DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000736	RECEIVED OCT 2 2017 OFFICE OF FOOD ADDITIVE SAFETY
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
   ☒ New ☐ Amendment to GRN No._____ ☐ Supplement to GRN No. _____
2. ☐ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*
3. Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: _____
4. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Sarah F. Kraak-Ripple	Manager, Regulatory Affairs

Organization *(if applicable)*: DuPont Nutrition & Health

Mailing Address *(number and street)*: 3329Agriculture Drive

City	State or Province	Zip Code/Postal Code	Country
Madison	Wisconsin	53716	United States of America

Telephone Number	Fax Number	E-Mail Address
6083952633	6083952630	sarah.kraak-ripple@dupont.com

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

Name of Contact Person	Position or Title

Organization *(if applicable)*:

Mailing Address *(number and street)*:

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Lactobacillus paracasei strain Lpc-37

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 13

Total number of pages 1088 309

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.

Lactobacillus paracasei Lpc-37 is intended to be used in yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snack and other foods and in supplement form including sachets, tablets and capsules. It is intended to be added to conventional foods at initial levels as high as 5x10^11 CFU/serving (i.e. 2x10^9 CFU/g) to ensure at least 1x10^10 CFU/250g serving throughout the shelf life of the product, and in dietary supplements to ensure at least 5x10^10 CFU/serving. The function of L. paracasei Lpc-37 is to serve as a probiotic microorganism to be consumed by the general population.

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 Sarah F. Kraak-Ripple *(name of notifier)*

has concluded that the intended use*(s)* of Lactobacillus paracasei strain Lpc-37 *(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. Sarah F. Kraak-Ripple *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

3329 Agriculture Drive, Madison, WI 53716 *(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)
(b) (6)	Sarah F. Kraak-Ripple	09/28/2017

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)
1	Insert Clear Lactobacillus paracasei Lpc-37: Acute Oral Toxicity Study in Rats - Up and Down Procedure	Submission
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	
	Insert Clear	

Add Continuation Page

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services,Food and Drug Administration, Office of Chief Information Officer, PRAStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Comprehensive GRAS Assessment

Of

Lactobacillus casei subsp. *paracasei* Lpc-37

For Usage Conditions for

General Recognition of Safety

For

Danisco USA, Inc.

Table of Contents

Part 1 – Signed statements and certification

DuPont Nutrition & Health
3329 Agriculture Drive
Madison, WI 53716
800 255 6837 Tel 608 395 2630 Fax

September 28, 2017
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
HFS-200,
5001 Campus Drive
College Park, MD 20740-3835

Rd: GRAS Notice – Exemption claim for the use of *Lactobacillus casei* subsp. *paracasei* Lpc-37

Dear Office of Food Additive Safety:

In accordance with the US Food and Drug Administration's (FDA) Substances Generally Recognized as Safe; Final Rule, (81 FR 54959) relating to the filing of notices for substances that are considered to be generally recognized as safe (GRAS), please accept this claim and the attached information, submitted in triplicate, for that purpose as it relates to the use of *Lactobacillus casei* subsp. *paracasei* Lpc-37. Specifically, we claim that the use of *Lactobacillus casei* subsp. *paracasei* Lpc-37 in yogurt and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and also in dietary supplement format including sachets, tablets and capsules at a level of no more than $5x10^{12}$ CFU/day, is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such uses are GRAS. No information used in this part of this notification is trade secret or confidential commercial information. In accordance with the requirements outlined in 21 CFR 170, Subpart E of the final rule, the following information is included with this exemption claim:

(i) Name and address of the Notifier:
Sarah F. Kraak-Ripple
3329 Agriculture Drive
Madison, WI 53716

(ii) Common or Usual Name of the Notified Substance:
Lactobacillus paracasei Lpc-37

(iii) Intended Conditions of Use:

Lactobacillus paracasei Lpc-37 is manufactured in compliance with current Good Manufacturing Practice as specified in 21 CFR Part 110. *Lactobacillus paracasei* Lpc-37 is intended to be used in yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and in supplement format including sachets, tablets and capsules. It is intended to be added to conventional foods at initial levels as high as $5x10^{11}$ cfu/250g serving (i.e. $2x10^{9}$ cfu/g) to ensure at least 1×10^{10} CFU/250g serving throughout the shelf life of the product and in dietary supplements to ensure at least 5×10^{10} CFU/serving.

(iv) Basis for the GRAS Determination:

This GRAS conclusion is based on scientific procedures (21 CFR 170.30 (a) and (b)) as discussed in the detailed description provided below.

(v) Availability to FDA of Data and Information that are the Basis of Determination:

The data and information forming the basis for this GRAS determination and the exemption claim asserted herein are available for FDA review and copying during customary business hours at the following address, or will be sent to FDA either in an electronic format that is accessible for FDA evaluation or on paper, upon request:

Sarah F. Kraak-Ripple
Manager, Regulatory Affairs
DuPont Nutrition & health
3329 Agriculture Drive
Madison, Wisconsin 53716
608-334-0342
sarah.kraak-ripple@dupont.com

(vi) No data or information contained in parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(vii) If applicable and necessary, as required by §170.270 I authorize FDA to send any trade secrets to the Food Safety Inspection Service (FSIS) of the U. S. Department of Agriculture.

(viii) I certify that, to the best of my knowledge, this GRAS notice for *Lactobacillus paracasei* Lpc-37 is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

Should you have any questions regarding the submission of this notice, please contact Sarah Kraak-Ripple of DuPont Nutrition & Health. Thank you for your prompt consideration of, and response to, this notice.

Sincerely,

(b) (6)

Sarah F. Kraak-Ripple
Manager, Regulatory Affairs
DuPont Nutrition & Health

Part 2 – Identity, method of manufacture, specifications, and physical or technical effect

A. Identity:

a. **Name of the GRAS organisms:** *Lactobacillus casei* subsp. *Paracasei* Lpc-37
The strain is also referred to in the Danisco Global Culture Collection (DGCC) 4981 and has been deposited in the ATCC Culture Collection as SD5275.

b. **Source of the GRAS organisms:** *L. paracasei* Lpc-37 was isolated from a diary source and was identified according to standard taxonomic guidelines.

i. **The taxonomic lineage is:**

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Lactobacillaaceae
Genus: Lactobacillus
Species: casei. Entrez Genome ID: 652 subsp. casei, 2032 subsp. paracasei. Number of genomes of this species sequenced: 37 subsp. casei, 42 subsp. paracasei (GOLD); 28 subsp. casei, 38 subsp. paracasei (NCBI).
Sub-Species: paracasei
Strain: Lpc-37

ii. **Description of the GRAS organisms:**
Lactobacillus paracasei is a member of the lactic acid bacteria (LAB) classification, a group related by the production of lactic acid as the major metabolic end product of carbohydrate metabolism and other physiological traits. LAB are Gram-positive and generally non-spore forming, catalase negative, and devoid of cytochromes. LAB are of nonaerobic habit but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative forming lactic acid as the major end product of sugar fermentation (Holzapfel et al., 2001). LAB is not a defined taxonomic group, rather it is a functional grouping, and thus, the boundaries are controversial. Among the core genera classified LAB are Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus (Axelsson, 2004). Most LAB are considered to be non-pathogenic and have a long history of use in fermented and non-fermented foods (Axelsson, 2004;

Douillard and De Vos, 2014). Comparative genomics has identified the genes in LAB involved in colonization, persistence, interaction and signaling and helped in the understanding of the response of LAB to their environment and their evolution (Douillard and De Vos, 2014). The long history of safe use in foods, their ubiquitous presence as a minor component in the bowel microflora, and their ability to inhibit the growth of pathogenic microorganisms leads to the presumption that most LAB are safe for use in foods.

Lactobacillus, the largest of the LAB genera, contains over 80 species. It is a non-pathogenic, rod-shaped, non-motile, and non-sporulating genus that is widespread in nature. Many Lactobacillus species have found applications in the food industry. The genus may be categorized into three groups, obligate homofermentative, facultative heterofermentative, and obligate heterofermentative (Axelsson, 2004).

L. paracasei Lpc-37 is a Gram-positive, non-spore forming, homofermentative rod that is a common inhabitant of the human intestinal tract (Mitsuoka, 1996, Kandler and Weiss, 1986). *L. paracasei* strains are also found naturally in fermented vegetables, milk and meat. Strains of this species are used in many food products including traditional fermented milks and cheese. Selected strains of this species are also used in probiotic foods and dietary supplements.

iii. Genomic Analysis:

Sequencing:

A draft genome sequence of *Lactobacillus paracasei Lpc-37* was obtained using published methods and deposited at the NCBI database under accession number AFYU00000000. The resulting genome draft yielded 151 contigs with 3,075,295 total basepairs in length and 128X average coverage. Alignment of the resulting draft to whole genome sequences of published *L. paracasei* strains show good overall genomic synteny and core similarity, with unique regions that can be utilized for strain differentiation (Broadbent et al., 2012).

RiboPrinter® analysis

RiboPrinter® analysis targets the 5S, 16S, and 23S regions plus intragenic spacers regions within the genome. This automated southern blot technology provides a genetic fingerprint that allows identification to the Genus and species level, but may also discriminate within a species. The Lpc-37 RiboPrinter® pattern matched those for *L. paracasei* within the RiboPrinter® database. See attached RiboPrinter® report in Appendix A.

Antibiotic resistance

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

Type of analysis conducted

In each case, a whole genome sequence of the manufactured strain was obtained and analysed for the mechanisms of HGT. Using the sequence, comparisons to known drug resistance markers could be done to determine their presence. When the mechanism of resistance was well documented and genomically located in the sequence, an evaluation of the flanking regions as well as the sequence identity was done. When a mechanism of resistance was not well understood, examination of all the known HGT mechanisms in that strain was completed to rule out a possibility of a resistance gene located in the vicinity. Note that not all drug resistances were evaluated. Only the genes responsible for the drug resistance over the EFSA breakpoint for clinically relevant antibiotics were investigated.

Analysis of *L. paracasei* Lpc-37 (DGCC 4981)

An antibiogram of Lpc-37 (DGCC 4981) was established using the ISO 10932 IDF223 method and VetMIC Lact-1 and 2 micro-dilution plates that included all antibiotics recommended by the FEEDAP. Recorded MICs are displayed in Table 1. MIC values are below or equal to the

Microbial Break Points (MBPs) defined for *Lactobacillus paracasei* (EFSA, 2012). According to these results, Lpc-37 (DGCC4981) does not bear acquired antibiotic resistance.

Genome summary

A proprietary genome sequence of *L. paracasei* Lpc-37 was obtained using published methods. The resulting genome draft yielded 151 contigs with 3,075,295 total basepairs in length and 128X average coverage. Alignment of the resulting draft to whole genome sequences of published *L.* paracasei strains show good overall genomic synteny and core similarity, with unique regions that can be utilized for strain differentiation (Broadbent et al., 2012).

Undesirable antibiotic resistance

Antibiogram of LQ10450 was established using ISO 10932 IDF223 method and VetMIC Lact-1 and 2 micro-dilution plates that include all antibiotics that are recommended by the FEEDAP. Recorded MICs are displayed in the table 1 below. All MIC values are below the Microbial Break Points (MBPs) defined for *Lactobacillus paracasei* (EFSA, 2012). According to these results, DGCC 4981 does not bear acquired antibiotic resistance.

Table 1: Antibiogram of *Lactobacillus paracasei* Lpc-37

APPENDIX : Antibiotic Susceptibility Profile

Method used : ISO 10932 IDF 223 with VetMIC Lact 1 and 2 microdilution plates

	Gentamycin	Kanamycin	Streptomycin	Tetracycline	Erythromycin	Clindamycin	Chloramphenicol	Ampicillin	Vancomycin	Virginamycin*
	Gm	Km	Sm	Tc	Em	Cl	Ch	Amp	Va	Vi*
DGCC 4981	MIC µg/ml									
	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.
Lactobacillus paracasei	4	64	32	1	0,12	0,12	4	1	>128	1
MBP for *Lactobacillus casei / paracasei***	32	64	64	4	1	1	4	4	NR***	4

* Virginamycin is no more included in the FEEDAP recommended list of antibiotics (june 2012) **EFSA Journal 2012;10(6):2740

NR***: not required

Production of biogenic amines

Histamines: In lactic acid bacteria, production of histamine results from the catabolism of histidine by a histidine decarboxylase. A specific detection method for histidine decarboxylase genes has been developed internally to DuPont based on the scientific literature and on the most updated genomic databases. Applied to DGCC 4981, the method failed to detect a histidine decarboxylase gene. Consequently, DGCC 4981 is unlikely to produce histamine.

