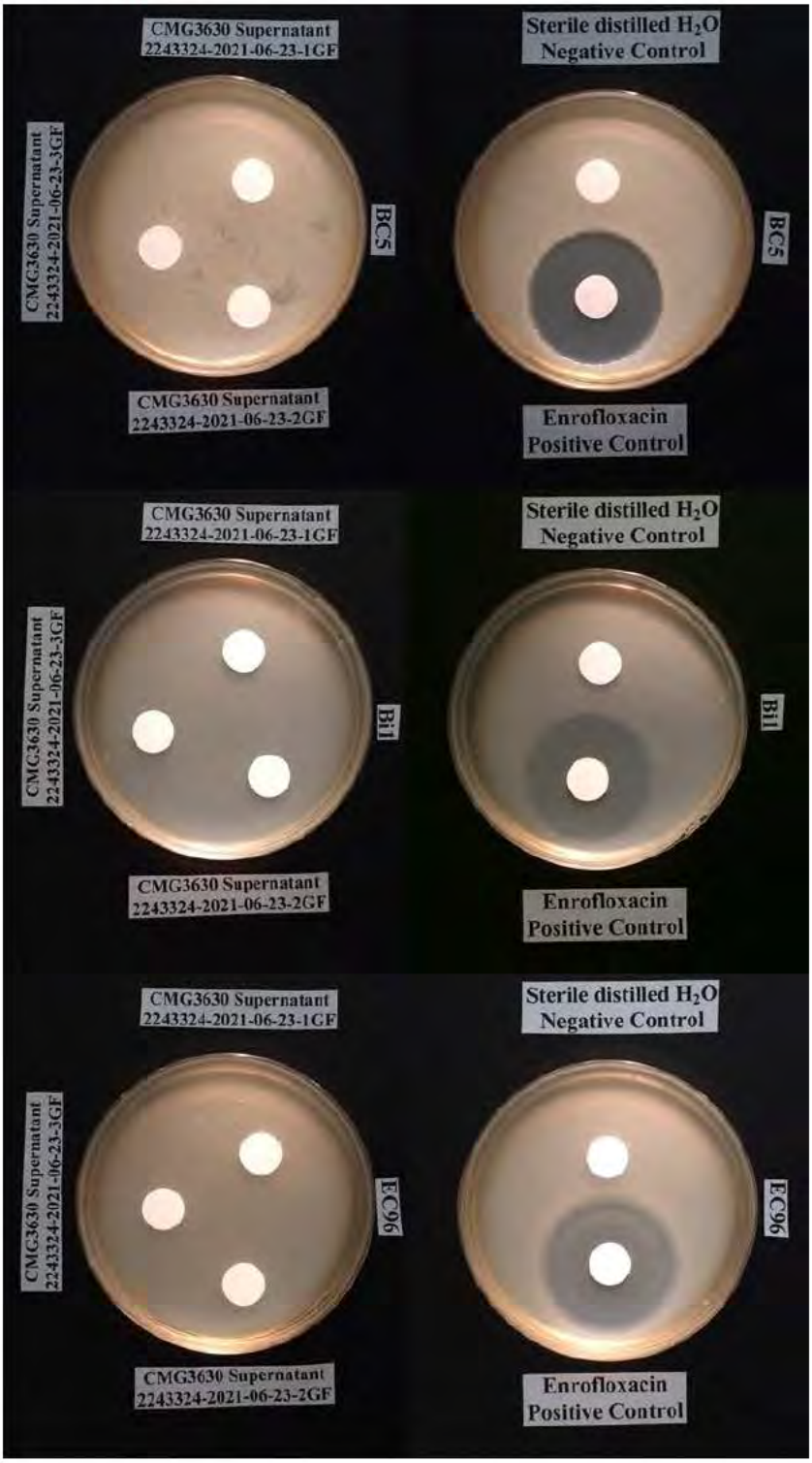
5.0. Reporting of Results

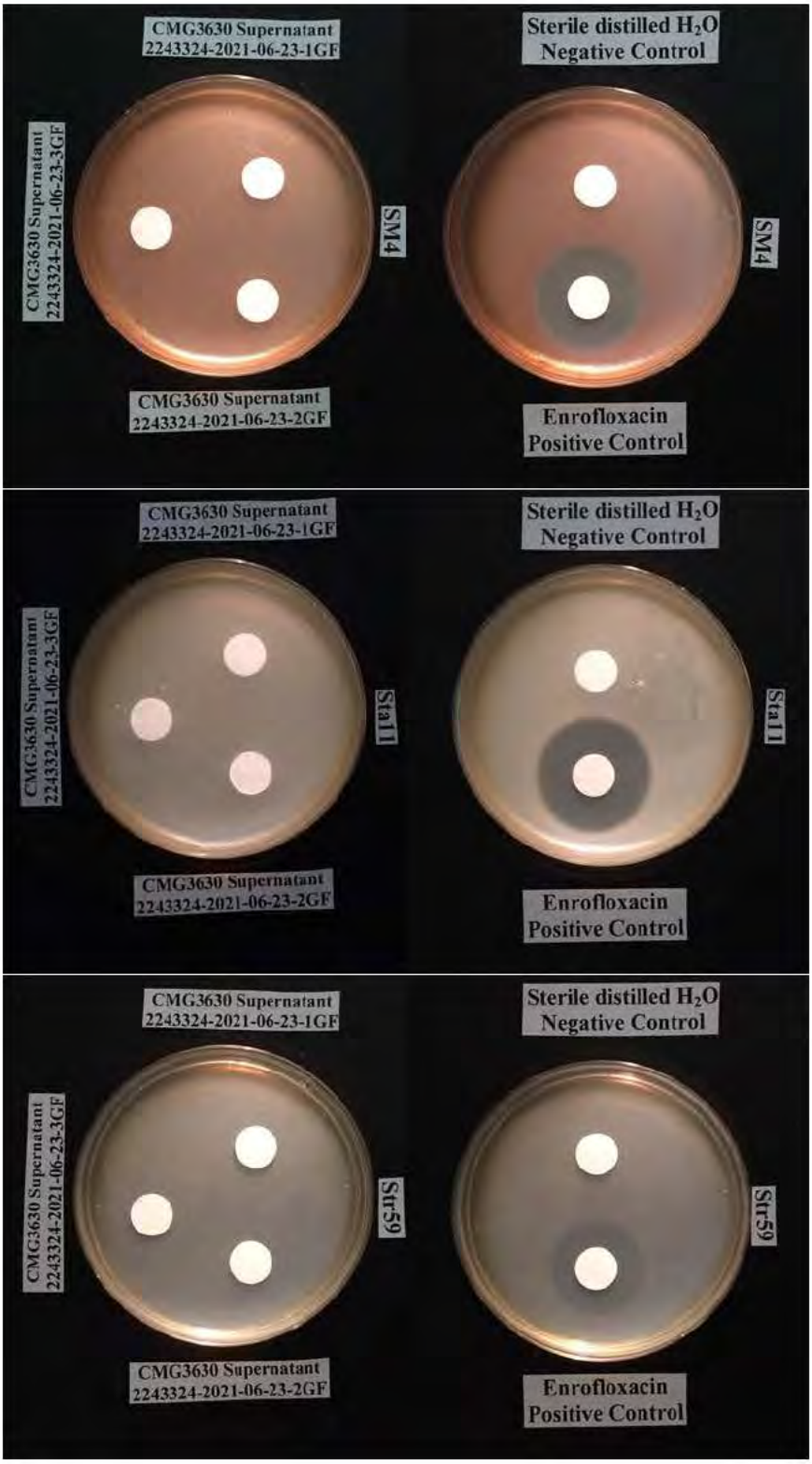
A report will be prepared by the Investigator.

6.0. Supernatant Sterility Testing

The sterility of the provided supernatant will be verified prior to use by streaking a loopful of the supernatant onto trypticase soy agar containing 5% sheep blood (BA) and incubating overnight at 36±2°C. Should contamination be observed, a portion of the supernatant will be filtered through a 0.22µm syringe filter and retested for sterility. Testing will be performed with a sterile supernatant.

8.0. Appendix 2: Photos





DETERMINATION OF ANTIMICROBIAL PROPERTIES OF *Lactobacillus reuteri* CMG3632

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

FINAL LABORATORY REPORT

Study Sponsor:

Elanco Animal Health
2500 Innovation Way
Greenfield, IN 46140
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Signature Page
(Page 1 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3632

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

FINAL STUDY REPORT

This protocol has been reviewed and approved by:

[redacted] 07/12/2021

Dharanesh Mahimapura Gangaiah
Person Responsible/Study Monitor
Elanco Animal Health

Date

Signature Page
(Page 2 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3632

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

FINAL STUDY REPORT

This protocol has been reviewed and approved by:

[redacted] 09JUL2021

Donald J. Bade
Primary Investigator
Microbial Research Incorporated

Date

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Executive Summary

A. Objective(s)/Purpose

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3632 provided by Elanco Animal Health Inc.

B. Study Overview

Three supernatant lots from culture of the CMG3632 *Lactobacillus reuteri* strain were tested against ATCC strains of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Bacillus circulans, Streptococcus pyogenes* and *Serratia marcescens*. Each organism was inoculated into molten trypticase agar and then was overlayed onto solidified TSA agar and allowed to harden. A paper disk saturated with the supernatant was placed onto the agar, allowed to incubate at 2-8°C overnight and then incubated overnight at 36±2°C. The plates were observed for zones of inhibition.

C. Results

No zones of inhibition were observed for CMG3632 strain culture supernatant lots or the sterile distilled water control.

D. Conclusions

There are no substances that are inhibitory to the organisms tested in CMG3632 *Lactobacillus reuteri* strain culture supernatants from Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF or Lot# 2243324-2021-06-23-3GF when tested according to the protocol.

1.0. Personnel

Person Responsible for Study / Sponsor's Monitor	
Dharanesh Mahimapura Gangaiah, Ph.D.	Elanco Animal Health 2500 Innovation Way Greenfield, IN 46140, U.S.A. Phone: (202) 631-6459 Email: dharanesh.mahimapura_gangaiah@elancoah.com
Sponsor Quality Assurance (QA)	
Jeffrey Voors	Elanco Animal Health 2500 Innovation Way Greenfield, IN 46140, U.S.A. Phone: (317) 408-5595 Email: voors.jeffrey.a@elancoah.com
Investigator	
Donald J. Bade	Microbial Research Incorporated (MRI) 2290 East Prospect Road Suite #1 Fort Collins, Colorado 80525 Phone: 970.221.4695 Fax: 970.221.0916
Archives – for source data, protocol, final report	
Responsible for archiving the final report and all source data	Elanco Animal Health Bldg. B 2500 Innovation Way Greenfield, Indiana 46140

2.0. Objective

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3632 provided by Elanco Animal Health Inc.

3.0. Materials and Methods

3.1. Test Article

Three independent CMG3632 strain culture supernatant lots including Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF and Lot# 2243324-2021-06-23-3GF were provided to MRI. The lots were received frozen from ELANCO Animal Health on June 24, 2021. The tubes were thawed and stored at 2-8°C until used.

3.2. Absence of Antimicrobial Production

Each of the three independent supernatant lots was tested as requested by Elanco.

3.3. Preparation of Culture Plates

The following six organisms were tested against each supernatant:

Table 1. List of Test Organisms and Dilutions

Organism	ATCC number	MRI code	Dilution tested
Staphylococcus aureus	6538	Sta 11	1:10
Escherichia coli	11229	EC 96	1:10
Bacillus cereus	2	BC 5	1:10
Bacillus circulans	4516	Bi 1	1:10
Streptococcus pyogenes	12344	Str 59	1:20
Serratia marcescens	14041	SM 4	1:10

1. Each organism was inoculated onto a trypticase soy agar (TSA) plate. The agar plates were incubated aerobically overnight at 36±2°C.
2. From the overnight TSA culture, a trypticase soy broth (TSB) was inoculated for each culture and incubated aerobically overnight at 36±2°C. This culture was used the same day as incubation was complete.
3. On the day of testing, TSA was prepared and allowed to cool to 44-49°C in a water bath until needed.
4. Uninoculated TSA was dispensed into petri dishes and allowed to harden.
5. Aliquots of TSA (9 mL and 9.5 mL) were dispensed into separate tubes and held in a 44-49°C water bath.
6. The organism, at the dilution in Table 1, was inoculated into the molten agar (i.e., 1 mL into 9 mL TSA for all but *Streptococcus pyogenes*, or 0.5 mL into 9.5 mL TSA for *Streptococcus pyogenes*), mixed and overlayed on the hardened TSA in the petri dish.
7. The agar was allowed to solidify.

3.4. Disk Preparation

1. Paper disks that were 12.7 mm in diameter (e.g., Fisher catalog number 09-927-541) were sterilized.
2. For each lot of the supernatant, 20µL was applied to at least 6 disks. The amount of material was enough to saturate the disk, but was not excessive.
3. A single disk of each lot of each supernatant, and a control disk, was place onto each of the inoculated agar plates for each organism. Multiple disks were placed onto a single plate.

3.5. Incubation

1. The inoculated agar plates were held aerobically at 2-8°C overnight without inverting.

2. After overnight 2-8°C incubation, the agar plates were incubated overnight at 36±2°C without inversion.

3.6. Interpretation

The plates were observed for a clear zone around the disk and if present, the zone diameter was recorded to the nearest 0.1 mm. If no zone was present, the zone was recorded as ≤12.7 mm. A visually clear zone around a disk with a diameter of ≥16 mm was interpreted to have the presence of antibacterial components in the supernatant. A supernatant that showed obvious antibacterial activity against three (or more) organisms was concluded that antimicrobial agents were present. Activity against less than three organisms was interpreted as insignificant activity.

3.7. Quality Control

A sterile disk was saturated with sterile distilled water according the procedure listed above and tested against each organism to assure no activity is observed for the test system.

As a positive control, a disk containing 20 µL of a 200 µg/mL solution of enrofloxacin was tested according to the procedures listed above and tested against each organism to confirm that the system would produce a zone of inhibition.

3.8. Supernatant Sterility Testing

The sterility of the provided supernatant was verified prior to use by streaking a loopful of the supernatant onto trypticase soy agar containing 5% sheep blood (BA) and incubating overnight at 36±2°C.

4.0. Disposition of the Research Material

All research material will be disposed using standard operational procedures (SOP) of autoclaving for the Microbiology Laboratory following approval of the final report.

5.0. Results

Each of the provided supernatants was sterile prior to testing.

No zones of inhibition were observed for the CMG3632 strain culture supernatant lots or the sterile distilled water control, as indicated in the table below:

Table 2. Zone Diameters from CMG3632 Supernatant and Control

Test Article Description	Test Article Lot number	Zone Diameter for the indicated organism (mm)					
		Staphylococcus aureus	*Escherichia coli*	*Bacillus cereus*	*Bacillus circulans*	*Streptococcus pyogenes*	*Serratia marcescens*
		ATCC 6538	ATCC 11129	ATCC 2	ATCC 4516	ATCC 12344	ATCC 14041
		MRI Sta 11	MRI EC 96	MRI BC -5	MRI Bi 1	MRI Str 59	MRI SM 4
CMG3632 Supernatant	2243324-2021-06-23-1GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
	2243324-2021-06-23-2GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
	2243324-2021-06-23-3GF	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7
Enrofloxacin	N/A	33.0	37.7	38.9	38.2	27.0	29.8
Sterile Water	N/A	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7	≤12.7

Following incubation, pictures were taken of each organism seeded into the agar onto which a saturated disk of each lot of CMG3632 strain culture supernatant, sterile water and enrofloxacin controls were placed according to the protocol. No zones of inhibition were observed for any of the provided supernatant lots. Zones of inhibition were observed for the enrofloxacin controls for all organisms.

6.0. Conclusion

There are no substances that are inhibitory to the organisms tested in CMG3632 *Lactobacillus reuteri* strain culture supernatants from Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF or Lot# 2243324-2021-06-23-3GF when tested according to the protocol.

7.0. Appendix 1: Protocol

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3632

Protocol

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

Study Sponsor:

ELANCO US Inc.
2500 Innovation Way
Greenfield, IN 46140
U.S.A.

Signature Page
(Page 1 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3632

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

Protocol

This protocol has been reviewed and approved by:

[redacted] 06/23/2021

Dharanesh Mahimapura Gangaiah — Date
Sponsor Management
Elanco Animal Health

Signature Page
(Page 2 of 2)

Determination of Antimicrobial Properties of *Lactobacillus reuteri* CMG3632

Elanco Study Number: MR2120-2

MRI Study Number: MR2120-2

Protocol

This protocol has been reviewed and approved by:

[redacted]

Donald J. Bade
Primary Investigator
Microbial Research Incorporated

23JUN2021
Date

Table of Contents

1.0. Personnel

Person Responsible for Study / Sponsor's Monitor	
Dharanesh Mahimapura Gangaiah, Ph.D.	Elanco Animal Health 2500 Innovation Way Greenfield, IN 46140, U.S.A. Phone: (202) 631-6459 Email: dharanesh.mahimapura_gangaiah@elancoah.com
Sponsor Quality Assurance (QA)	
Jeffrey Voors	Elanco Animal Health 2500 Innovation Way Greenfield, IN 46140, U.S.A. Phone: (317) 408-5595 Email: voors.jeffrey.a@elancoah.com
Investigator	
Donald J. Bade	Microbial Research Incorporated (MRI) 2290 East Prospect Road Suite #1 Fort Collins, Colorado 80525 Phone: 970.221.4695 Fax: 970.221.0916
Archives – for source data, protocol, final report	
Responsible for archiving the final report and all source data	Elanco Animal Health Bldg. B 2500 Innovation Way Greenfield, Indiana 46140

2.0. Objective

Determination of the Antimicrobial Properties of *Lactobacillus reuteri* CMG3632 provided by Elanco Animal Health Inc.

3.0. Materials and Methods

3.1. Test Article

Three independent CMG3632 strain culture supernatant lots including Lot# 2243324-2021-06-23-1GF, Lot# 2243324-2021-06-23-2GF and Lot# 2243324-2021-06-23-3GF. The test article will be kept under 2-8°C until it is required for the testing.

3.2. Absence of Antimicrobial Production

Each of the three independent lots of supernatant will be tested as requested by Elanco.

3.3. Preparation of Culture Plates

The following six organisms will be tested against each supernatant:

Organism	ATCC number	MRI code	Dilution tested
Staphylococcus aureus	6538	Sta 11	1:10
Escherichia coli	11229	EC 96	1:10
Bacillus cereus	2	BC 5	1:10
Bacillus circulans	4516	Bi 1	1:10
Streptococcus pyogenes	12344	Str 59	1:20
Serratia marcescens	14041	SM 4	1:10

1. Inoculate each organism onto a trypticase soy agar (TSA) plate. Incubate the agar plate overnight at 36±2°C. Hold the culture at 2-8°C for up to 7 days.
2. From the overnight TSA culture, inoculate a trypticase soy broth (TSB) of each culture. Incubate the inoculated TSB overnight at 36±2°C. Use this culture the same day as incubation is complete.

   On the day of testing, prepare >350 mL of TSA and allow to cool to 44-49°C in a water bath until needed.
3. Dispense 15 mL of uninoculated TSA into a minimum of 12 petri dishes. Allow to harden.
4. Dispense a minimum of ten 9 mL and two 9.5 mL aliquots of TSA into separate tubes and hold in a 44-49°C water bath.
5. Dilute the organism at the dilution in the above table into the molten agar (i.e., 1 mL into 9 mL TSA for all but *Streptococcus pyogenes*, or 0.5 mL into 9.5 mL TSA for *Streptococcus pyogenes*), mix and overlay the hardened agar in the petri dish.
6. Allow to solidify.