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

literature and on the most updated genomic databases. Applied to DGCC 4981, the method failed to detect a tyrosine decarboxylase gene. Consequently, DGCC 4981 is unlikely to produce tyramine.

Genetic safety

The genome of *L. paracasei* Lpc-37 was analyzed for bacteriocins, toxin genes, and genes associated with hemolysin production. First, the "Virulence, Disease and Defense" subsystem feature in RAST (Rapid Annotation using Subsystem Technology, http://rast.nmpdr.org/rast.cgi) was mined. Next, the annotations of the genome were mined for key words using the Geneious 6.1.8 viewer. Suspect genes were confirmed using BLAST protein (*blastp*) in NCBI. Finally, local searches were performed using Geneious 6.1.8 with the custom Basic Local Alignment Search Tool (BLAST) function. The following databases were used:

- **BAGELdb**. The database for bacteriocins (http://bagel.molgenrug.nl/).
- **NCBI_bacteriocindb**. 138,176 proteins that are a result from a "bacteriocin" search in *Gene* in the National Center for Biotechnology Information (NCBI).
- **T3db**. A collection of toxin genes from the Toxin and Toxin-Target Database (http://www.t3db.ca/).
- **DBETHdb**. A collection of 229 bacterial endotoxins from 26 pathogenic bacteria (http://www.hpppi.iicb.res.in/btox/).
- **Pioneer_toxin_2016db.** A collection of 7,639 toxin protein sequences from an internal database at DuPont Pioneer.

The protein sequences of Lpc-37 annotations were compared to all of these databases. As noted in the guidelines from European Food Safety Authority in regards to allergen presence, results that match at least 35% of sequence identies in a sliding 80 amino acid window were considered suspect and analysed further. Searches from the various collections were refined based on target, as the searches can broadly incorporate elements that are not related to the query (for example, if "bacteriocin" is in the title of the reference organism). Suspect proteins were assessed using *blastp* and UniProt (www.uniprot.org).

Summary

- A colicin V and a class IIb bacteriocin set comprised of 2 separate proteins were identified along with three prebacteriocins.
- A *dinJ-yafQ* and a *ChpAB* toxin-antitoxin (TA) system were located, but both are not harmful to hosts.
- Prophage holins were also located, but these are not harmful when on the bacterial chromosome.
- Three genes that are associated with hemolysin was identified, but were shown to have transport functions only and do not produce the hemolysin protein.

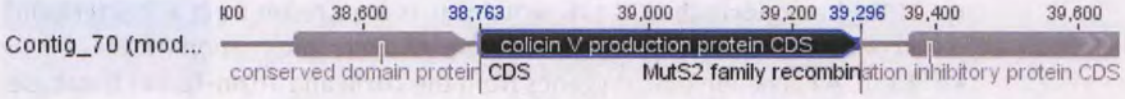
Results

Bacteriocins

Colicin V

Colicins are antimicrobial peptides that are most often encoded in *E. coli* (Gillor et al., 2008), but have been shown to be present in some Gram-positive microbes like Lactobacillus (Trivedi et al., 2014). A colicin V gene was identified in the chromosome of Lpc-37 and matches to genes in other strains of *L. paracasei*. Previous research showed that colicins aid with pathogenesis when employed in pathogenic organisms (Gillor et al., 2008). Another study has shown that colicins can lyse eukaryotic cells, especially cancerous cells, but are dependent on type and mechanism. Colicin V was isolated and determined to be similar to class II Gram-positive bacteriocins (Fath et al., 1994), which are not toxic to mammalian cells (Nes et al., 2015).

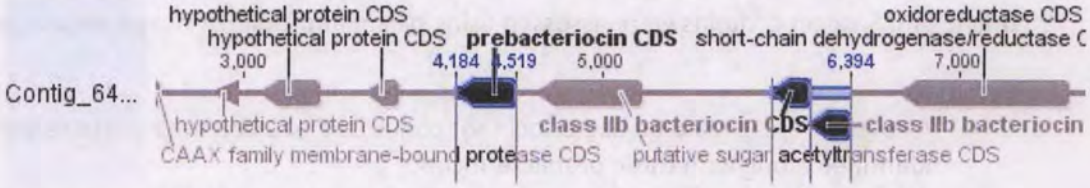
Figure 1: Contig 70



Type IIb bacteriocins

Several genetic factors for type IIb bacteriocin production were identified. This class of bacteriocins is defined by having two separate peptides less than 10 kDa in mass (Drider et al., 2006). Type IIb bacteriocins have not been shown to be toxic to eukaryotic cells, but actually contribute to probiotic qualities of organisms (Gillor et al., 2008).

Figure 2: Contig 64



Prebacteriocins

Three pre-bacteriocins were identified that protect cells from their own bacteriocin proteins (Fimland et al., 2002).

Toxin production

dinJ-yafQ toxin-antitoxin (TA) system

There are two genes that encode the *dinJ-yafQ* toxin-antitoxin system. TA systems are intracellular regulatory mechanisms that are thought to enable distinct functions like gene

regulation, growth control, and programmed cell death (Magnuson, 2007). As such, they pose no danger to hosts. The *yafQ* gene is an endoribonuclease that inhibits translation by cleaving RNAs as part of a type II toxin-antitoxin system (Prozorov et al., 2010, Wang and Wood, 2011). The system is involved specifically in apoptosis and broadly in biofilm creation.

Figure 3: Contig 40

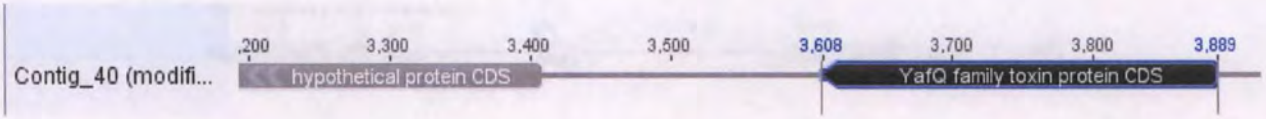
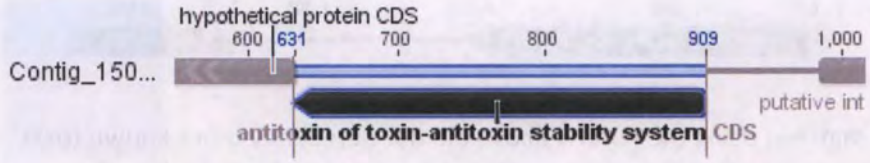


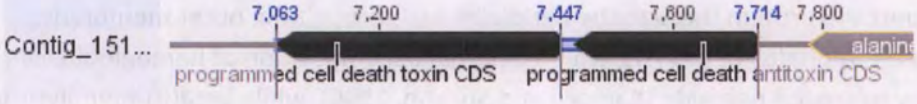
Figure 4: Contig 150



ChpAB

Two genes which comprise a TA system similar to pemK, have been shown in *E. coli* to be key in plasmid maintenance (Masuda et al., 1993).

Figure 5: Contig 151



Holin-like toxins

Holins are typically used in conjunction with endolysins by bacteriophage to lyse bacterial cells (Wang et al., 2000). Holin proteins accumulate inside cells with endolysin until activation, when they form pores in the cell membrane and kill the cell (Wang et al., 2000). A previous study showed λ bacteriophage holins to lyse eukaryotic cells in research for cancer treatment when chemically transfected into the cells via plasmid vectors (Agu et al., 2006). Two holin genes and an endolysin were identified in Lpc-37 and have been identified previously in prophage of other probiotic Lactic Acid Bacteria (Ventura et al., 2003). BLAST analysis of the two Lpc-37 holin-like proteins shows very little similarity (14% identity) to λ-holins and confirms both holin genes to be present in 28 other strains of *L. casei* subsp. *paracasei*. Because previous acute toxicology studies have shown to be negative (Mukerji, 2015), it is concluded that these holins are not toxic to eukaryotic cells while part of the *L. paracasei* chromosome, even when secreted extracellularly.

Figure 6: Contig 68

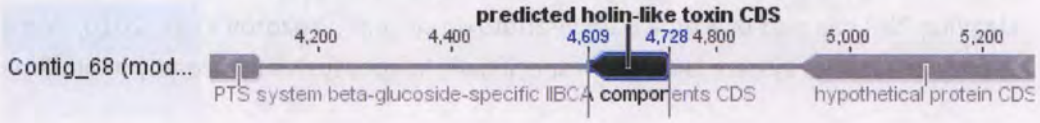


Figure 7: Contig 57

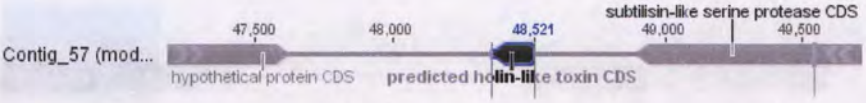
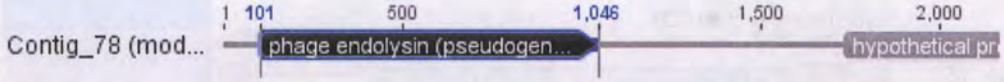


Figure 8: Contig 78



Local BLAST matches using the criteria above did not detect any other known toxin producing genes, only genes involved in transport and normal cellular functions.

Hemolysin

Three genes were located in Lpc-37 with protein sequences related to hemolysins that also match known genes in other Lactobacillus species. Studies show that four separate genes are necessary for hemoloysin production and excretion in *E. coli* (Wagner et al., 1983). Two genes, *hylA* and *hylC* synthesize active hemolysin proteins. The genes *hylBa* and *hylBb* work to transport the protein through the periplasm and through the outer membrane, respectively. Alpha(α)-hemolysis, which is partial decomposition of hemoglobin, can be caused by hydrogen peroxide (Barnard and Stinson, 1996), while beta(β)-hemolysis is the complete lysis of blood cells and gamma(γ)-hemolysis means bacteria have no effect on blood cells. Lpc-37 was plated on 5% sheep's blood agar and resulted in a discolouring indicating that the strain is α-hemolytic. No β-hemolysis, or complete lysis of blood cells was seen. Further, *hylA* and *hylC* protein sequences from *Aquifex aeolicus* and *E. coli,* respectively, had no matches in Lpc-37. It is thus concluded that the genes noted above are involved in cellular transportation, and not in the production of virulent hemolysin.

Conclusions

- Three bacteriocin producing genes and three prebacteriocins were identified. These all target other bacteria and are not a danger to humans.
- Two toxin-antitoxin systems that only target Lpc-37 were located. Two holin genes and an endolysin were also identified, but do not affect human cells. These factors are not a danger to humans. Lpc-37 did not show any adverse effects when tested by acute toxicity testing.
- Lpc-37 is α-hemolytic, meaning that hydrogen peroxide produced by the bacterium causes partial hemolysis of red blood cells. The bacterium does not produce hemolysin, which would cause β-hemolysis, or complete lysis of blood cells.

Lactic Acid Production

The overgrowth of commensal microorganisms capable of producing D-lactate during chronic antibiotic exposure in individuals with intestinal failure has been reported to result in D-lactate acidosis (Hudson et al., 1990). However, the consumption of D-lactate producing bacteria has a long history of safe use because D-lactate is readily metabolized in humans (Ewaschuk et al., 2005; Hudson et al., 1990) and toxicity has not been reported in normal individuals with functional small intestines. Thus, ingestion of probiotics that produce a racemic mix of lactate does not pose a significant risk.

L. paracasei Lpc-37 only produces L(+) lactic acid.

B. Method of manufacture:

Danisco operates multiple DuPont Nutrition and Health culture production and blending facilities in the Unites States, Europe and Asia.

The Danisco USA Inc. Madison, Wisconsin manufacturing site is aligned with DuPont Nutrition and Health. The site consists of two adjacent buildings (Culture Plant/Freeze Dry, Natural Extracts Plant) at 3322 and 3326 Agriculture Drive, Madison, Wisconsin, USA 53716.

The Danisco USA Inc. Rochester, New York manufacturing site is aligned with DuPont Nutrition and Health. The site consists of two adjacent buildings (Live Culture Inoculants Production Plant (LCI) and Direct Fed Microbials Plant (DFM)) at 1700 Lexington Ave, Rochester, New York, USA 14606.

Other DuPont Nutrition and Health cultures production/blending sites are located in France (Dange, Epernon, Sassenage, and Vinay), Germany (Niebull), and China (Beijing).

For a number of Danisco bacterial culture products, production may involve more than one manufacturing facility.