3.4. Disk Preparation

1. Sterilize paper disks that are 12.7 mm in diameter (e.g., Fisher catalog number 09-927-541).
2. Apply 20µL of the supernatant dilution to at least 6 disks. The amount of material should be enough to saturate the disk, but not in excess. Adjust the amount of material accordingly and apply to all disks.
3. Place a single disk of the supernatant on each of the 6 inoculated agar plates. Multiple disks may be placed onto a single plate.

3.5. Incubation

1. Hold the inoculated agar plates at 2-8°C overnight without inverting.
2. After overnight 2-8°C incubation, incubate the plates overnight at 36±2°C without inversion.

3.6. Interpretation

Examine the plates for a clear zone around the disk. If a zone is present, record the diameter of the zone of inhibition to the nearest 0.1 mm. A visually clear zone around a disk with a diameter of ≥16 mm indicates the presence of antibacterial components in the supernatant. If a supernatant shows obvious antibacterial activity against three (or more) organisms, it is concluded that antimicrobial agents are present. Activity against less than three organisms will be interpreted as insignificant activity.

Prepare a picture of each plate using a digital camera.

3.7. Quality Control

A sterile disk will be saturated with sterile distilled water according the procedure listed above and tested against each organism to assure no activity is observed for the test system.

As a positive control, a disk containing 20µL of a 200 µg/mL solution of enrofloxacin will be tested according to the procedures listed above and tested against each organism to confirm that the system would produce a zone of inhibition.

4.0. Disposition of the Research Material

All research material will be disposed using standard operational procedures (SOP) of autoclaving for the Microbiology Laboratory. The method of disposal will be documented in the final report.

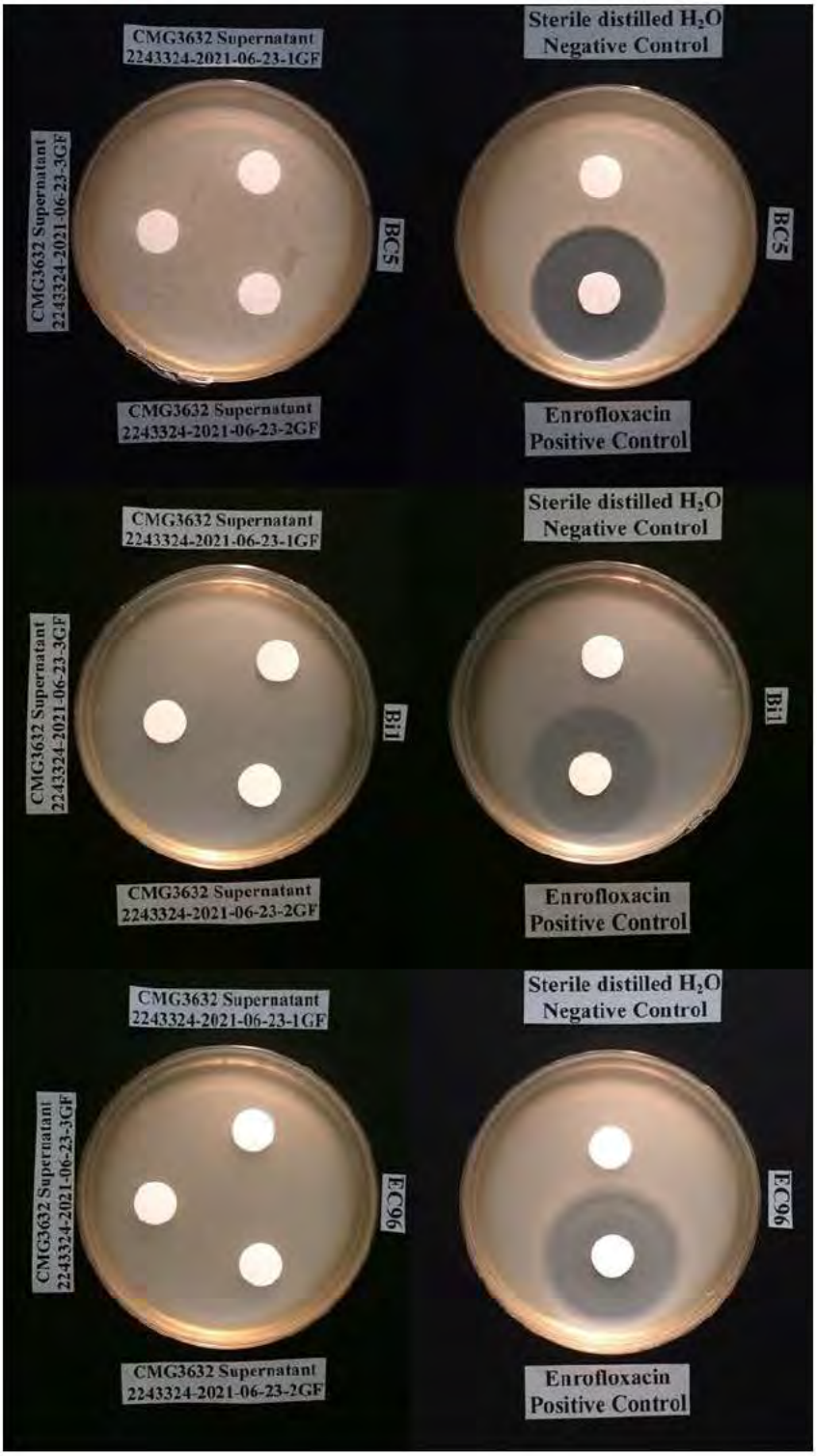
5.0. Reporting of Results

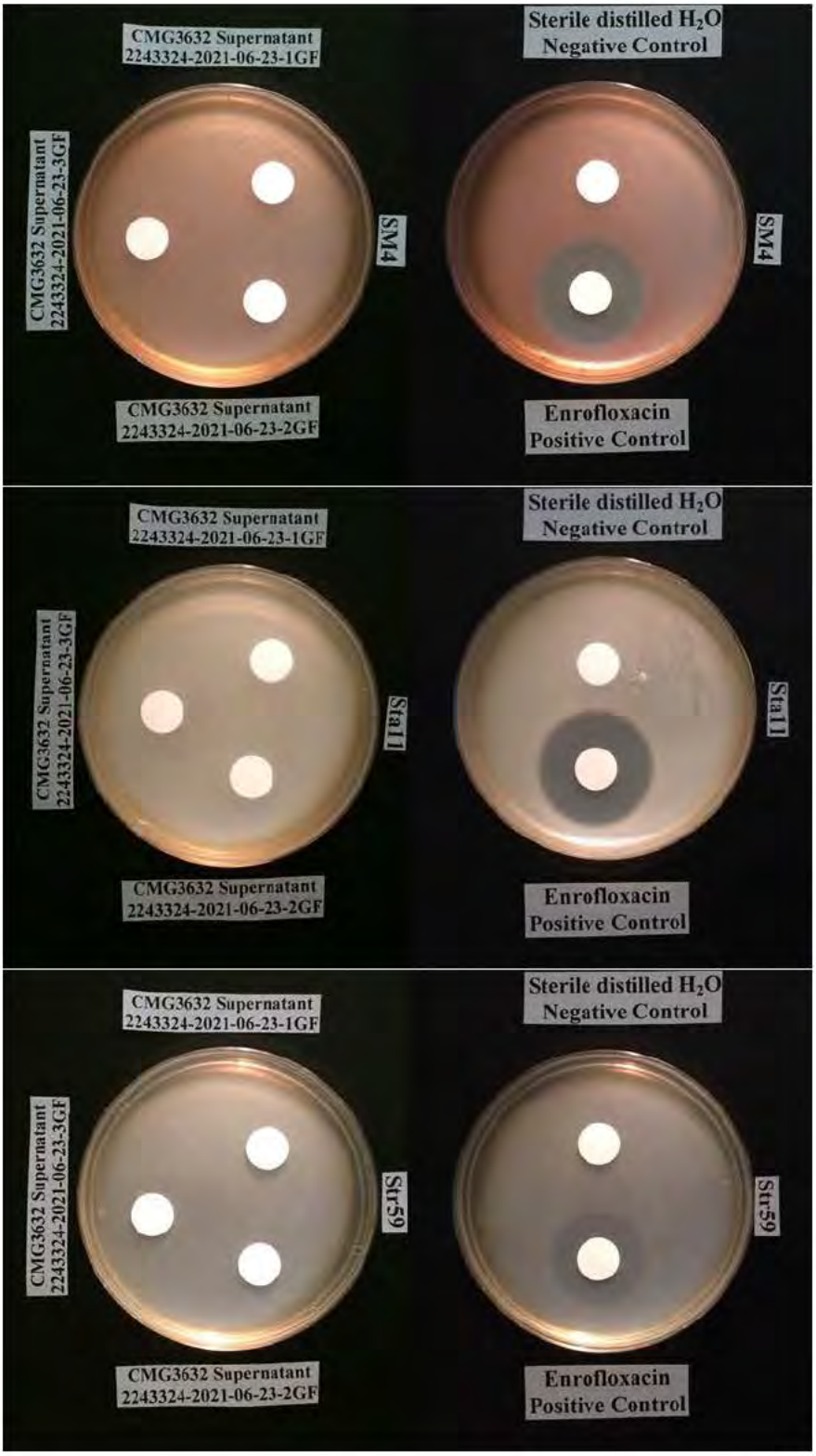
A report will be prepared by the Investigator.

6.0. Supernatant Sterility Testing

The sterility of the provided supernatant will be verified prior to use by streaking a loopful of the supernatant onto trypticase soy agar containing 5% sheep blood (BA) and incubating overnight at 36±2°C. Should contamination be observed, a portion of the supernatant will be filtered through a 0.22µm syringe filter and retested for sterility. Testing will be performed with a sterile supernatant.