L. paracasei Lpc-37 strain production is initiated in the Rochester, NY facility, where fermentation occurs starting from the culture seed through large scale fermentation. The bacteria are harvested and concentrated into pellet form, and then freeze dried in a qualified facility.

The milling and bulk packaging for *Lactobacillus paracasei* Lpc-37 take place in the Madison, WI facility.

The Danisco Rochester plant manufacturing process, for production of cultures, is a batch type fermentation process where a blend of proteins, carbohydrate, and other vitamins and minerals are blended with water, sterilized, and then inoculated with the selected bacteria. Each fermentation product has a defined growth medium and fermentation growth conditions (pH, temperature).

L. paracasei Lpc-37 is manufactured in compliance with the U.S. Food and Drug Administration's current Good Manufacturing Practice guidelines (21 CFR 117) in FDA regulated and inspected facilities. All ingredients utilized are food grade or approved for use by the FDA (Appendix B). The manufacturing process is summarized below.

The source organism used is *L. paracasei* Lpc-37. The cultures are maintained in the culture bank of Danisco USA Inc. as frozen 1mL vials at -180°C. Danisco USA Inc. independently verifies the identity of each organism. Each seed lot in the culture bank is fully characterized to insure the identity of the seed strains. From the seed vials, Danisco USA Inc. produces concentrated starter for the industrial fermentation.

As the bacteria fermentation products produced by Danisco are destined to be either directly consumed or used as starter cultures for food fermentations such as yogurt manufacture, Danisco takes great care to ensure the quality of the product. These quality control processes begin with the identification, storage and handling of the bacteria seed stocks.

Genus and species designation for each bacterial species have been determined by 16S rRNA testing. For identification on strain level, a specific DNA-fingerprinting technique is applied that ensures identity of the seed stocks. The fingerprinting technique is applied prior to preservation of every strain.

A Master Seed repository is maintained for each of the bacterial strains at the Danisco Global Culture Collection (DGCC) in Niebull, Germany. The repository is a collection of purified, tested, and qualified Master Seed stocks derived from single strain isolates stored at -180°C in liquid nitrogen to maintain long term cell viability.

The microbiological quality of the Master Seeds is determined by microbiological testing for microbiological contamination at the DGCC.

Testing and release of Master Seed vial lots are performed to insure the Master Seeds meet the specifications listed within are absolute acceptance criteria. If a Master Seed vial lot fails any of the required tests, the lot is placed on QC hold to prohibit use and the lot is subsequently destroyed.

Working seed

Working seeds are prepared under controlled conditions from master seed stock maintaining effective acceptance criteria at DGCC. All Working Seeds are prepared under controlled conditions from Master Seed stock meeting established acceptance criteria and each new lot of Working Seeds is held in "quarantine" at liquid nitrogen temperature pending QC testing (strain identity and purity as described for the Master Seeds) and release. If the Working Seed vial lot fails any of the required tests, the lot is placed on QC hold and destroyed. Qualified, tested Working Seed stocks are stored at -76°C until use in production fermentation.

The use of tandem Master and Working seed inventories reduces the risk of genetic drift over time due to excessive sub-culturing of strains and insures the integrity of the strain collection.

All steps in the preparation of Master and Working seed are documented in a specified database, allowing traceability of every seed preparation down to each single batch of raw material used.

Fermentation process

The fermentation begins by withdrawing one of the working seed vials and scaling-up via a series of fermentations until a commercial size batch is complete. The fermentation starts off in a 100mL vessel, then transferred sequentially to a 6 L vessel, then to 300L vessel and finally to the largest vessel where fermentation is completed.

As each organism produces organic acids during metabolism, an ammonium hydroxide base must be injected into the medium to maintain pH at the proper set point to maintain the optimum pH during growth.

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

At the Danisco Madison plant, there are two methods to measure growth in the fermenter. First, flow meters on the ammonium hydroxide feed lines to the fermenters measure the volume of base used to maintain optimum growth pH of the culture. The base addition rate is proportional to the acid developed in the fermentation, which is proportional to cell growth rates.

Second, the pH in the fermenter is monitored on digital display and on recording charts. By consulting these charts, the growth characteristic of a given fermentation can be determined.

Fermenters are normally cooled to stop the fermentation when the pH and base addition data indicate that the fermentation has entered stationary phase. Cooled fermentate is pumped through continuous flow centrifuges and the bacteria are concentrated. Cryoprotectant is added to cooled concentrate and the mixture is then pelletized by immersion of concentrate droplets in liquid nitrogen. These concentrate pellets are then freeze-dried.

Batches of concentrated bacteria are freeze-dried in a qualified facility.

Milling process

The milling process takes place entirely in the Danisco Madison facility. The freeze-dried pellets are milled according to standard procedures utilizing a Fitzpatrick mill fitted with a mesh screen operating at 2000 rpm. Production batch records contain mill charge and appropriate operator sign-off.

Blending process

The blending process is performed in the Madison, WI facility under 21 CFR 111 cGMPs. Blending can occur by either blending in Marion and/or V-blender mixers, or by utilizing Intermediate Bulk Containers (IBCs). The processes are slightly different, but are used interchangeably due to available resources.

Freeze dried pellets are milled according to standard procedures utilizing a Fitzpatrick mill fitted with a mesh screen. The milled pellets, along with approved excipients are added to the blender. All ingredients added to the blender, both milled pellets and excipients, and are documented on production batch record containing traceability information and appropriate operator sign off. Milling and ingredient addition is performed in a controlled environment.

The blender is allowed to mix for an established amount of time prior to packaging to ensure homogeneity.

Product is dispensed out of blender and through metal detector prior to packaging.

Packaging

Bulk packaging of the product is carried out in a controlled environment within the Danisco Madison facility. The HVAC system consists of an air-handling unit with air-cooled direct expansion type condenser including ducted heater for reheating. Pressure relief dampers operate in conjunction with the fresh air intake system maintaining the whole area at a positive pressure to prevent contaminant infiltration to the packaging room. The area design conditions are as follows:

HEPA filter is used in the packaging room for high performance in these demanding operating conditions as the final filter for particulate removal when clean air is required.

Dry Bulb Temperature	72° F
Relative Humidity	≤ 35% RH

Quality Systems

The Danisco Madison plant has fully implemented HACCP plans, Standard Operating Procedures and Quality Control programs to ensure the quality of each product. Danisco Madison has numerous certifications, including ISO FSSC 22000 food safety certification, ISO 9001 Quality Management System certification, and NSF Dietary Supplements cGMP certification.

A quality control laboratory is maintained on site. Quality control personnel are qualified by training and experience to test products and to release product based on specifications. In addition, a third-party laboratory with ISO 17025 certification, located in Madison WI, performs QC testing for Danisco Madison facility under contract.

The Quality Control unit utilizes a SAP computer quality control system for the specification, quality control data entry and product release. No product can be released for use without acceptance by the Quality Control unit according to specified acceptance criteria.

Each bacteria fermentation product must meet specifications and must have a confirmation of identity (compared to the Master Seed) by 16S rDNA sequence analysis or RiboPrinter® analysis for release of the product. Microbiological testing is performed by trained QC microbiologists in the Madison plant laboratory and certified external laboratory using standard methods.

Cleaning and quality testing of the process rooms and equipment are under the control of Manufacturing and Quality Assurance, following the established SOPs. Fermentation rooms are isolated from the freeze-drying processes and access is controlled. Materials cannot enter the milling and blending process areas prior to cleaning, sanitation and subsequent surface testing for cleanliness via ATP testing. Room access is controlled by appropriate signage, and additional protective gowning must be worn in processing rooms where product is potentially exposed. Operator sign-off for clean, sanitation and testing is required on the lot batch ticket. Quality Assurance is responsible for review of completed batch tickets.

Process rooms are segregated from other manufacturing areas with appropriate closures. Room air quality is controlled via HEPA air filtration of incoming air and maintenance of positive pressure in the process rooms relative to adjacent processing areas. HEPA filtration operation is monitored for performance; air quality is monitored monthly by Quality Assurance. Operators may not bring materials into process areas where HEPA filtration is not functioning to specification. Operators sign-off on the lot batch ticket for temperature and humidity and record the temperature and humidity on the batch ticket. Quality Assurance is responsible for review of completed batch tickets.

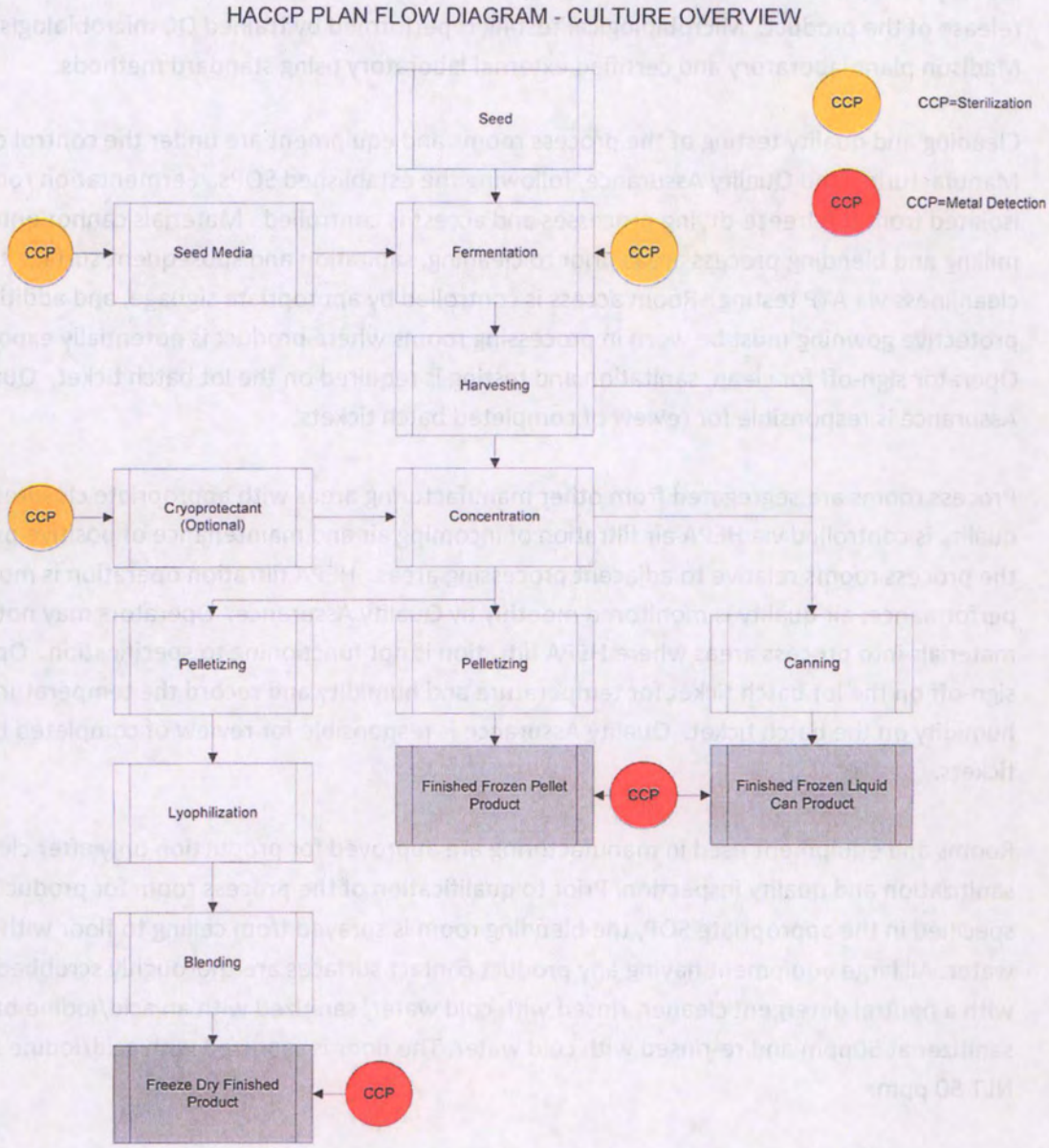
Rooms and equipment used in manufacturing are approved for production only after cleaning, sanitization and quality inspection. Prior to qualification of the process room for production, as specified in the appropriate SOP, the blending room is sprayed from ceiling to floor with 145-160°F water. All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

Process rooms and equipment are tested by Quality Assurance following cleaning and sanitation for microbial contamination and test results are entered on the batch tickets with Quality Assurance sign-off. ATP and Microbiological swabs are taken after cleaning and sanitation. Room and equipment surfaces must be negative by test to qualify for use in production.

Batch records are maintained as per Standard Operating Procedures and are provided to Quality Assurance for each lot produced. Quality Assurance is responsible for batch ticket review.

A schematic overview of the manufacturing process is presented in Figure 9 below. Specifications are listed in Table 2.

Figure 9: Manufacturing Flow Diagram



C. Specifications for food-grade material

Table 2: Product Specifications

Parameter	Specification	Method
Description	White to cream-colored free-flowing powder	
Particle Size		
Color	Beige	
Odor	Characteristic	
Taste	Characteristic	
Viable cell count	≥ 4.00 x 10^{11} CFU/g	ISO 7889/IDF117
Proximates (per 100 g)		
Carbohydrates (g)	48.1	
Protein (g)	19.1	
Moisture	5.4	
Fats (g)	0.9	
Fiber (g)	2.5	
Sodium (mg)	2480	
Heavy metals		
Lead	< 1 ppm	AOAC 984.27
Cadmium	< 3 ppm	AOAC 984.27
Mercury	< 3 ppm	AOAC 984.27
Microbiological purity		
Non-lactic Cell Count	< 5,000/g	ISO 13559
Enterococci (CFU/g)	< 100/g	CMMEF, Current Ed.
Coliform (MPN)	Negative by test in 10 g	AOAC 966.24
Escherichia coli (MPN)	Negative by test in 0.3 g	AOAC 966.24
Staphylococcus (coagulase +)	Negative by test in 40 g	AOAC 975.55
Salmonella	Negative in 40 g	AOAC 2004.3
Listeria	Negative in 25 g	AOAC 999.06
Molds and Yeast	< 200 CFU/g	USP

Batch analysis

Certificates of analysis of 4 non-consecutive batches of finished product are included in Appendix C. These indicate that the manufacturing process consistently meets product specifications and is not contaminated.

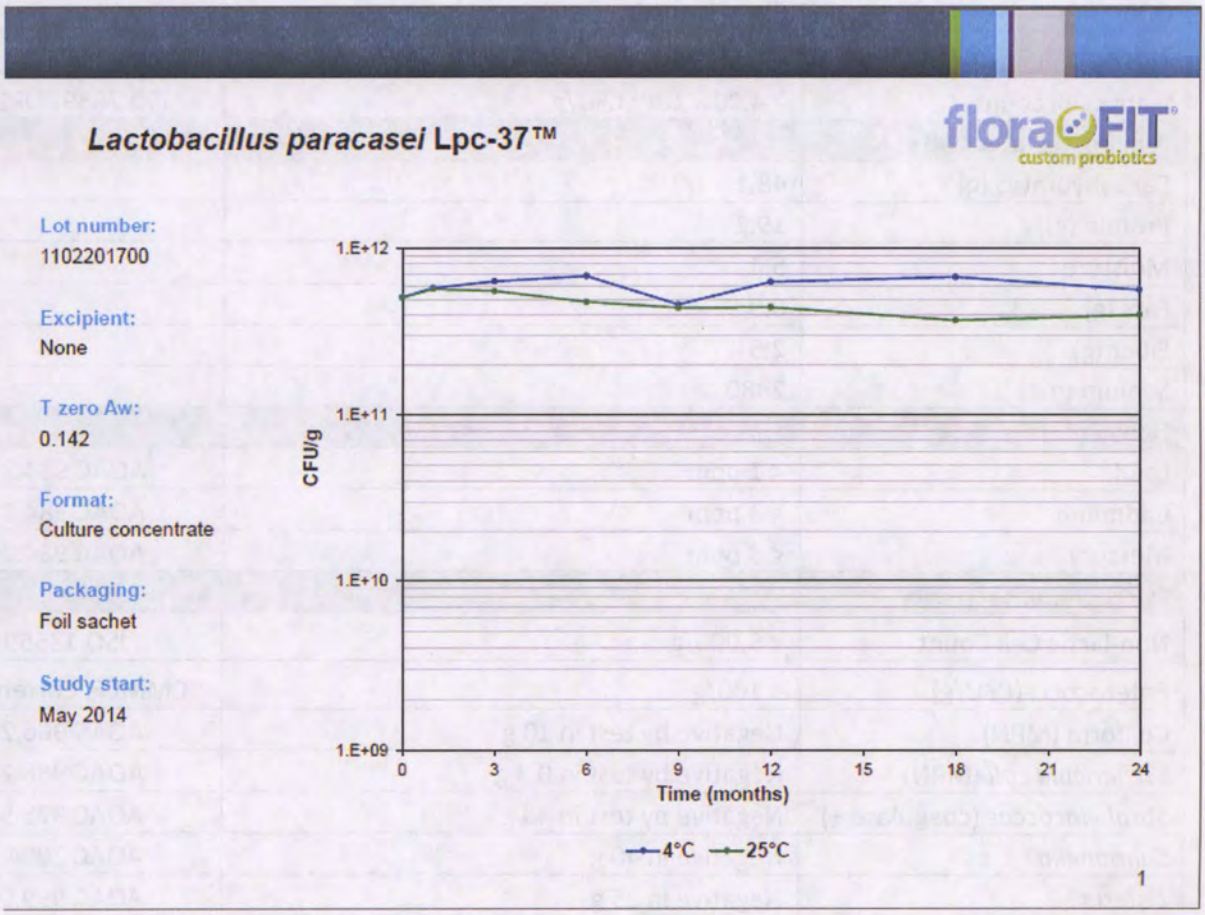
Enumeration

Enumeration is performed to obtain the total bacterial cell count per gram in a sample. The results of this test are used to determine if a sample has the required number of bacteria to qualify for an intermediate or final product.

Stability

The stability of *L. paracasei* Lpc-37 was analyzed at refrigerated (4°C) and at room temperature (~23°C) over a 24-month period by monitoring viable cell counts at regular intervals.

ytFigure 10: Stability diagram



GMO Status

DuPont Nutrition & Health certifies that *L. paracasei* Lpc-37 is conventional (non-GMO). Any culture strain used in the manufacture of these products or any culture strain contained as part of this product has itself not been genetically modified according to Directive 2001/18/EC neither subject to the labeling requirement of (EC) 1830/2003 nor to the authorization procedure of Regulation (EC) 1829/2003 (Appendix D)

Allergens

The *L. paracasei* Lpc-37 is negative for wheat, other cereals containing gluten, crustacean shellfish, eggs, fish, peanuts, soybeans, milk (including lactose), nuts, celery, mustard, sesame seed, sulfur dioxide and sulfites, lupin and molluscs (Appendix D).

Part 3 – Dietary exposure

A. Current dietary exposure of *L. paracasei* Lpc-37

Danisco has proposed the use of *L. paracasei* Lpc-37 in yogurt and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and also in supplement format including sachets, tablets and capsules. It is intended to be added to conventional foods at levels sufficient to ensure at least $1x10^{10}$ CFU/serving throughout the shelf of the product and in dietary supplements to ensure at least $5x10^{10}$ CFU/serving.

B. Intended human food uses (estimated daily intake)

Considering the average individual consumes only about 20 servings/day of all foods combined (Millen et al., 2005), a conservative estimate of the total estimated daily intake at $5x10^{11}$ CFU/serving times 10 servings/day would estimate a maximum intake of $5x10^{12}$ CFU/person/day of either conventional food or dietary supplement. It is unlikely that a consumer would consume 10 servings of foods containing Lpc-37 and the number of CFU will decline over the shelf-life of the food. It is likely the maximum ingestion is thus less than the $5x10^{12}$ CFU/day and well within the levels that have been shown to be safe.

Part 4 – Self-limiting levels of use

There is no self-limiting level of use for Lpc-37, and use will be restricted to those food types that can support viability of Lpc-37 throughout the shelf-life of the product.

Part 5 - Experience based on common use in food before 1958

Lactic acid bacteria have long been considered safe and suitable for human consumption. Very few instances of infection have been associated with these bacteria and several published studies have addressed their safety. (Borriello et al., 2003; Gueimonde et al., 2004)

Lactobacillus species have historically been considered safe and suitable for human consumption with several published studies addressing its safety. (Aguirre and Collins, 1993; Gasser, 1994; Salminen et al., 1998) *Lactobacillus paracasei* has been included as one of the many microorganisms intentionally added to food that should be regarded as safe based on EFSA's comprehensive assessment of safety. A list of qualifying microorganisms was compiled to represent those that meet the criteria of Qualified Presumption of Safety (QPS) and do not raise safety concerns (EFSA, 2007). This QPS list has been updated frequently and the *L. paracasei* listing is included. The most recent update indicates no safety concerns, and so the listing of *L. paracasei* remains in the 2013 QPS update (EFSA, 2013).

Lactobacillus paracasei has also been documented as recognized as having a technical role in fermented food products. *Lactobacillus* species have a long history of safe use when consumed as part of dairy food and supplement products, with thirty-plus *Lactobacillus* species listed in IDF Bulletin No. 377: *Inventory of Microorganisms with a Documented History of Use in Food* (Mogensen et al., 2002). A more recent IDF Bulletin No. 455, *Safety Demonstration of Microbial Food Cultures in Fermented Food Products*, provides an update to the aforementioned inventory of microbial species, taking a global perspective versus the original focus of European dairy products. The updated inventory lists a reorganization of the *Lactobacillus* species included, with eighty-four species of *Lactobacillus* listed, and references *L. paracasei* subsp. *paracasei* as originally documented in 1970 (Bourdichon et al., 2012).

Part 6 - Narrative

A. Review of safety information

1. History of consumption of *L. paracasei*

As indicated previously, the *L. paracasei* strain Lpc-37 was isolated from a dairy source and identified according to standard taxonomic guidelines. Lpc-37 has been in commercial for more than 15 years, and is a lyophilized bacteria fermentation product that is produced in accordance with cGMP as provided for in 21 CFR 110 and 21 CFR 111. Danisco USA, Inc sells Lpc-37 for inclusion in food and dietary supplement products globally. Lpc-37 has been sold to food and dietary supplement companies worldwide, including those in North America, Central America, South America, China, South Africa, European countries and Asia/Pacific countries.

2. Regulatory History of *L. paracasei* and related lactobacilli

In 2008, PURAC submitted a GRAS notification (GRN 240) to the US. Food and Drug Administration (FDA) for a food ferment solution as a food ingredient (PURAC, 2008). The subject of the notice was corn, cane, or beet sugar cultured with *Lactobacillus paracasei* subsp. *paracasei*, *Bacillus coagulans* and *Proprionibacterium freudenreichii* subsp. *Shermanii* for use as a flavoring and antimicrobial agent for meat and poultry products at levels of 2.0 – 4.8%, including beef, lamb and goat, organ meats, pork, veal; fresh poultry (chicken, duck, other poultry, turkey), meat products (bacon, frankfurters, ham, luncheon meats, processed meat products), and poultry products (processed poultry products). During processing, the microorganisms are removed so the final amount in the meat product is negligible. The FDA reviewed the GRAS notification GRN240 and responded that it had no questions (CFSAN, 2008).

In 2011, PURAC submitted a GRAS notification (GRN 378) to the US. Food and Drug Administration (FDA) for a food ferment solution as food ingredient (PURAC, 2011). The subject of the notice was cultured dairy sources, sugars, wheat, malt, and fruit- and vegetable-based sources fermented by *Lactobacillus paracasei* subsp. *paracasei* and other probiotic bacteria including *Streptococcus thermophilus, Bacillus coagulans, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus sakei, Lactobacillus bulgaricus* and *Proprionibacterium freudenreichii* subsp. *shermanii* or mixtures of these microorganisms for use as antimicrobial agents in a variety of food categories typically at levels of 0.1 to 4.5%, including meat and poultry, but excepting infant formula and infant foods. The use of the microorganisms was primarily as a processing aid. During processing, the microorganisms are removed so the final amount in the dairy products is negligible. The FDA reviewed the GRAS notification GRN 378 and responded that it had no questions (CFSAN, 2012).

3. Safety of lactic acid bacteria and Lactobacillus species

Lactobacillus paracasei

In phase one of a two-phase study, nine healthy adults consumed capsules containing $1x10^{10}$ CFU of each *L. gasseri* 177 and E16B7, *L. acidophilus* 821-3, *L. paracasei* 317, and *L. fermentum* 338-1-1, for five consecutive days. Data on gut health, blood parameters and liver and kidney function were collected. In phase two of this study, five additional healthy adults consumed $1x10^{10}$ CFU of *L. acidophilus* for five consecutive days. In both phases of the study, the feeding of high doses of different *Lactobacillus* strains did not induce any severe adverse effects in the gastrointestinal tract and/or abnormal values of blood indices (Hütt et al., 2011).