8.0. Appendix 2: Photos





Annex 9
Fermentation Medium

Annex

Fermentation Medium Ingredients

Ingredient	Function	Reference for human food use
Dipotassium phosphate (K2HPO4)	Nutrient	21 CFR 182.6285
Magnesium sulfate heptahydrate (MgSO4 * 7H2O)	Nutrient	21 CFR 184.1443
Manganese sulfate monohydrate (MnSO4 * H2O)	Nutrient	21 CFR 184.1461
Pea protein	Nutrient	Generally recognized as safe for use in a variety of foods, GRN 000609
Citric acid diammonium salt	Nutrient	21 CFR 184.1140 Ammonium citrate dibasic
FNI 100 AG Yeast extract (Lallemand)	Nutrient	21 CFR 184.1983. (Bakers yeast extract)
Sucrose	Nutrient	21 CFR 184.1854.
Ferrous sulfate heptahydrate (FeSO4*7H2O)	Nutrient	21 CFR 184.1315. .
Betaine	Nutrient	FEMA GRAS No: 22
Thiamine HCl	Vitamin	21 CFR 184.1875.
D-Biotin	Vitamin	21 CFR 182.8159
Calcium hydroxide (Ca(OH)2)	Neutralizer	21 CFR 182.1205
Polysorbate 80 (from non-animal source)	Emulsifier	21 CFR 178.3400
D-Lactose monohydrate	Formulation ingredient	21 CFR 168.122
Sodium acetate trihydrate	pH Control	21 CFR 184.1721
Hydrogen chloride	pH Control	21 CFR 182.1057
Carbon dioxide		
Nitrogen	Nitrogen source	
Reverse osmosis deionized water		

Annex 10
GMP Certificate

SGS Systems & Services Certification Pty Ltd
10/585 Blackburn Road,
Notting Hill, Victoria 3168, Australia

Certificate Of Registration

BioSource Cultures & Flavors

S66 W14328 Janesville Road,
Muskego, Wisconsin, UNITED STATES, 53150

is registered as meeting the requirements of the

SQF Food Safety Code for Manufacturing, Edition 8.1

Certified HACCP Based Food Safety Plans

Certification Details:

Date of Decision: 15-Jan-2020
Date of Audit: 18-Dec-2019
Certificate Number: 35209

Date of Expiry: 03-Mar-2021
Date of Next Audit: 18-Dec-2020
Certificate Type: Initial Certification

Registration Schedule:

Scope of Registration (Food Sector Categories and Products)

19. Food Ingredient Manufacture:
Ingredient or additive (ie. fermented sausage)

31. Manufacture of Dietary Supplements:
Probiotics

SQF INSTITUTE
One world. One standard.
SQF Institute is a division of the Food Marketing Institute (FMI).

JAS-ANZ
www.jas-anz.org/register

Authorized by

Issuing Officer

Annex 11
Certificates of Analysis Strain 3630

eurofins | Food Integrity & Innovation

Report Number:	**3015021-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3037648-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3038178-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3038386-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3038687-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References

Elements by ICP Mass Spectrometry (ICP_MS_S)

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Testing Location(s)

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

Released on Behalf of Eurofins by

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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2918.01

These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Eurofins.

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Report Number:	**3039915-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3041026-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	<10 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3041219-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3043440-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-A**	**Eurofins Sample:**	**9868719**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	5.8e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**3015022-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3037649-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3038179-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038387-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038686-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References

Elements by ICP Mass Spectrometry (ICP_MS_S)

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Testing Location(s)

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

Released on Behalf of Eurofins by

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

ilac-MRA A2LA ACCREDITED
2918.01

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Report Number:	**3039916-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041027-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	10 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Madison WI 53704
800-675-8375

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Report Number:	**3041220-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3043441-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-B**	**Eurofins Sample:**	**9868720**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	6.8e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3015024-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3037651-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038181-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038389-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038683-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References

Elements by ICP Mass Spectrometry (ICP_MS_S)

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Testing Location(s)

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
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Released on Behalf of Eurofins by

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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Report Number:	**3039918-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041029-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	30 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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Madison WI 53704
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Report Number:	**3041222-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Madison WI 53704
800-675-8375

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Report Number:	**3043443-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3630-D**	**Eurofins Sample:**	**9868722**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3630-D	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	6.2e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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Madison WI 53704
800-675-8375

S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777 ● P: 414 422 9085; F: 414 422 9086 ● info@biosource.us.com

Certificate of Analysis

Date: 2/25/2021
Customer: ELANCO
Product: L. reuteri 3630
Lot #: 201123LRE3630
Lab Report #: BQL201208, 3120523-0 (Eurofins)
Purchase Order #: Email

Microbiological Analyses

Test	Method	Spec Limits	Results
Lactic Acid Bacteria Counts	BQL-LAB-001.3	Not specified	270.8 x 10^9 cfu/g
Enterococcus	CMMEF, 4th Ed	< 100 CFU/g	<10
Enterobacteriaceae	USPN 2021	< 10 cfu/g	< 10
E. coli	USPE 2022	Absent/10 g	Absent
Yeast & Mold	USPM 2021	< 100 cfu/g	< 10
CP Staphylococcus	USPA 2022	Absent/10 g	Absent
Salmonella	USPS 2022	Absent /25g	Absent
Listeria	FDA BAM Ch. 10	Not Detected /25 g	Not detected
Appearance/Color	Visual	White to Tan powder	Pass
Identification	Riboprinter	>0.85 Similarity Index	0.9
Arsenic	AOAC 2011.19 and 993.14	NMT 1 ppm	194 ppb
Cadmium	AOAC 2011.19 and 993.14	NMT 0.3 ppm	115 ppb
Lead	AOAC 2011.19 and 993.14	NMT 1 ppm	45.7 ppb
Mercury	AOAC 2011.19 and 993.14	NMT 0.05 ppm	<5.00 ppb

Manufacturing Date
11/23/2020

Best Before Date
11/23/2021

Country of Origin
US

Ingredients
Lactobacillus reuteri and no excipient

Recommended Storage Conditions
Store frozen at -20°C or colder

Approved by: Jerry Stoecklein, Quality Director

S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777 ● P: 414 422 9085; F: 414 422 9086 ● info@biosource.us.com

Rev. 3 – 9/07/21

Certificate of Analysis

Date: 9/7/2021
Customer: ELANCO
Product: L. reuteri 3630
Lot #: 210713LRE14(ET3(TRE))
Lab Report #: BQL210730, 411817173 (Merieux)
Purchase Order #: 4800018988

Microbiological and Analytical Analyses

Test	Method	Spec Limits	Results
Lactic Acid Bacteria Counts	BQL-LAB-001.3	Not specified	255.0 x 10^9 cfu/g
Non-Lactics	ISO 13559	< 5000 CFU/g	2100 cfu/g
Enterococcus	CMMEF, 4th Ed	< 100 CFU/g	<10 cfu/g
Enterobacteriaceae	USPN 62	< 10 cfu/g	< 10 cfu/g
E. coli	USPE 62	Negative/10 g	Negative/10g
Yeast & Mold	USPM 2021	< 100 cfu/g	< 10 cfu/g
CP Staphylococcus	USPA 2022	Negative/25 g	Negative/25g
Salmonella	USPS 2022	Negative /10g	Negative/10g
Listeria	FDA BAM Ch. 10	Negative /25 g	Negative/25g
Appearance/Color	Visual	White to Tan powder	Pass
Identification	Riboprinter	>0.85 Similarity Index	0.97
Arsenic	AOAC2015.01Mod<2232>	NMT 1 ppm	0.06 ppm
Cadmium	AOAC2015.01Mod<2232>	NMT 0.3 ppm	0.037 ppm
Lead	AOAC2015.01Mod<2232>	NMT 1 ppm	0.15 ppm
Mercury	AOAC2015.01Mod<2232>	NMT 0.05 ppm	<0.005 ppm

Manufacturing Date
07/13/2021

Best Before Date
07/13/2022

Country of Origin
US

Ingredients
Lactobacillus reuteri and no excipient

Recommended Storage Conditions
Store frozen at -20°C or colder

Approved by: Jerry Stoecklein, Quality Director

Annex 12
Certificates of Analysis Strain 3632

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Report Number:	**3015016-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3037642-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3037643-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Suitability of Test Method	
Staphylococcus Suitability Result	Pass**

Method References	**Testing Location**
Suitability of Test Method (USPA_SOT)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038173-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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800-675-8375

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Report Number:	**3038381-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3038692-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References | **Testing Location**

Elements by ICP Mass Spectrometry (ICP_MS_S)

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location(s) | **Released on Behalf of Eurofins by**

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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Report Number:	**3039910-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041021-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	<10 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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Madison WI 53704
800-675-8375

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Report Number:	**3041213-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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800-675-8375

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Report Number:	**3041214-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Suitability of Test Method	
Bile-Tolerant Gram-Neg Bacteria Suitability Result	Pass**

Method References	**Testing Location**
Suitability of Test Method (USPN_SOT)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3043421-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-A**	**Eurofins Sample:**	**9868714**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-A	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	4.8e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3015017-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3037644-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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Report Number:	**3038174-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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800-675-8375

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Report Number:	**3038382-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Madison WI 53704
800-675-8375

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Report Number:	**3038691-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	Result
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References

Elements by ICP Mass Spectrometry (ICP_MS_S)

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Testing Location(s)

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

Released on Behalf of Eurofins by

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of Eurofins.

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Report Number:	**3039911-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041022-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	80 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041215-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
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Report Number:	**3043422-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-B**	**Eurofins Sample:**	**9868715**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-B	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	2.2e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
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Report Number:	**3015018-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3037645-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038175-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038383-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038690-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References

Elements by ICP Mass Spectrometry (ICP_MS_S)

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Testing Location(s)

Food Integrity Innovation-Madison

Eurofins Food Chemistry Testing Madison, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

Released on Behalf of Eurofins by

Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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Report Number:	**3039912-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
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Report Number:	**3041023-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	<10 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3041216-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
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Report Number:	**3043423-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

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Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-C**	**Eurofins Sample:**	**9868716**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-C	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	3.4e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
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Report Number:	**3015020-0**
Report Date:	**28-Sep-2020**
Report Status:	**Final**

Certificate of Analysis

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Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
L. reuteri 1E1 PCR Identification	
Organism Tested	Lactobacillus reuteri
Result	Present

Method References	**Testing Location**
L. reuteri 1E1 PCR Identification (PCRID)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Internal DuPont Method, "General Method for Genetic Identity of Probiotics using End-Point PCR." Received 07Mar2018

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3037647-0**
Report Date:	**18-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

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Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Staphylococcus	
Staphylococcus Aureus	Absent /1 g

Method References	**Testing Location**
Staphylococcus (USPA_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
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Report Number:	**3038177-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

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Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Salmonella USP	
Salmonella	Absent /10 g

Method References	**Testing Location**
Salmonella USP (USPS_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038385-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
E. Coli	
Escherichia Coli	Absent /1 g

Method References	**Testing Location**
E. Coli (USPE_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the suitability of the test method results, conditions stipulated are adequate for detecting the presence of the specified microorganism.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Report Number:	**3038688-0**
Report Date:	**19-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Elements by ICP Mass Spectrometry	
Arsenic	<10.0 ppb
Cadmium	<5.00 ppb
Lead	<5.00 ppb
Mercury	<5.00 ppb

Method References — **Testing Location**

Elements by ICP Mass Spectrometry (ICP_MS_S)

Food Integrity Innovation-Madison
3301 Kinsman Blvd Madison, WI 53704 USA

Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL, (Modified).
Paquette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).

Testing Location(s) — **Released on Behalf of Eurofins by**

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Edward Ladwig - President Eurofins Food Chemistry Testing Madison

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Report Number:	**3039914-0**
Report Date:	**20-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Yeast and Mold Count	
Combined Yeast and Mold Count	<10 CFU/g

Method References	**Testing Location**
Yeast and Mold Count (USPM_61)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 61.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
800-675-8375

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Report Number:	**3041025-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Non-Lactic Count	
Non-Lactic Count	<10 CFU/g

Method References	**Testing Location**
Non-Lactic Count (ISONLC)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA
ISO 13559:2002	

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
2102 Wright Street
Madison WI 53704
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Report Number:	**3041218-0**
Report Date:	**21-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Enterobacterial count (MPN	
Bile-Tolerant Gram-Negative Bacteria	Absent /1 g

Method References	**Testing Location**
Enterobacterial count (MPN (USPN_62)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

USP current revision, Chapter 62.
To satisfy the requirements of the USP, the suitability of Test Method must be completed on each matrix.
**Based on the results of the preparatory test, the detection limit stipulated is adequate for the enumeration of the specified microorganisms.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

Eurofins Food Chemistry Testing US, Inc.
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Report Number:	**3043439-0**
Report Date:	**22-Oct-2020**
Report Status:	**Final**

Certificate of Analysis

Elanco US Inc.

2500 Innovation Way
Greenfield Indiana 46140 United States

Sample Name:	**Lactobacillus reuteri 3632-E**	**Eurofins Sample:**	**9868718**
Project ID	ELANCO_ANI-20200918-0027	**Receipt Date**	18-Sep-2020
PO Number	4100746960	**Receipt Condition**	Cold on Wet Ice or Ice Packs
Lot Number	3632-E	**Login Date**	18-Sep-2020
Sample Serving Size		**Date Started**	18-Sep-2020
		Sampled	Sample results apply as received

Analysis	**Result**
Total Probiotic Enumeration	
Total Probiotic Enumeration	3.3e9 CFU/g

Method References	**Testing Location**
Total Probiotic Enumeration (TPRO)	**Food Integ. Innovation-Madison NE** 2102 Wright Street Madison, WI 53704 USA

Compendium of Methods for the Microbiological Examination of Foods (Probiotics) 5th Edition, Chapter 20, American Public Health Association: Washington, D.C., (2015). Modified.

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integ. Innovation-Madison NE	**Shannon Jacoby - Business Unit Manager**

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Certificate of Analysis

Date: 2/25/2021
Customer: ELANCO
Product: L. reuteri 3632
Lot #: 201123LRE3632
Lab Report #: BQL201208, 3121727-0 (Eurofins)
Purchase Order #: Email

Microbiological and Analytical Analyses

Test	Method	Spec Limits	Results
Lactic Acid Bacteria Counts	BQL-LAB-001.3	Not specified	430.0 x 10^9 cfu/g
Non-Lactics	ISO 13559	< 5000 CFU/g	300 cfu
Enterococcus	CMMEF, 4th Ed	< 100 CFU/g	<10 cfu
Enterobacteriaceae	USPN 2021	< 10 cfu/g	< 10
E. coli	USPE 2022	Absent/10 g	Absent
Yeast & Mold	USPM 2021	< 100 cfu/g	< 10
CP Staphylococcus	USPA 2022	Absent/10 g	Absent
Salmonella	USPS 2022	Absent /25g	Absent
Listeria	FDA BAM Ch. 10	Not Detected /25 g	Not detected
Appearance/Color	Visual	White to Tan powder	Pass
Identification	Riboprinter	>0.85 Similarity Index	0.89
Arsenic	AOAC 2011.19 and 993.14	NMT 1 ppm	282 ppb
Cadmium	AOAC 2011.19 and 993.14	NMT 0.3 ppm	195 ppb
Lead	AOAC 2011.19 and 993.14	NMT 1 ppm	103 ppb
Mercury	AOAC 2011.19 and 993.14	NMT 0.05 ppm	6.38 ppb

Manufacturing Date
11/23/2020

Best Before Date
11/23/2021

Country of Origin
US

Ingredients
Lactobacillus reuteri and no excipient

Recommended Storage Conditions
Store frozen at -20°C or colder

Approved by: Jerry Stoecklein, Quality Director

S66 W14328, Janesville Road, P.O Box 777, Muskego, WI 53150-0777 ● P: 414 422 9085; F: 414 422 9086 ● info@biosource.us.com

Certificate of Analysis

Date: 8/20/2021
Customer: ELANCO
Product: L. reuteri 3632
Lot #: 210712LRE15(ET5(TRE))
Lab Report #: BQL210730, 411840628 (Merieux)
Purchase Order #: 4800018988

Microbiological and Analytical Analyses

Test	Method	Spec Limits	Results
Lactic Acid Bacteria Counts	BQL-LAB-001.3	Not specified	180.0 x 10^9 cfu/g
Non-Lactics	ISO 13559	< 5000 CFU/g	<100
Enterococcus	CMMEF, 4th Ed	< 100 CFU/g	<10
Enterobacteriaceae	USPN 2021	< 10 cfu/g	< 10
E. coli	USPE 2022	Absent/10 g	Absent
Yeast & Mold	USPM 2021	< 100 cfu/g	< 10
CP Staphylococcus	USPA 2022	Negative/25 g	Negative
Salmonella	USPS 2022	Negative /10g	Negative
Listeria	FDA BAM Ch. 10	Negative /25 g	Negative
Appearance/Color	Visual	White to Tan powder	Pass
Identification	Riboprinter	>0.85 Similarity Index	0.97
Arsenic	AOAC 2011.19 and 993.14	NMT 1 ppm	0.11
Cadmium	AOAC 2011.19 and 993.14	NMT 0.3 ppm	0.052
Lead	AOAC 2011.19 and 993.14	NMT 1 ppm	0.19
Mercury	AOAC 2011.19 and 993.14	NMT 0.05 ppm	<0.005