In a randomized, double-blind, placebo-controlled study, 48 healthy adults consumed *L. paracasei* LC01at a level of $1x10^{10}$ CFU/day for four weeks to evaluate the effect of the bacterium on human intestinal microflora. Real-time PCR and biochemical analyses was used to determine the intestinal bacterial composition, and the concentration of short-chain fatty acids and ammonia of fecal samples. After the four-week treatment and a two-week washout period, a significant inhibition in fecal *Escherichia coli* and an increase in *Lactobacillus, Bifidobacterium,* and *Roseburia intestinalis* were observed. This, combined with the fact that there were no adverse events during the study period, led the authors to conclude that human consumption of *L. paracasei* exerts positive effects on the intestinal microflora of young adults. (Zhang et. al., 2013)

In this double-blind, placebo-controlled, parallel dose-response study, the immunomodulating effect of *L. paracasei* CR431 and *B. lactis* BB-12 was studied in 75 healthy young adults, using phagocytic activity of blood cells, fecal IgA and cytokine production in *in-vitro*-stimulated blood as the main read-outs. Although there were no solid effects on the immune function of young healthy adults supplemented with high doses of *L. paracasei* and *B. lactis,* no adverse events were reported in this study and the authors concluded that it cannot be concluded that that the tested bacteria exert no health-promoting effects. (Christensen et al., 2006)

In a randomized, triple-blinded study, placebo-controlled study was conducted in healthy adults to evaluate the effects of daily consumption of fermented probiotic mill on immune response towards influenza virus type H1N1, H3N2 and Flu-B. Sixty participants consumed 100 mL daily of fermented milk products containing either 10^9 CFU of *L. paracasei* 43 or a placebo. After two weeks of consumption, the subjects received an intramuscular injection with 0.5 mL of affluenza vaccine. The results showed that drinking the fermented milk containing *L. paracasei* improved the seroconversion rate of total antibodies against H1N1 and H3N2 viral antigens, showing that a probiotic product may enhance response rate to influenza vaccine. Although additional safety parameters were not reported, it was mentioned that drinking fermented milk is already marketed and the sensory properties are acceptable by general consumers. (Trachootham et al., 2017)

Verdenelli et al. (2011) conducted a study to investigate the effect of different kinds of food products enriched with a combination of two potential probiotic strains, *L. rhamnosus* IMC 501® and *L. paracasei* IMC 502® on bowel habits healthy adults. The study was a double-blind, placebo feeding study in which 47 healthy adults participated for 12 weeks, consuming one or more food products enriched with a combination of the two strains at a minimal level of 10^9 CFU in each food. This study demonstrated that the consumption of the two potential probiotic strains tested, was well tolerated and exerted a beneficial effect on the bowel habits of healthy adults.

In a randomized, double-blind, controlled, cross-over study, Riezzo et al. (2012) evaluated the effects of artichokes enriched with *L. paracasei* IMPC 2.1 at $2x10^{10}$ CFU on treatment preference, symptom profile and short-chain fatty acid (SCFA) production in 20 constipated adults compared with ordinary artichokes over two separated 15-day feeding periods. In this study, 80% of the patients preferred probiotic-enriched artichokes to ordinary ones. For symptom profile, satisfactory relief of symptoms was significantly higher during the probiotic enriched artichoke period and no adverse events were noted during the study period.

In a 21-day, randomized, double-blind, active controlled parallel study by West et al. (2012) it was determined that a symbiotic supplement of three capsules a day, containing *L. paracasei* 431® at $4.6x10^8$ CFU, *B. lactis* BB-12® at $6x10^8$ CFU, *L. acidophilus* LA-5® at $4.6x10^8$ CFU, *L. rhamnosus* LGG® at $4.6x10^8$ CFU, elicits favorable changes in colonic microbiota in healthy, physically active males. It was reported that there were five episodes of mild GI symptoms that included flatulence and stomach rumbles in both the test group and the control group during the supplementation. It was reported that both supplements were otherwise well tolerated.

The aim of a double-blind, randomized, cross-over study by Perrin et al. (2014) was to compare the effect of a powder form of *L. paracasei* NCC2461 to the effect of a blend of *L. acidophilus* ATCC SD5221 and *B. lactis* ATCC SD5219 in patients with allergic rhinitis to grass pollen. A total of 28 subjects completed the study which consisted of two phases of 4 weeks feeding of *L. paracasei* at $1x10^{10}$ CFU/day. While no effect was observed on nasal congestion, a significant decrease in nasal pruritus was seen with the *L. paracasei* treatment. The authors also noted that consumption was well-tolerated and there was no noticeable clinical issue and no formulation related adverse events during the study.

To confirm the anti-allergic effects of *L. paracasei* NCC 2461 in grass pollen allergic subjects exposed to natural doses of allergens during the pollen season, Nembrini et al. (2015) set up a double-blind, randomized, placebo-controlled, parallel study with 131 pollen allergic adults. Each subject, having a clinical history of allergic rhinitis to grass pollen, consumed a daily sachet containing $5x10^9$ CFU of *L. paracasei* for 8 weeks. In this study, no significant differences were observed in allergic rhinitis symptoms scores, quality of life, or specific IgE levels between the treatment group and the control group. Additional safety parameters were not reported.

In a randomized, double-blind, placebo-controlled, crossover study to determine the impact of a probiotic on the intestinal microbial ecology, 34 healthy adults consumed a daily capsule of $2.4x10^{10}$ CFU

of *L. paracasei* DG for 4 weeks. The capsules were well tolerated by all participants and no adverse events were reported. The results showed significant increased evacuations after probiotic supplementation but not after the placebo. The probiotic intervention showed a rebalancing effect on short chain fatty acids, particularly butyrate. The authors believe that the fecal butyrate concentrations could represent an important biomarker to identify subjects who may benefit from probiotic treatment (Ferrario et al., 2014).

A multi-center, randomized, double-blind, placebo-controlled trial with 100 healthy adults in three different cities was carried out to evaluate the tolerance, safety, gut colonization, and immunomodulatory effects of three probiotics. The test subjects were divided into five separate groups and received a daily treatment of a placebo, $9x10^9$ CFU of *L. paracasei* CNCM I-4034, $9x10^9$ CFU of *B. breve* CNCM I-4035, $9x10^9$ CFU of *L. rhamnosus* CNCM I-4036, or $9x10^9$ CFU of *B. breve* CNCM I-4035/*L. rhamnosus* CNCM I-4036 blend for 30 days. Gastrointestinal symptoms, defecation frequency and stool consistency were not altered by probiotic intake, and there were no relevant changes in blood and serum parameters. An immunomodulatory effect was seen, however through increased levels of anti-inflammatory molecules. No adverse events occurred during and after treatment. The authors concluded that the intake of these three bacterial strains was safe ad exerted a varying degree of immunomodulatory effects (Plaza-Diaz et al., 2013).

Jespersen, et al. (2015) set up a randomized, double-blind, placebo-controlled, two-arm, parallel-group study to investigate the effect of *L. paracasei* 431 on immune response to influenza vaccination and respiratory symptoms in healthy adults. The study tested 1104 subjects at two centers in Germany and Denmark. Each subject received an acidified milk drink containing $\geq 1x10^9$ CFU of *L. paracasei* or a placebo acidified milk drink for 6 weeks, 3 weeks before and 3 weeks after a challenge with the seasonal influenza vaccination. There was a total number of 2212 adverse events in 914 subjects reported. Of these, 41 events in 34 of the subjects (21 in the probiotic group and 20 in the placebo group), were assessed as study product related. The most prevalent of the product-related adverse events were gastrointestinal disorders (48% of events) and nasopharyngitis (29%). In total, 373 events in 344 subjects (186 in the probiotic group and 187 in the placebo group) were assessed as vaccine related. Five adverse events were defined as serious, however none of these were assessed to be related to the study product or vaccine. This study did not show an observable effect on the components of the immune response to influenza vaccination, but a reduction in the duration of upper respiratory symptoms was seen.

In a randomized, double-blind, cross-over with parallel groups study, Balzaretti et al. (2015) determined, using 8 healthy women, consuming 24 billion CFU daily of *L. paracasei* LPC-S01 (DSM 26760) for 7 days, that it is a safe bacterial strain for human consumption, which does not contain any acquired antibiotic resistance, does not produce biogenic amines and can be administered in high number to healthy people without adverse events.

In a 90-day oral toxicity study done in rats, Jia et al. (2011) found that *L. paracasei* GM080 did not lead to any general organ or systemic toxicity when fed to rats at dietary concentrations as high as 5.0 g/kg

body weight (approximately equivalent to $1x10^{10}$ CFU/kg bw). The *L. paracasei* was fed at various levels, suspended in water, to 40 healthy male and female Sprague-Dawley rats by gavage for 90 days. The rats were observed twice daily for abnormalities, physical appearance and morality. Blood was collected post-mortem for hematology and clinical chemistry studies. No mortality or treatment related adverse clinical reactions were found during this study and there was no general organ or systemic toxicity seen. Isolated statistically significant changes of some hematology and clinical chemistry parameters in the treatment groups were observed, however the changes were found to be not dose-responsive and were within the laboratory's historical normal range of controls and were not considered to be of toxicological significance.

Lee et al. (2017) studied the consumption of a yogurt containing *L. paracasei* 431®at $12x10^8$, *B. lactis* BB-12®at $12x10^8$ CFU/day, 0.0175%/day, and heat-treated *L. plantarum* nF1. The study was a randomized, open-label, placebo-controlled study of 152 non-diabetic adults for 12 weeks and no adverse events were reported from the participants. The study results demonstrated that consumption of yogurt containing these cultures could enhance the immune response, particularly in immunocompromised populations.

Saito et al. (2017) conducted a randomized, double-blind, placebo-controlled, parallel-group study as well as a safety examination under excessive consumption study using 54 adults consuming up to $10x10^{11}$ CFU/day of *L. paracasei* K71 for 4 weeks. All adverse events reported in each trial were determined to be unrelated to the intake of the test compound and it was determined that this culture is safe at high consumption levels and it can be a dietary approach to enhance mucosal immune function.

In another multi-center, randomized, double-blind, placebo controlled study, 41 adult males consumed a daily sachet containing $5x10^9$ CFU/g *L. paracasei* B21060 plus 1243g arabinogalctan plus 700 mg oligo-fructosaccharides plus 500 mg L-glutamine for 6 months. The intent of this study was to inprove the quality and quantity of spermatozoa in idiopathic oligoasthenoteratospermia (iAOT) patients. The researchers concluded from this study that the sachets taken constitutes a safe therapy for improving the volume of the ejaculate and the quality/quantity of spermatozoa in iOAT patients. (Maretti & Cavallini, 2017)

Wattanarat et al. (2015) compared the salivary HNP1-3 levels between probiotic treatment group and a control group in a randomized, double-blind, placebo-controlled, parallel groups trial on 60 healthy school children. *L. paracasei* SD1 was spray-dried into skimmed milk powder. The children then drank a reconstituted skimmed milk product containing $7.5x10^8$ CFU of the *L. paracasei* or a control reconstituted skimmed milk product per day for six months. Measurements of salivary HNP1-3, MS and LB levels were taken at T0 (baseline), T3 (after three months of intervention), T6 (after six months of intervention), and at T12 (Six months after cessation of intervention). There were no adverse side effects from the probiotics or the milk powder intake reported and it was concluded that the test product can temporarily enhance salivary HNP1-3 levels and decrease the numbers of MS, while an

increase in LB counts. The authors concluded that the increment of pit and fissure caries, but not of smooth surface caries was diminished by probiotic supplementation in the form of milk powder.