Manufacturing Date
07/12/2021

Best Before Date
07/12/2022

Country of Origin
US

Ingredients
Lactobacillus reuteri and no excipient

Recommended Storage Conditions
Store frozen at -20°C or colder

Approved by: Megan Jacobson, Quality Manager

Annex 13
Dietary Supplement Label Database Report

DSLD ID	Product Name	Serving Size	Daily Value Target Group	Ingredient	DSLD Ingredient Categories	Amount Per Serving	Amount Per Serving Unit	% Daily Value per Serving
12221	RepHresh Pro-B	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri RC-14	bacteria	2.5E9	CFU	
45780	Activz Complete Natural Vanilla Berry Flavor	26.0 Gram(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
45795	Activz 9 A Day+	9.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
45823	Activz 9 A Day+	9.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
53010	Bioray NDF Natural Detox Formula	52.0 Drop(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
53011	Bioray NDF Natural Detox Formula	52.0 Drop(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
54029	Bioray NDF Plus	52.0 Drop(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
54036	Bioray NDF Plus	52.0 Drop(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
62371	Mass Probiotics GoLive Berry Pomegranate	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62371	Mass Probiotics GoLive Berry Pomegranate	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62371	Mass Probiotics GoLive Berry Pomegranate	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62561	Mass Probiotics GoLive Probiotic + Prebiotic Orange Tangerine	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62561	Mass Probiotics GoLive Probiotic + Prebiotic Orange Tangerine	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62561	Mass Probiotics GoLive Probiotic + Prebiotic Orange Tangerine	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62739	Mass Probiotics Kids! GoLive Probiotic + Prebiotic Berry-Licious Fruit Punch	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age	L. reuteri SD-5865	bacteria	0.0		
62739	Mass Probiotics Kids! GoLive Probiotic + Prebiotic Berry-Licious Fruit Punch	9.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age	L. reuteri SD-5865	bacteria	0.0		
62970	Mass Probiotics GoLive Probiotic + Prebiotic Spa	12.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62970	Mass Probiotics GoLive Probiotic + Prebiotic Spa	12.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
62970	Mass Probiotics GoLive Probiotic + Prebiotic Spa	12.6 Gram(s)	Adults and children 4 or more years of age; Children less than 4 years of age; Preg	L. reuteri SD-5865	bacteria	0.0		
64994	Renew Life Women's Care Flush & Be Fit Afternoon Pack 2	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
74379	Nature's Bounty Cardio-Health	1.0 Capsule(s)	Adults and children 4 or more years of age	Lactobacillus reuteri NCIM	bacteria	0.0		
74383	Nature's Bounty Optimal Solutions Cardio-Health Probiotics	1.0 Capsule(s)	Adults and children 4 or more years of age	Lactobacillus reuteri NCIM	bacteria	0.0		
180668	Bioray Primary Detox	1.0 mL	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182254	Hyperbiotics Glucose Support	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182262	Hyperbiotics Immune	2.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182275	Hyperbiotics PRO-15	1.0 Pearl Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182294	Hyperbiotics PRO-15 Advanced Strength	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182306	Hyperbiotics PRO-Dental Natural Mint Flavor	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182310	Hyperbiotics PRO-Kids ENT Strawberry Vanilla	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
182332	Hyperbiotics PRO-Moms	1.0 Tablet(s)	Pregnant and lactating	L. reuteri	bacteria	0.0		
182338	Hyperbiotics PRO-Women	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
187631	Swanson Probiotic for Digestive Health	1.0 Veggie Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
188635	Swanson Ultra Potency Probiotic	1.0 Veggie Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
201971	SimplyBiotix Oral & Sinus Cinnamon Stick Flavor	1.0 Lozenge(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
203226	Swanson L. Reuteri Plus	1.0 Veggie Capsule(s)	Adults and children 4 or more years of age	Lactobacillus reuteri	bacteria	0.0		
203256	Swanson Ultra Probiotic Complex	1.0 Veggie Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
204212	DNA Miracles Probiotics Extra	1.73 Gram(s)	Infants	Lactobacillus reuteri	bacteria	0.0		
206644	BioGaia Protectis Drops with Vitamin D	5.0 Drop(s)	Adults and children 4 or more years of age; Children less than 4 years of age	L. reuteri DSM 17938	bacteria	0.0		
206644	BioGaia Protectis Drops with Vitamin D	5.0 Drop(s)	Adults and children 4 or more years of age; Children less than 4 years of age	L. reuteri DSM 17938	bacteria	0.0		
208684	Health Plus Turmeric Joint Curcumin	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	100.0	mg	
210254	RepHresh Pro-B Probiotic	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri RC-14	bacteria	0.0		
213733	ProBioCare Men's 50 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213737	ProBioCare Men's 50 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213738	ProBioCare Women's 50 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213743	ProBioCare Women's 50 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213746	ProBioCare 75 Billion Probiotic Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213747	ProBioCare Women's 100 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
213748	ProBioCare Men's 100 Billion Probiotics Delayed Release	1.0 Vegetable Capsule(s)	Adults and children 4 or more years of age	L. reuteri SD-5865	bacteria	0.0		
224705	Natures Plus GI Natural Pro Biotic Mega	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
228701	1 Body Probiotic 30 Billion CFU	2.0 Vegetarian Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
230474	Dr. David Williams Probiotic Advantage Oral Sinus Natural Cinnamon Flavor	1.0 Lozenge(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
233108	Bronson Probiotic 50 Billion CFU plus Prebiotic	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
239564	Codeage Hair Renewal	4.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
239567	Codeage Teeth & Gums	3.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
239644	BioGaia Protectis Tabs Lemon Flavored	1.0 Tablet(s)	Adults and children 4 or more years of age	Lactobacillus reuteri DSM	bacteria	0.0		
240405	NewRhythm Probiotics	2.0 Veggie Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
241309	ChildLife Clinicals Clinical Formulas Kidney/Urinary Health Natural Berry Flavor	1.6 Gram(s)	Adults and children 4 or more years of age	Lactobacillus reuteri 1E1	bacteria	1.0	mg	
242686	Nature's Way Fortify Optima Daily Probiotic	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri 1E1	bacteria	0.0		
242945	Nature's Way Primadophilus Reuteri	1.0 Capsule(s)	Adults and children 4 or more years of age	Lactobacillus reuteri UALr	bacteria	0.0		
243535	Renew Life Brenda Watson's Vital Woman Flush & Be Fit	1.0 Packet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
246331	Rephresh Pro-B	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri (RC-14)	bacteria	0.0		
249420	Bioray Mind Zeal	1.0 mL	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
249584	Bioray NDF Natural Detox Formula	2.0 Dropper(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0		
249586	Bioray NDF Plus	2.0 Dropper(s)		L. reuteri	bacteria	0.0		

249596	Bioray Primary Detox	1.0 mL	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
249766	NaturesPlus GI Natural Pro-Biotic Mega	1.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
251453	BiOptimizers CogniBiotics	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
251672	Nature's Way Primadophilus Reuteri	1.0 Teaspoon(s)	Adults and children 4 or more years of age; Children less than 4 years of age	Lactobacillus reuteri UALr	bacteria	0.0	
251672	Nature's Way Primadophilus Reuteri	1.0 Teaspoon(s)	Adults and children 4 or more years of age; Children less than 4 years of age	Lactobacillus reuteri UALr	bacteria	0.0	
252141	Custom Probiotics L. Reuteri	0.1 Gram(s)		L. reuteri	bacteria	0.0	
255929	Health Plus Turmeric Joint Curcumin	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	100.0	mg
257128	Purity Labs Probiotic-16	1.0 Pearl Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
257250	1 Body Probiotic 30 Billion CFU	2.0 Veggie Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
257664	Hyperbiotics Glucose Support	1.0 Tablet(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
258133	Bronson Women's Probiotic 50 Billion CFU plus Prebiotic	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
258339	NATURELO Whole Food Multivitamin for Men	4.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
259244	Bronson Brain + Mood Probiotic 50 Billion CFU Plus Prebiotic	2.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
262840	NATURELO Premium Supplements Whole Food Multivitamin for Men 50+	4.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
262841	NATURELO Premium Supplements Whole Food Multivitamin for Women 50+	4.0 Capsule(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	
263178	Vitamin Bounty Women's Pro-Daily 10 Billion CFU	2.0 Veggie Cap(s)	Adults and children 4 or more years of age	L. reuteri	bacteria	0.0	

National Institutes of Health
Office of Dietary Supplements

Dietary Supplement Label Database

Search

'L. reuteri'

Filters

SUBMIT FILTERS

Clear Filter(s)

Market Status

- All Labels
- On Market
- Off Market

Search term (found anywhere on label): 'L. reuteri' Clear Search

Active filters:

Market Status On Market

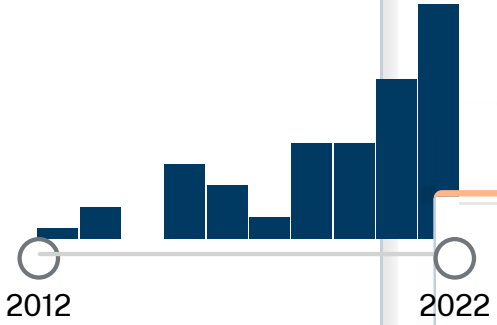
Product Type Non-Nutrient/Non-Botanical Other Combinations

74 labels found (<1% of database).

Not finding what you are searching for?

Off Market

Date Added to DSLD



Sort by: Best Match

LABEL TABLE

DOWNLOAD

Product

Product Name

Product Type

- [ ] Amino Acid/Protein
- [ ] Botanical
- [ ] Fiber and Other Nutrients
- [ ] Omega 3 and Other Fatty Acids
- [ ] Vitamin

Show 6 more

Ingredient

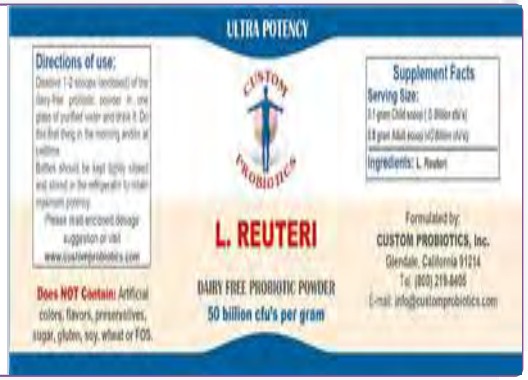
ON MARKET

Custom Probiotics L. Reuteri

Brand: Custom Probiotics

Net Contents:

Date Added to DSLD: 10/2021



ON MARKET

Swanson L. Reuteri Plus

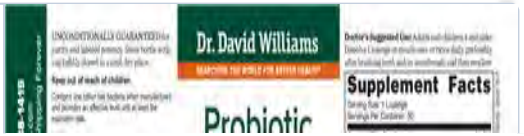
Brand: Swanson

Net Contents:

Date Added to DSLD: 05/2019

ON MARKET

Dr. David Williams Probiotic Advantage



Ingredient Name

Ingredient Category

- Amino Acids
- Animal Parts or Sources
- Blends
- Botanicals
- Sugars

Show 13 more

Brand

Brand Name

Additional Filters

Intended Target Group

- All Adults and Children 4 Years

Oral Sinus Natural Cinnamon Flavor

Brand: Dr. David Williams

Net Contents: 50.0 Lozenge(s)

Date Added to DSLD: 07/2020

ON MARKET

Health Plus Turmeric Joint Curcumin

Brand: Health Plus

Net Contents:

Date Added to DSLD: 11/2021

ON MARKET

Health Plus Turmeric Joint Curcumin

Brand: Health Plus

Net Contents:

Date Added to DSLD: 10/2019

ON MARKET

Designs for Health Probiotic Synergy

Brand: Designs for Health

Net Contents: 60.0 BIO-tract Probiosphere(s)

D t Add d t DSLD 07/2013

and Above

- [ ] Children 1 to 4 Years
- [ ] Infants
- [ ] Pregnant and Lactating

Supplement Form

- [ ] Bars
- [ ] Capsules
- [ ] Softgel Capsules
- [ ] Tablets and Pills
- [ ] Gummies and Jellies

Show 5 more

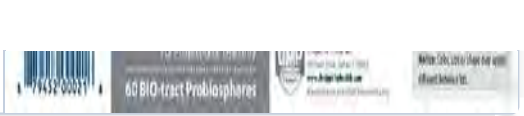
Dietary Claim

Claim Types

- [ ] Nutrient
- [ ] Structure/Function
- [ ] Approved Health
- [ ] Qualified Health
- [ ] All Other