In a 12-week, double-blind, randomized, placebo-controlled trial, 60 children with Perennial Allergic Rhinitis (AR) were given either $2x10^9$ CFU/capsule *L. paracasei* HF.A00232 as a supplementary agent to levocetirizine or a placebo capsule with the levocetirizine for eight weeks and a shift to usage of levocetirizine as a rescue treatment during the last four weeks of the trial. Parameters that were evaluated included nasal, throat and eye TSS, TSS and levocetirizine use, and were recorded daily. Physical examinations which included a Pediatric Rhinoconjunctivitis Quality of Life Questionnaires (PRQLQs), and blood samples were obtained for evaluation at baseline, week 8, and week 12. No serious adverse events were recorded in either group and the vital signs and physical examination of all systems revealed no differences between the two groups. No add-on effect from the *L.* paracasei as a supplement to levocetirizine was seen in the first eight weeks, however several individual symptoms, including sneezing, itchy nose and swollen puffy eyes showed significant improvement at the end of the study in the probiotic group. The authors concluded that this may continuously improve the life quality of children with AR, with an approximate 56% reduction in levocetirizine usage. (Lin et al., 2014)

Wang & Wang (2015) conducted a double-blind prospective, randomized, placebo-controlled study on 220 children with moderate-to-severe Atopic Dermatitis (AD). The study was to assess the effects of *L.* paracasei and *L. fermentum*, and their mixture on the disease severity, quality of life and immune biomarkers of these children. The children were divided into groups and received *L. paracasei* GMNL-133 at $2x10^9$ CFU/day or *L. fermentum* GM090 at $2x10^9$ CFU/day or *L. paracasei/L. fermentum* mix at $4x10^9$ CFU/day or a placebo for three months. Changes in severity scoring of atopic dermatitis (SCORAD), Family Dermatology Life Quality Index (FDLQI), and Children's Dermatology Life Quality Index (CDLQI) scores in the different groups and at different visits were evaluated. Skin prick tests, levels of IgE, IFN-ɣ, IL-4, TGF-β, and TNF-α, and urine biomarkers were also evaluated. In the study, the children who received any of the probiotic products, showed lower SCORAD scores than the placebo group and the difference remained out to four months after discontinuing the probiotics. The authors noted that there were no group differences in bowel cramps, fecal frequency, and gastroenteritis. They also concluded that exposure to these probiotics or a combination of these probiotics is an effective intervention for reducing the severity of AD and can improve the quality of life of patients.

A study by Neto et al. (2013), was aimed to evaluate some effects of symbiotic supplementation on inflammatory markers and the body composition of elderly at risk of frailty. This double-blind study lasted three months and followed 17 elderly individuals who were divided randomly into two groups. One group received *L. paracasei* at 10^8-10^9 CFU/day, *L. rhamnosus* at 10^8-10^9 CFU/day, *L. acidophilus* at 10^8-10^9 CFU/day, *B. lactis* at 10^8-10^9 CFU/day, and 6g *frutooligossacarides*/day, while the second group received a placebo for the three months. The subjects were analyzed for anthropometric measurements, bioelectric impedance with vectorial analysis, IL-6, and TNF-α. A comparison between groups did not show any difference for the variables investigated, however, electrical impedance demonstrated that the majority of the test group individuals maintained or improved their tissue hydration, when compared to the placebo group after supplementation, leading the authors to conclude

that consumption of this probiotic combination demonstrated a trend towards a preservation of hydration status in apparently healthy elderly individuals. Additional safety parameters were not reported.

Table 3: Studies on *Lactobacillus paracasei*

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
Two-phase feeding	9 healthy adults (20-68 y)	*Lactobacillus paracasei* 317 at $1x10^{10}$ CFU/day, *Lactobacillus gasseri* 177 and E16B7 at $1x10^{10}$ CFU/day, *Lactobacillus acidophilus* 821-3 at $1x10^{10}$ CFU/day, *Lacobacillus fermentum* 338-1-1 at $1x10^{10}$ CFU/day	5 days	Did not induce any severe adverse events in the gastrointestinal tract and/or abnormal values of blood indices.	Hütt et al., 2011
Randomized, double-blind, placebo-controlled	48 healthy adults (20-28 y)	*Lactobacillus paracasei* LC01 at $1x10^{10}$ CFU/day	4 weeks	A significant inhibition of *E. coli* and an increase in *Lactobacillus, Bifidobacterium,* and *Roseburia intestinalis* was seen. No adverse events reported.	Zhang et al., 2013
Double-blind, placebo-controlled, parallel dose-response	75 healthly, young adults (18-40 y)	*Lactobacillus paracasei* CRL-431® and *Bifidobacterium lactis* BB-12® at 10^8-10^{11} CFU/day	3 weeks	No adverse events reported and no solid effects on the immune function were seen.	Christensen et al., 2006
Randomized, triple-blinded, placebo controlled	60 healthy adults (18-45 y)	*Lactobacillus paracasei* 431 at 10^9 CFU/day	6 weeks	Improved seroconversion rate of total antibodies against H1N1 and H3N2 viral antigens. Additional safety parameters were not reported.	Trachootham et al., 2017
Double-blind, placebo	47 healthy adults (23-65 y)	*Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® at $>10^9$ CFU/day	12 weeks	The consumption of these strains was well-tolerated and a positive effect on improved bowel habits was seen.	Verdenelli et al., 2011

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
Randomized, double-blind, controlled, cross-over	20 constipated adults (24-53 y)	*Lactobacillus paracasei* IMPC 2.1 at $2x10^{10}$ CFU/day	2x15 days	No adverse events were noted and satisfactory relief of symptoms was significantly higher during the study period.	Riezzo et al., 2012
Randomized, double-blind, active controlled parallel	22 healthy, physically active males (27-40 y)	*Lactobacillus paracasei* 431® at $4.6x10^8$ CFU/day, *Bifidobacterium lactis* BB-12® at $6x10^8$ CFU/day, *Lactobacillus acidophilus* La-5® at $4.6x10^8$ CFU/day, *Lactobacillus rhamnosus* LGG® at $4.6x10^8$ CFU/day and two prebiotics (raftiline and raftilose)	21 days	Consumption was well-tolerated. Favorable changes in colonic microbiota was seen.	West et al., 2012
Randomized, double-blind, cross-over	28 adults with allergic rhinitis to grass pollen (18-35 y)	*Lactobacillus paracasei* NCC 2461 at $1x10^{10}$ CFU/day or *Lactobacillus acidophilus* ATCC SD5221 and *Bifidobacterium lactis* ATCC SD5219 at $1x10^{10}$ CFU/day	2x4 weeks	There was no noticeable clinical issue and no formulation-related adverse event during the study; consumption was well-tolerated. A reduction in nasal pruritus was seen.	Perrin et al., 2014
Randomized, double-blind, placebo-controlled, parallel	131 pollen-allergic adults (18-65 y)	*Lactobacillus paracasei* NCC 2461 at $5x10^9$ CFU/day	8 weeks	No significant difference was seen between the treatment and control groups. Additional safety parameters were not reported.	Nembrini et al., 2015
Randomized, double-blind, placebo-controlled, cross-over	34 healthy adults (23-55 y)	*Lactobacillus paracasei* DG at $\geq 2.4x10^{10}$ CFU/day	4 weeks	A measurable impact on the fecal microbiota was seen. Consumption was well tolerated; no adverse events were reported.	Ferrario et al., 2014

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
Multi-center, randomized, double-blind, placebo-controlled	100 healthy adults (26-30 y)	*Lactobacillus paracasei* CNCM I-4034 at $9x10^9$ CFU/day, *Bifidobacterium breve* CNCM I-4035 at $9x10^9$ CFU/day, *Lactobacillus rhamnosus* CNCM I-4036 at $9x10^9$ CFU/day	30 days	Safe and tolerable, no serious adverse events occurred. An increased level of anti-inflammatory molecules was seen.	Plaza-Diaz et al., 2013
Randomized, double-blind, placebo-controlled, two arm parallel group	1104 healthy adults (18-60 y)	*Lactobacillus paracasei* 431 at $\geq 10^9$ CFU/day	6 weeks	A reduction in upper respiratory symptoms was seen, however, 2212 adverse events reported in 914 subjects, 41 events in 34 subjects were assessed as study product related (gastrointestinal disorders and nasopharyngitis), 5 serious adverse events were reported but none were assessed to be study product related.	Jespersen et al., 2015
Randomized, double-blind, cross-over with parallel groups	8 healthy adult women	*Lactobacillus paracasei* LPC-S01 (DMS 26760) at $2.4x10^{10}$ CFU/day	1 week	The strain is a safe bacterial strain for human consumption, which can be administered in high number to healthy people without adverse events.	Balzaretti et al., 2015

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
90-day oral toxicity study	40 healthy male and female Sprague-Dawley rats	*Lactobacillus paracasei* GW080 up to 5.0g/kg bw ($1x10^{10}$ CFU/kg bw)	90 days	No general organ or systemic toxicity was seen. No effects were of toxicological significance.	Jia et al., 2011
Randomized, open-label, placebo-controlled	152 non-diabetic adults (>60 y)	*Lactobacillus paracasei* 431® at $12x10^8$, *Bifidobacterium lactis* BB-12® at $12x10^8$ CFU/day, 0.0175%/day, heat-treated *Lactobacillus plantarum* NF1	12 weeks	An enhanced immune response was seen and no adverse events reported.	Lee et al., 2017
Randomized, double-blind, placebo-controlled, parallel group	54 adults (20-64 y)	*Lactobacillus paracasei* K71 at $6\text{-}10x10^{11}$ CFU/day	4 weeks	Safe at high levels of consumption and can be a dietary approach to enhance mucosal immune function.	Saito et al., 2017
Multi-center, randomized, double-blind, placebo-controlled	41 adult males (30-43 y)	*Lactobacillus paracasei* B21060 at $5x10^9$ CFU/day, plus arabinogalctan (1243 mg), plus oligo-fructosaccharides (700 mg), plus L-glutamine (500 mg)	6 months	Shown to be a safe therapy for idiopathic oligosthenoterospermia (iOAT) patients.	Maretti & Cavallini, 2017
Randomized, double-blind, placebo-controlled, two parallel groups	60 children (13-15 y)	*Lactobacillus paracasei* SD1 at $7.5x10^8$ CFU/day	6 months	A temporary enhancement of salivary HNP1-3 levels was seen. No adverse side effects were reported.	Wattanarat et al., 2015
Randomized, double-blind, placebo-controlled	60 children (6-13 y)	*Lactobacillus paracasei* HF.A00232 at $5x10^9$ with Levocetirizine	8 weeks	My continuously improve the life quality of children with Allergic Rhinitis. No serious side effects reported.	Lin et al., 2014

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
Randomized, double-blind, placebo-controlled	220 children with Atopic Dermatitis (1-18 y)	*Lactobacillus paracasei* GMNL-133 at $2x10^9$ CFU/day or *Lactobacillus fermentum* GM090 at $2x10^9$ CFU/day or *Lactobacillus paracasei/Lactobacillus fermentum* mix at $4x10^9$ CFU/day	3 months	Determined to be an effective intervention for reducing the severity of Atopic Dermatitis and improving the quality of life for patients. There were no group differences in adverse events seen.	Wang & Wang, 2015
Randomized, double-blind	17 healthy elderly individuals (60-74 y)	*Lactobacillus paracasei* at 10^8-10^9 CFU/day, *Lactobacillus rhamnosus* at 10^8-10^9 CFU/day, *Lactobacillus acidophilus* at 10^8-10^9 CFU/day, *Bifidobacterium lactis* at 10^8-10^9 CFU/day, and 6g *frutooligossacarides*/day	3 months	No significant differences seen in inflammation or body composition although a subtle trend towards an improve hydration status was seen. Additional safety parameters were not reported.	Neto et al., 2013

Safety of *Lactobacillus paracasei* Lpc-37

L. paracasei Lpc-37 was administered by oral gavage to five fasted female rats at a dose of 5000mg/kg. The rats were then observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The rats were necropsied to detect grossly observable evidence of organ or tissue damage. There were no incidents of mortality, clinical abnormalities, or overall body weight losses. Gross findings were limited to a large spleen identified in one animal, which was considered non-specific. It was concluded that under the conditions of this study, *L. paracasei* Lpc-37 was not considered acutely toxic via the oral route of exposure in female rats (Mukerji, 2015).

L. paracasei Lpc-37 was included in a five-strain formulation, investigated for its ability to stabilize the intestinal microbiota during and after antibiotic therapy (Engelbrektson et al., 2006, Engelbrektson et al., 2009). In this randomized, double blind, placebo-controlled, parallel group study, 40 healthy adult patients consumed a capsule containing $5x10^9$ CFU/day of *L.paracasei* Lpc-37, $5x10^8$ CFU/day of *B. bifidum* Bb-02, $5x10^9$ CFU/day of *B. lactis* Bi-07, $5x10^9$ CFU/day of *B. lactis* Bl-04, and $5x10^9$ CFU/day of *L. acidophilus* NCFM, or a placebo capsule for a period of 48 days. The study showed a reduction in antibiotic-induced disturbance of the total microbiota population and no adverse events were reported.