Show 1 more

Date Added to DSLD: 07/2013



ON MARKET

Designs for Health Probiotic Supreme

Brand: Designs for Health

Net Contents:

Date Added to DSLD: 06/2013

ON MARKET

Designs for Health Probiotic Synergy

Brand: Designs for Health

Net Contents:

Date Added to DSLD: 06/2013

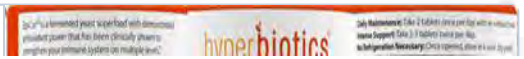
ON MARKET

Renew Life Brenda Watson's Vital Woman Flush & Be Fit

Brand: Renew Life Brenda Watson's Vital Woman

Net Contents:

Date Added to DSLD: 02/2021



SUBMIT FILTERS

Clear Filter(s)

ON MARKET

Hyperbiotics Immune

Brand: Hyperbiotics

Net Contents: 60.0 Patented, Time-Release Tablet(s)

Date Added to DSLD: 10/2018

ON MARKET

Hyperbiotics PRO-15 Advanced Strength

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018

ON MARKET

Swanson Ultra Potency Probiotic

Brand: Swanson

Net Contents:

Date Added to DSLD: 02/2019

ON MARKET

Swanson Ultra Probiotic Complex

Brand: Swanson

Net Contents: 60.0 Veggie DRcap(s)

Date Added to DSLD: 05/2019

ON MARKET

Bioray NDF Plus

Brand: Bioray

Net Contents:

Date Added to DSLD: 06/2021

ON MARKET

Purity Labs Probiotic-16

Brand: Purity Labs

Net Contents:

Date Added to DSLD: 12/2021

ON MARKET

Renew Life Women's Care Flush & Be Fit Afternoon Pack 2

Brand: Renew Life

Net Contents:

Date Added to DSLD: 09/2016

ON MARKET

Bioray NDF Plus

Brand: Bioray

Net Contents:

Date Added to DSLD: 11/2015

ON MARKET

Swanson Probiotic for Digestive Health

Brand: Swanson

Net Contents:

Date Added to DSLD: 02/2019

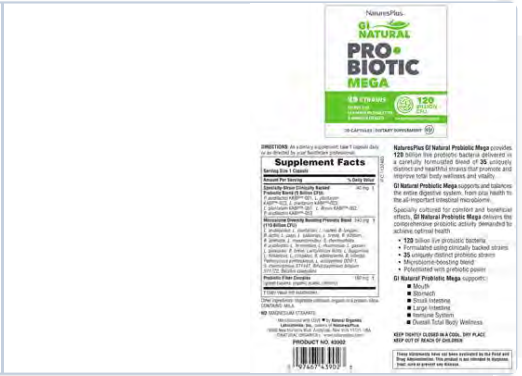
ON MARKET

Natures Plus GI Natural Pro Biotic Mega

Brand: Natures Plus GI Natural

Net Contents:

Date Added to DSLD: 06/2020



ON MARKET

Bronson Probiotic 50 Billion CFU plus

Prebiotic

Brand: Bronson

Net Contents:

Date Added to DSLD: 10/2020

Items per page: 20 1 – 20 of 74

The inclusion of a product and its label in the DSLD is not an endorsement of that product or a guarantee of accuracy by the National Institutes of Health's Office of Dietary Supplements. Read the full disclaimer.

National Institutes of Health
Office of Dietary Supplements

Dietary Supplement Label Database

Search

'L. reuteri'

SEARCH

See search tips

Filters

SUBMIT FILTERS

Clear Filter(s)

Market Status

- All Labels
- On Market
- Off Market

Search term (found anywhere on label): 'L. reuteri' Clear Search

Active filters:

Market Status On Market

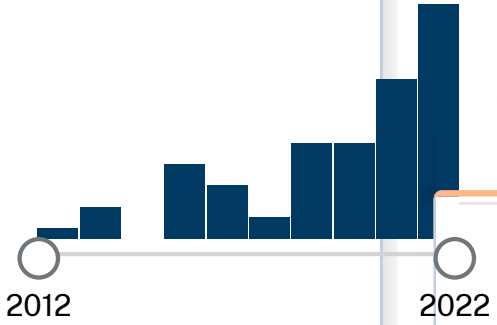
Product Type Non-Nutrient/Non-Botanical Other Combinations

74 labels found (<1% of database).

Not finding what you are searching for?

Off Market

Date Added to DSLD



Product

Product Name

Product Type

- [ ] Amino Acid/Protein
- [ ] Botanical
- [ ] Fiber and Other Nutrients
- [ ] Omega 3 and Other Fatty Acids
- [ ] Vitamin

Show 6 more

Ingredient

Sort by:

LABEL | TABLE

DOWNLOAD

ON MARKET

Bioray Mind Zeal

Brand: Bioray

Net Contents:

Date Added to DSLD: 07/2021

ON MARKET

Codeage Teeth & Gums

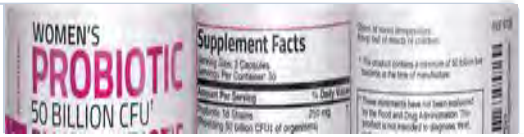
Brand: Codeage

Net Contents:

Date Added to DSLD: 11/2020

ON MARKET

Bronson Women's Probiotic 50 Billion



Ingredient Name

Ingredient Category

- Amino Acids
- Animal Parts or Sources
- Blends
- Botanicals
- Sugars

Show 13 more

Brand

Brand Name

Additional Filters

Intended Target Group

- All Adults and Children 4 Years

CFU plus Prebiotic

Brand: Bronson

Net Contents: 60.0 Vegetarian Capsule(s)

Date Added to DSLD: 10/2021

ON MARKET

Bioray NDF Natural Detox Formula

Brand: Bioray

Net Contents:

Date Added to DSLD: 11/2015

ON MARKET

Bioray NDF Natural Detox Formula

Brand: Bioray

Net Contents:

Date Added to DSLD: 10/2015

ON MARKET

Hyperbiotics Glucose Support

Brand: Hyperbiotics

Net Contents: 60.0 Patented, Time-Release Tablet(s)

and Above

- [ ] Children 1 to 4 Years
- [ ] Infants
- [ ] Pregnant and Lactating

Supplement Form

- [ ] Bars
- [ ] Capsules
- [ ] Softgel Capsules
- [ ] Tablets and Pills
- [ ] Gummies and Jellies

Show 5 more

Dietary Claim

Claim Types

- [ ] Nutrient
- [ ] Structure/Function
- [ ] Approved Health
- [ ] Qualified Health
- [ ] All Other

Show 1 more

Tablet(s)

Date Added to DSLD: 10/2018

ON MARKET

Hyperbiotics PRO-Women

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018

ON MARKET

SimplyBiotix Oral & Sinus Cinnamon Stick Flavor

Brand: SimplyBiotix

Net Contents:

Date Added to DSLD: 05/2019

ON MARKET

Bioray NDF Natural Detox Formula

Brand: Bioray

Net Contents:

Date Added to DSLD: 06/2021

ON MARKET

SUBMIT FILTERS

Clear Filter(s)

ON MARKET

1 Body Probiotic 30 Billion CFU

Brand: 1 Body

Net Contents: 60.0 Acid-Resistant Capsule(s)

Date Added to DSLD: 12/2021

ON MARKET

Bronson Brain + Mood Probiotic 50 Billion CFU Plus Prebiotic

Brand: Bronson

Net Contents:

Date Added to DSLD: 01/2022

ON MARKET

NATURELO Premium Supplements Whole Food Multivitamin for Women 50+

Brand: NATURELO Premium Supplements

Net Contents:

Date Added to DSLD: 12/2021

ON MARKET

Activz Complete Natural Vanilla Berry Flavor

Brand: Activz

Net Contents: 13.7 oz.; 390.0 Gram(s)

Date Added to DSLD: 05/2015

ON MARKET

Bioray NDF Plus

Brand: Bioray

Net Contents:

Date Added to DSLD: 11/2015

ON MARKET

Bioray Primary Detox

Brand: Bioray

Net Contents:

Date Added to DSLD: 09/2018

ON MARKET

Hyperbiotics PRO-Kids ENT Strawberry Vanilla

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018

ON MARKET

Hyperbiotics PRO-Moms

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018

ON MARKET

1 Body Probiotic 30 Billion CFU

Brand: 1 Body

Net Contents:

Date Added to DSLD: 06/2020

ON MARKET

Bioray Primary Detox

Brand: Bioray

Net Contents:

Date Added to DSLD: 06/2021

ON MARKET

NATURELO Whole Food Multivitamin

for Men

Brand: NATURELO

Net Contents:

Date Added to DSLD: 10/2021

Items per page: 20 21 – 40 of 74

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Dietary Supplement Label Database

National Institutes of Health
Office of Dietary Supplements

National Institutes of Health
Office of Dietary Supplements

Dietary Supplement Label Database

Search

'L. reuteri'

SEARCH

See search tips

Filters

SUBMIT FILTERS

Clear Filter(s)

Market Status

- All Labels
- On Market
- Off Market

Search term (found anywhere on label): 'L. reuteri' Clear Search

Active filters:

Market Status On Market

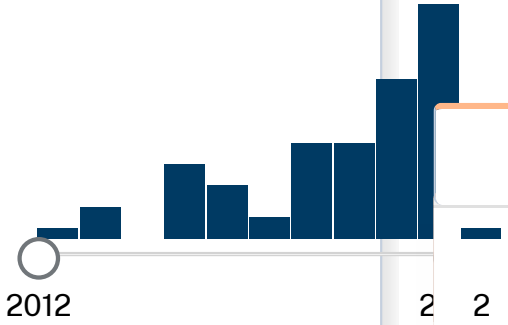
Product Type Non-Nutrient/Non-Botanical Other Combinations

74 labels found (<1% of database).

Not finding what you are searching for?