L. paracasei Lpc-37 was the main probiotic component in a placebo-controlled cross-over study with 15 healthy adults and 15 patients with atopic dermatitis (AD) (Roessler et al., 2012). The probiotic combination of the probiotics *L. paracasei* Lpc-37, *L. acidophilus* 74-2 and *B. animalis* subsp. *lactis* DGCC 420 (*B. lactis* 420) resulted in a significant increase in lactobacilli. Each group of subjects consumed $7.8x10^{10}$ CFU/day of Lpc-37 for eight weeks. There was a significant reduction in the genotoxic potential of faecal water in patients with AD. All 30 patients completed the study with no adverse effects reported.

L. paracasei Lpc-37 was included in a double blind, randomized, placebo-controlled study in a developing area of India. Three hundred and seventy-two pre-school children, ages two to five years, were administered either probiotic strains *L. paracasei* Lpc-37, *B. lactis* HN019 or placebo for a period of nine months. Study participants were monitored for diarrhea, fever, weight gain and linear growth. The children consumed $2x10^9$ CFU/day to $5x10^9$ CFU/day. This study showed a significant reduction in the incidence of diarrhea and fever during the "wet season" and a reduction in the incidence of fever in August. No adverse events were reported (Hemalatha et al., 2014).

The influence of *L. paracasei* Lpc-37 ingested at a level of 10^{10} CFU/day alone and in combination with a calcium supplement on faecal lactobacilli colonization and beneficial health effects were studied in a double-blind, placebo-controlled, cross-over study with 32 men and women (Trautvetter et al., 2011). The patients consumed the supplements for a period of four weeks. There was a significant increase in faecal concentration of *L. paracasei* seen by the end of the study. No adverse events were reported.

Table 6: Studies on *Lactobacillus paracasei* Lpc-37

Study Design	Subjects	Strain/Dose	Duration	Results	Reference
Acute Oral Toxicity Trial	5 Femal Crl:DC(SD) Rats	$3.35x10^{12}$ CFU/Kg	14 days	No incident of mortality, clinical abnormalities, or overall body weight losses.	Mukerji, 2015
Randomized, double blind, placebo controlled, parallel	40 Healthy Adults	*Lactobacillus paracasei* Lpc-37 at $5x10^9$ CFU/day plus *Bifidobacterium bifidum* Bb-02 at $5x10^8$ CFU/day plus Bifidobacterium *lactis* Bi-07 at $5x10^9$ CFU/day plus *Bifidobacterium lactis* Bl-04 at $5x10^9$ CFU/day plus *Lactobacillus acidophilus* NCFM at $5x10^9$ CFU/day	48 days	Reduced antibiotic-induced disturbance of the total microbiota population. No adverse events were reported.	Engelbrektson et al., 2006; Engelbrektson et al., 2009
Placebo controlled, cross-over	15 healthy adults, 15 patients with atopic dermatitis (AD)	*Lactobacillus paracasei Lpc-37 at $7.8x10^{10}$ CFU/day; Lactobacillus acidophilus 74-2 at $5.8x10^6$ CFU/day; Bifidobacterium animalis* subsp. *lactic* DGCC 420 at $1.2x10^7$ CFU/day	8 weeks	Significantly reduces the genotoxic potential of faecal water in AD patients. No adverse events were reported.	Roessler et al., 2012
Double blind, randomized, placebo controlled	372 pre-school children ages 2 - 5	*Lactobacillus paracasei* Lpc-37 at 2-$5x10^9$ CFU/day; *Bifidobacterium animalis*subsp. *Lactis* HN019 at 2-5x109 CFU/day	9 months	Significantly reduced incidence of diarrhea and fever during the "wet season" and reduced incidence of	Hemalatha et al., 2014

				fever in August. No adverse events were reported.	
Double blind, placebo controlled, cross-over	32 men and women	*Lactobacillus paracasei* Lpc-37 at 10^{10}CFu/day and calcium supplement	4 weeks	Faecal concentration of *L. paracasei* increased significantly. No adverse events were reported.	Trautvetter et al., 2011

Lactic acid bacteria and lactobacilli

Lactic acid bacteria (LAB) and lactobacilli have a long history of safe use in foods (Bernardeau et al., 2008; Salminen et al., 1998). Lactobacilli are intrinsically resistant to some antibiotics. Because this antibiotic resistance is not transferable and LAB are sensitive to many antibiotics in common clinical use they present no particular safety concern. Lactobacillemia induced by food, particularly fermented dairy products, is extremely rare and only occurs in predisposed patients (Bernardeau et al., 2008).

The Food and Agriculture Organization and World Health Organization expert consultation reported that "lactobacilli have a long history of use as probiotics without established risk to humans, and this remains the best proof of their safety....no pathogenic or virulence properties have been found for lactobacilli"(FAO/WHO, 2002). The safety of probiotic bacteria was recently reviewed (Sanders et al., 2010; Sanders et al., 2007). Taken as a whole, any probiotic strain, including members of the genera *Lactococcus, Lactobacillus*, and *Bifidobacterium* is considered safe, as long as the strain is devoid of any transferable antibiotic resistance genes.

Infections in humans by these genera are extremely rare. There have been 180 cases of lactobacillemia and 69 cases of infective endocarditis attributed to lactobacilli reported during the past 30 years (Borriello et al., 2003). In most cases of endocarditis, dental surgery occurred in the days or weeks preceding the disease. These infections resulted from native sources of these genera and not from consumption of probiotics products. Two cases of *Lactobacillus* infection were linked with probiotic consumption (Borriello et al., 2003). Increasing consumption of probiotic lactobacilli has not led to an increase in such opportunistic infections in consumers (Salminen et al., 2002). Thus, the risk of infection by these genera is in the "negligible" range, taking into account that exposure to them is universal and persistent, not only through probiotic products but also as common colonizers of the human body (the digestive tract and oral and vaginal cavities). This lack of pathogenicity extends across all age groups (including preterm infants and pregnant women) (Lin et al., 2005, Saavedra et al., 2004). However, further investigation is warranted for probiotic use in at-risk human populations such as severely immunocompromised subjects, neonates, or hospitalized patients (Snydman, 2008).

In a comprehensive, evidence-based review and meta-analysis of the literature regarding the safety of probiotics, 622 peer-reviewed research articles were evaluated (Hempel et al., 2011). Of these, 235 studies reported only nonspecific safety statements such as "well tolerated" but did not indicate specific adverse events or what kinds of events were monitored. The remaining 387 studies predominantly investigated *Lactobacillus*, alone or in combination with other genera, most often *Bifidobacterium*. These studies were pooled to evaluate the relative risks (RR) of use of probiotics, active or lyophilized, single ingredients or in combination, in all delivery vehicles when used to improve health. The following key relative risk results germane to the current report are listed along with 95% confidence intervals (CI), p value, and the number of randomized clinical trials (RCT) included in the pool.

There was **no evidence of increased risk from interventions with probiotics** compared to control groups

based on the number of participants with adverse events (RR 0.98, CI: 0.93 – 1.04, p=0.537, 121 RCT)

based on the number of adverse-event incidences (RR 1.00, CI: 0.93 – 1.07, p=0.999, 208 RCT)

"**None of the case series, controlled clinical trials, or parallel and crossover RCT reported an infection caused by the administered probiotics**" though few reported that they monitored for this.

There was no indication participants using probiotic organisms experienced more:

Gastrointestinal events (RR 1.03, CI: 0.89 – 1.18, p=0.693, 126 RCT)

Infections (RR 1.00, CI: 0.87 – 1.16, p=0.967, 65 RCT)

Or other adverse events (RR 1.01, CI: 0.91 – 1.12, p=0.923, 131 RCT)

Stratified by probiotic genus, there was **no indication that participants using *Lactobacillus* experienced an increased risk**. (RR 0.98, CI: 0.87 – 1.11, p=0.785)

Stratified by age, there was no indication of increased risk of adverse events for children, adults, or elderly.

Although case studies have reported serious adverse events in health compromised, not generally healthy participants, **subgroup analyses of RCT did not show an increased risk of adverse events** in either:

Medium health-compromised participants (RR 1.03, CI: 0.94 – 1.13, p=0.491)

Critically ill patients (RR 0.79, CI: 0.51 – 1.22, p=0.286)

There was **no indication that consumption of probiotics lead to hospital admission or lengthened hospitalization**. Most of these studies were based on *Lactobacillus* interventions. (RR 1.06, CI: 0.97 – 1.16, p=0.201, 66 RCT)

There was no indication that consumption of probiotics increased the risk of adverse events in individuals concomitantly taking:

Antibiotics (RR 1.07, CI: 0.94 – 1.23, p=0.271)

Corticosteroids (RR 1.04, CI: 0.88 – 1.22, p=0.650)

The strength of these conclusions is somewhat mitigated by the inconsistency between the results of RCT and case studies, the lack of systematic reporting of adverse events, and poor documentation in the studies evaluated. The authors conclude the RCT-based evidence does not indicate an increased risk of adverse events. "The available evidence in RCTs does not indicate an increased risk; however, rare adverse events are difficult to assess and despite the substantial number of publications, the current literature is not well equipped to answer questions on the safety of probiotic interventions with confidence."

Lactic acid bacteria have long been considered safe and suitable for human consumption. Very few instances of infection have been associated with these bacteria and several published studies have addressed their safety (Aguirre and Collins, 1993; Gasser, 1994; Gueimonde et al., 2004; Salminen et al., 1998). Lactobacilli have long been considered to be non-pathogenic despite a small number of

opportunistic infections where immunocompromised hosts with underlying health issues experienced infection. A 36-year-old male (with uncontrolled diabetes) was admitted to a hospital because of chorea caused by hyperglycemia. Although he had no signs of infection, he had elevated levels of C-Reactive Protein (CRP). Upon further investigation, he was diagnosed with splenic abscess. The fluid drained from the spleen was found to contain Gram-positive rods which were identified through 16S rRNA to be *L. paracasei.* Contributing factors to this disease is the use of steroids, alcoholism and diabetes. This only the second case of this rare disease reported being caused by a rare organism. The patient underwent surgery to remove the spleen as well as antibiotic treatments and was released with a full recovery. (Doi et al., 2011)

4. Adverse events in clinical trials

In any published clinical trials thus far, no adverse events have been associated with the administration of *L. paracasei* Lpc-37 and no serious adverse events have been reported.

B. Inconsistent information

DuPont Nutrition & Health (formerly Danisco) and an external expert has reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with this conclusion of GRAS status.

C. Expert Panel Evaluation

DuPont Nutrition & Health has concluded that *Lactobacillus paracasei* Lpc-37 is GRAS for use in conventional foods and dietary supplements on the basis of scientific procedures. This GRAS conclusion is based on the totality of evidence generally available in the public domain pertaining to the safety of Lpc-37, and discussed herein, and on consensus of an expert who is qualified by scientific training and experience to evaluate the safety of food ingredients. Based on the publicly available scientific data from the literature, a decision tree for determining the safety of microbial cultures to be consumed by humans and animals shown in Appendix E (Pariza et al., 2015), and additional supporting data generated by DuPont Nutrition & Health, the company has concluded that *Lactobacillus paracasei* Lpc-37 is safe and suitable for use in yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and in supplement format including sachets, tablets, and capsules. Therefore, it can be considered Generally Recognized As Safe (GRAS). In addition, the safety determination, including the origins of the production organism, the production process and materials, and safety of the product were reviewed by an external expert in the field, Dr. Michael W. Pariza, who concurred with the company's conclusion that the product is GRAS (Appendix F).

D. Common knowledge elements of GRAS conclusions

The first common knowledge element for a conclusion of GRAS status is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals for the safety assessment. The studies on which this GRAS conclusion has been based, have been published in the scientific literature.

The second common knowledge element required for a GRAS determination is that consensus among qualified scientists about the safety of the substance with its intended use must be demonstrated. It is agreed that there is adequate data in the scientific literature to conclude that *L. paracasei* Lpc-37 is a common component of food sources for man and animals, and is nutritionally efficacious without any evidence of adverse effects.

Finally, *L. paracasei* is consumed as a dietary supplement in the United states and internationally, *L. paracasei* is QPS in Europe, and *L. paracasei* is in common use as a food preparation in fermented food.

E. Final conclusion

Based on scientific procedures, the above data and the information presented herein, DuPont Nutrition & Health has concluded the intended uses of *L. paracasei* Lpc-37 is GRAS when consumed in conventional foods and dietary supplements at levels of up to $5x10^{11}$ CFU/serving. Danisco believes it does not present a significant or unreasonable risk of illness or injury at this levels for these uses. General recognition of Danisco's GRAS determination is supported by the consensus rendered by an independent Expert Panel, qualified by experience and scientific training to evaluate the proposed uses for *L. paracasei* Lpc-37.