Off Market

Date Added to DSLD



Sort by: Best Match

LABEL TABLE

DOWNLOAD

Product

Product Name

Product Type

- [ ] Amino Acid/Protein
- [ ] Botanical
- [ ] Fiber and Other Nutrients
- [ ] Omega 3 and Other Fatty Acids
- [ ] Vitamin

Show 6 more

Ingredient

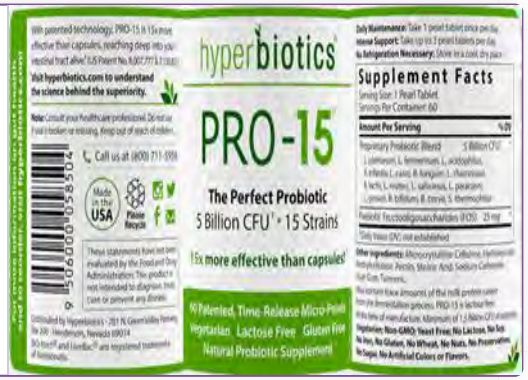
ON MARKET

Hyperbiotics PRO-15

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018



ON MARKET

Hyperbiotics PRO-Dental Natural Mint Flavor

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 10/2018

ON MARKET

Codeage Hair Renewal

Brand: Codeage

Net Contents: 120.0 Capsule(s)

Date Added to DSLD: 11/2020

Brand

Brand Name

Additional Filters

Intended Target Group

- [ ] All Adults and Children 4 Years

ON MARKET

BiOptimizers CogniBiotics

Brand: BiOptimizers

Net Contents: 60.0 Veggie Cap(s)

and Above

- [ ] Children 1 to 4 Years
- [ ] Infants
- [ ] Pregnant and Lactating

Supplement Form

- [ ] Bars
- [ ] Capsules
- [ ] Softgel Capsules
- [ ] Tablets and Pills
- [ ] Gummies and Jellies

Show 5 more

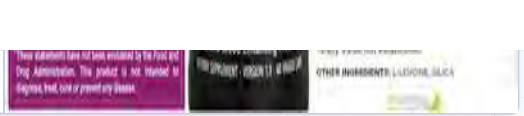
Dietary Claim

Claim Types

- [ ] Nutrient
- [ ] Structure/Function
- [ ] Approved Health
- [ ] Qualified Health
- [ ] All Other

Show 1 more

Date Added to DSLD: 07/2021



ON MARKET

Hyperbiotics Glucose Support

Brand: Hyperbiotics

Net Contents:

Date Added to DSLD: 09/2021

ON MARKET

NATURELO Premium Supplements Whole Food Multivitamin for Men 50+

Brand: NATURELO Premium Supplements

Net Contents:

Date Added to DSLD: 12/2021

ON MARKET

Vitamin Bounty Women's Pro-Daily 10 Billion CFU

Brand: Vitamin Bounty

Net Contents:

Date Added to DSLD: 11/2021

ON MARKET

SUBMIT FILTERS

Clear Filter(s)

ON MARKET

Activz 9 A Day+

Brand: Activz

Net Contents: 4.0 oz.; 114.0 Gram(s); 135.0 Capsule(s)

Date Added to DSLD: 05/2015

ON MARKET

Activz 9 A Day+

Brand: Activz

Net Contents: 8.0 oz.; 228.0 Gram(s); 270.0 Capsule(s)

Date Added to DSLD: 05/2015

ON MARKET

Nature's Way Fortify Optima Daily Probiotic

Brand: Nature's Way

Net Contents: 30.0 Delayed-Release Veg. Capsule(s)

Date Added to DSLD: 01/2021

ON MARKET

Nature's Bounty Cardio-Health

Brand: Nature's Bounty

Net Contents: 60.0 Capsule(s)

Date Added to DSLD: 05/2017

ON MARKET

Nature's Bounty Optimal Solutions Cardio-Health Probiotics

Brand: Nature's Bounty Optimal Solutions

Net Contents: 90.0 Capsule(s)

Date Added to DSLD: 06/2017

ON MARKET

Rephresh Pro-B

Brand: Rephresh

Net Contents: 30.0 Capsule(s)

Date Added to DSLD: 03/2021

ON MARKET

ProBioCare Women's 50 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents: 60.0 Vegetable Capsule(s)

Date Added to DSLD: 02/2020

ON MARKET

Mass Probiotics GoLive Probiotic + Prebiotic Orange Tangerine

Brand: Mass Probiotics

Net Contents: 10.0 Packet(s); 3.4 oz.; 96.0 Gram(s)

Date Added to DSLD: 07/2016

ON MARKET

Mass Probiotics Kids! GoLive Probiotic + Prebiotic Berry-Licious Fruit Punch

Brand: Mass Probiotics

Net Contents: 10.0 Packet(s); 3.4 oz.; 9.6 Gram(s)

Date Added to DSLD: 07/2016

ON MARKET

ProBioCare 75 Billion Probiotic Delayed Release

Brand: ProBioCare

Net Contents: 30.0 Vegetable Capsule(s)

Date Added to DSLD: 02/2020

ON MARKET

RepHresh Pro-B

Brand: RepHresh

Net Contents: 30.0 Capsule(s)

Date Added to DSLD: 08/2012

Items per page: 20 41 – 60 of 74

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Dietary Supplement Label Database

National Institutes of Health

Office of Dietary Supplements

National Institutes of Health
Office of Dietary Supplements

Dietary Supplement Label Database

Search

'L. reuteri'

SEARCH

See search tips

Filters

SUBMIT FILTERS

Clear Filter(s)

Market Status

- All Labels
- On Market
- Off Market

Search term (found anywhere on label): 'L. reuteri' Clear Search

Active filters:

Market Status On Market

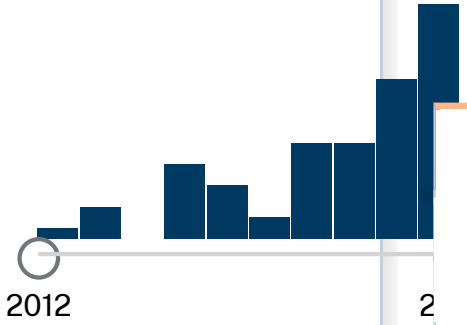
Product Type Non-Nutrient/Non-Botanical Other Combinations

74 labels found (<1% of database).

Not finding what you are searching for?

Off Market

Date Added to DSLD



Sort by: Best Match

LABEL TABLE

Product

Product Name

Product Type

- [ ] Amino Acid/Protein
- [ ] Botanical
- [ ] Fiber and Other Nutrients
- [ ] Omega 3 and Other Fatty Acids
- [ ] Vitamin

Show 6 more

Ingredient

ON MARKET

ProBioCare Men's 50 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents:

Date Added to DSLD: 02/2020

ON MARKET

ProBioCare Men's 100 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents:

Date Added to DSLD: 02/2020

ON MARKET

Mass Probiotics GoLive Berry

Ingredient Name

Ingredient Category

- Amino Acids
- Animal Parts or Sources
- Blends
- Botanicals
- Sugars

Show 13 more

Brand

Brand Name

Additional Filters

Intended Target Group

- All Adults and Children 4 Years

Pomegranate

Brand: Mass Probiotics

Net Contents: 10.0 Packet(s); 3.4 oz.; 96.0 Gram(s)

Date Added to DSLD: 07/2016

ON MARKET

Mass Probiotics GoLive Probiotic + Prebiotic Spa

Brand: Mass Probiotics

Net Contents:

Date Added to DSLD: 07/2016

ON MARKET

BioGaia Protectis Drops with Vitamin D

Brand: BioGaia

Net Contents:

Date Added to DSLD: 09/2019

ON MARKET

ProBioCare Men's 50 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents: 60.0 Vegetable Capsule(s)

and Above

- [ ] Children 1 to 4 Years
- [ ] Infants
- [ ] Pregnant and Lactating

Supplement Form

- [ ] Bars
- [ ] Capsules
- [ ] Softgel Capsules
- [ ] Tablets and Pills
- [ ] Gummies and Jellies

Show 5 more

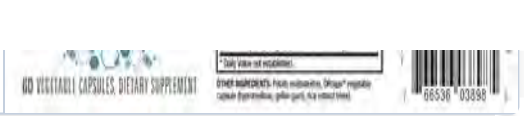
Dietary Claim

Claim Types

- [ ] Nutrient
- [ ] Structure/Function
- [ ] Approved Health
- [ ] Qualified Health
- [ ] All Other

Show 1 more

Date Added to DSLD: 02/2020



ON MARKET

RepHresh Pro-B Probiotic

Brand: RepHresh

Net Contents:

Date Added to DSLD: 12/2019

ON MARKET

ProBioCare Women's 50 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents:

Date Added to DSLD: 02/2020

ON MARKET

ProBioCare Women's 100 Billion Probiotics Delayed Release

Brand: ProBioCare

Net Contents:

Date Added to DSLD: 02/2020

ON MARKET

SUBMIT FILTERS

Clear Filter(s)

ON MARKET

ChildLife Clinicals Clinical Formulas Kidney/Urinary Health Natural Berry Flavor

Brand: ChildLife Clinicals Clinical Formulas

Net Contents: 1.7 Ounce(s); 48.0 Gram(s)

Date Added to DSLD: 01/2021

ON MARKET

DNA Miracles Probiotics Extra

Brand: DNA Miracles

Net Contents:

Date Added to DSLD: 06/2019

ON MARKET

BioGaia Protectis Tabs Lemon Flavored

Brand: BioGaia

Net Contents:

Date Added to DSLD: 12/2020

ON MARKET

Nature's Way Primadophilus Reuteri

Brand: Nature's Way

Net Contents: 5.0 Oz(s); 141.75 Gram(s)

Date Added to DSLD: 06/2021

ON MARKET

Nature's Way Primadophilus Reuteri

Brand: Nature's Way

Net Contents:

Date Added to DSLD: 01/2021

Items per page: 20 61 – 74 of 74

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Dietary Supplement Label Database

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Affiliates: HHS NIH ODS

Version 8.1.4 | October 2021