This declaration is made in accordance with FDA's standard for food ingredient safety, i.e. Reasonable certainty of no harm under the intended conditions of use.

> The External Expert offers the following conclusion:
> "..., I conclude that DuPont's *Lactobacillus paracasei* Lpc-37 product, manufactured consistent with cGMP and meeting food grade specifications, is Generally Recognized As Safe (GRAS) for the use in dairy foods and dietary supplement products. It is my professional opinion that other qualified experts would also concur in this conclusion."

Part 7 - List of supporting data and information in GRAS notice

All data and information, listed below, except one, used in accordance with the above document is generally available.

The study that is not generally available; Mukerji, P., 2015 is used as a supporting paper for safety of the organism.

Agu, C. A., Klein, R., Schwab, S., König-Schuster, M., Kodajova, P., Ausserlechner, M., Binishofer, B., Bläsi, U., Salmons, B., Günzburg, W.H., and Hohenadl, C., 2006. The cytotoxic activity of the bacteriophage λ-holin protein reduces tumour growth rates in mammary cancer cell xenograft models. The journal of gene medicine, 8(2), 229-241.

Aguirre, M. and Collins, M.D., 1993. Lactic acid bacteria and human clinical infection. J. Appl. Bacteriol 75, 95-107.

Aires, J., Doucet-Populaire, F. and Butel, M.J., 2007. Tetracycline resistance mediated by tet(W), Tet(M), and tet(O) genes of Bifidobacterium isolates from humans. Appl. Environ. Microbiol 73, 2751-2754.

Axelsson, L., 2004. Lactic acid bacteria: Classification and physiology, in: S. Salminen, W.A. von (Eds.), Lactic acid bacteria: Microbiology and functional aspects. Marcel Dekker, New York, pp. 1-73.

Balzaretti, S., Taverniti, V., Rohdini, G., Marcolegio, G., Minuzzo, M., Remagni, M.C., Fiore, W., Arioli, S. and Guglielmetti, S., 2015. The vaginal isolate *Lactobacillus paracasei* LPC-S01 (DSM 26760) is suitable for oral administration. Frontiers in Microbiol, September 2015, vol. 6, article 952.

Barnard, J.P. and Stinson, M.W., 1996. The alpha-hemolysin of *Streptococcus gordonii* is hydrogen peroxide. *Infection and immunity,* 64(9), 3853-3857.

Bernardeau, M., Vernoux, J.P., Henri-Dubernet, S. and Gueguen, M., 2008. Safety assessment of dairy microorgnaisms: The Lactobacillus genus. Int J Food Microbiol 126, 278-285.

Borriello, S.P., Hammes, W.P., Holzapfel, W., Marteau, P., Schrezenmeir, J., Vaara, M., and Valtonen, V., 2003. Safety of probiotics that contain lactobacilli or bifidobacteria. Clin Infect Dis, 36(6), 775-780.

Bourdichon, F., Boyaval, P., Casaregola, S., Dupont, J., Farrokh, C., Frisvod, J.C., Hammes, W.P., Huys, G., Jany, J.L., and Laulund, S., 2012. The 2012 inventory of microbial species with technological beneficial role in fermented food products. Bulletin of the International Dairy Federation 455, 22-61.

Broadbent, J.R., Neeno-Eckwall, E.C., Stahl, B., Tandee, K., Cai. H., Morovic, W., Horvath, P., Heidenreich, J., Barrangou, R., and Steele, J.L., 2012. Analysis of the *Lactobacillus casei* Supragenome and Its

Influence in Species Evolution and Lifestyle Adaption. BMC Genomics, 13:533. Doi: 10.1186/1471-2164-13-533.

CFSAN 2008. Agency Response to GRAS Notice No. GRN 000240, FDA.

CFSAN 2012. Agency Response to GRAS Notice No. GRN 000378, FDA.

Christensen, H.R., Larsen, C.N., Kæstel, P., Rosholm, L.B., Sternberg, C., Michaelsen, K.F. and Frøkiær, H., 2006. Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. FEMS Innunol Med Microbiol 47 (2006) 380-390.

Colomer-Lluch, M., Jofre, J. and Muniesa, M., 2011. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. PLoS. One 6, e17549.

Doi, A., Nakajo, K., Kamiya, T. and Ohkusu, K., 2011. Splenic Abscess caused by *Lactobacillus paracasei.* J. Infect. Chemother 17:122-125.

Douillard, F.P. and De Vos, W.M., 2014. Functional genomics of lactic acid bacteria: from food to health. Microb. Cell Fact 13 Suppl 1, S8.

Drider, D., Fimland, G., Héchard, Y., McMullen, L.M. and Prévost, H., 2006. The continuing story of class IIa bacteriocins. Microbiology and molecular biology reviews, 70(2), 564-582.

EFSA Opinion of the Scientific Committee, 2007. Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. Question No EFSA-Q-2005-293. The EFSA J 587, 1-16.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAPP), 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal, 10(6), 2740.

EFSA Panel on Biological Hazards, B., 2013. Scientific Opinion on the Maintenance on the list of QPS biological agents intestinally added to food and feed (2013 update). EFSA Journal, 11(11), 3449.

Engelbrektson, A.L., Korzenik, J.R., Sanders, M.E., Clement, B.G., Leyer, G., Klaenhammer, T.R., and Kitts, C.L., 2006. Analysis of treatment effects on the microbial ecology of the human intestine. FEMS Microbiol Ecol 57(2), 239-250.

Engelbrektson, A.L., Korzenik, J.R., Pittler, A., Sanders, M.E., Klaenhammer, T.R., Leyer, G., and Kitts, C.L., 2009. Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. J Med Microbiology 58, 663-670.

Ewaschuk, J.G., Naylor, J.M., and Zello, G.A., 2005. D-lactate in human and ruminant metabolism. J. Nutr 135, 1619-1625.

FAO/WHO Report of Joint Expert Consultation, 2002. Health and Nutrition Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. FAO Food and Nutrition Paper 85.

Fath, M.J., Zhang, L.H., Rush, J. and Kolter, R., 1994. Purification and characterization of colicin V from *Escherichia coli* culture supernatants. Biochemistry, 33(22), 6911-6917.

Ferrario, C., Taverniti, V., Milani, C., Fiore, W., Laureati, M., De Noni, I., Stuknyte, M., Chouaia, B., Riso, P. and Guglielmetti, S., 2014. Modulation of fecal clostidiales bacteria and butyrate by probiotic intervention with *Lactobacillus paracasei* DG varies among healthy adults. J. Nutr. 144: 1787-1796.

Fimland, G., Eijsink, V.G. and Nissen-Meyer, J., 2002. Comparative studies of immunity proteins of pediocin-like bacteriocins. Microbiology, 148(11), 3661-3670.

Gasser, F., 1994. Safety of lactic acid bacteria and their occurrence in human clinical infections. Bulletin de l'Institut Pasteur 92, 45-67.

Gillor, O., Etzion, A. and Riley, M.A., 2008. The dual role of bacteriocins as anti- and probiotics. Applied microbiology and biotechnology, 81(4), 591-606.

Gueimonde, M., Ouwehand, A.C. and Salminen, S., 2004. Safety of probiotics. Food & Nutrition Research 48, 42-48.

Hemalatha, R, O., Ouwehand, A.C., Forssten, S.D., Babu Geddan, J.J., Sriswan Mamidi, R., Bhaskar, V. and Radhakrishna, K.V, 2014. A community-based randomized double blind controlled trial of *Lactobacillus paracasei* and *Bifidobacterium lactis* on reducing risk for diarrhea and fever in preschool children in an urban slum in India. Eur J Nutr Food Safety 4(4), 325-341.

Hempel, S., Newbery, S., Ruelaz, A., Wang, Z., Miles, J.N.V., Suttorp, M.J., Johnsen, B., Shanman, R., Slusser, W., Fu, N., Smith, A., Roth, E., Polak, J., Motala, A., Perry, T. and Shekelle, P.G., 2011. Safety of probiotics to reduce risk and prevent or treat disease. Evidence Report/Technology Assessment No. 200. (Prepared by the Southern California Evidence-based Practice Center under Contract No. 290-2007-10062-I), Rockville, MD.

Holzapfel, W.H., Haberer, P., Geisen, R., Bjorkroth J. and Schollinger, U., 2001. Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin. Nutr 73, 365S-373S.

Hudson, M., Pocknee, R. and Mowat, N.A., 1990. D-lactic acidosis in short bowel syndrome – and examination of possible mechanisms. Q. J. Med 74, 157-163.

Hütt, P., Kõll, P., Stsepetova, J., Alvarez, B., Mändar, R., Krogh-Andersen, K., Marcotte, H., Hammarström, L. and Mikelsaar, M., 2011. Safety and persistence of orally administered human Lactobacillus sp. Strains in healthy adults. Beneficial Microbes, 2(1): 79-90.

Jespersen, L., Tarnow, I., Eskesen, D., Morberg, C.M., Michelsen, B., Bügel, S., Dragsted, L.O., Rijkers, G.T. and Calder, P.C., 2015. Am J Clin Nutr 101:1188-96.

Jia, X., Wang, W., Song, Y. and Li, N., 2011. A 90-day oral toxicity study on a new strain of *Lactobacillus paracasei* in rats. Food and Chem Tox 49, 1148-1151.

Kandler, O. and Weiss, N. 1986. Genus Lactobacillus, p. 1209-1234. P.H.A. Sneath, N.S. Mair, M.E. Sharpe & Holt, J.G. (ed.). Bergey's manual of systematic bacteriology, vol. 2. Williams and Wilkins, Baltimore, Maryland.

Lee, A., Lee, Y.J., Yoo, H.J., Kim, M., Chang, Y., Lee, D.S. and Lee, J.H., 2017. Consumption of dairy yogurt containing *Lactobacillus paracasei* ssp. *paracasei, Bifidobacterium animalis* ssp. *lactis* and heat-treated *Lactobacillus plantarum* improves immune function including natural killer cell activity. Nutrients 9, 558.

Lin, W.A., Fu, L-S., Lin, H-K., Shen, C-Y. and Chen, Y-J., 2014. Evaluation of the effect of *Lactobacillus paracasei* (HF.A00232) in children (6-13 years old) with perennial allergic rhinitis: a 12-week, double-blind, randomized, placebo-controlled study. Peds and neonatology 55, 181-188.

Lin, H.C., Su, B.H., Chen, A.C., Lin, T.W., Tsai, C.H., Yeh, T.F. and Oh, W. 2005. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Ped 1-4.

Magnuson, R.D., 2007. Hypothetical functions of toxin-antitioxin systems. Journal of Bacteriology, 189(17), 6089-6092.

Maretti, C. and Cavallini, G., 2017. The association of a probiotic with a prebiotic (Flortec, Braco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: a pilot study. Andrology 5, 439-444.

Marshall, B.M., Ochieng, D.J. and Levy, S.B., 2009. Commensals: underappreciated reservoir of antibiotic resistance. Microbe 4, 231-238.

Masuda, Y., Miyakawa, K., Nishimura, Y., and Ohtsubo, E., 1993. chpA and chpB, *Escherichia coli* chromosomal homologs of the pem locus responsible for stable maintenance of plasmid R100. Journal of Bacteriology, 175(21), 6850-6856.

Mathur, S. and Singh, R., 2005. Antibiotic resistance in food lactic acid bacteria—a review. Int. J. Food Microbiol 105, 281-295.

Millen, A.E., Midthune, D., Thompson, F.E., Kipnis, V. and Subar, A.F., 2005. The National Cancer Institute diet history questionnaire: validation of pyramid food servings. Am. J. Epidemiol 163, 279-288.

Mitsuoka, T. 1996. Intestinal Flora and Human Health. Asia Pacific J. clin. Nutr. 5:2-9

Mogensen, G., Salminen, S., O'Brien, J., Holzapfel, A., Shortt, C., Fonden, R., Miller, G.D., Donohue, D., Playne, M., Crittenden, R., Salvadori, B. and Zink, R., 2002. Inventory of microorganisms with a documented history of use in food, Health Benefits and Safety Evaluation of Certain Food Components., 377 ed.

Mukerji, P., 2015. *Lactobacillus paracasei* Lpc-37: Acute oral toxicity study in rats – up-and-down procedure. Internal study. Project ID: DuPont-20999-834.

Nawaz, M., Wang, J., Zhou, A., Ma, C., Wu, X., Moore, J.E., Millar, B.C. and Xu, J., 2011. Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr. Microbiol 62, 1081-1089.

Nembrini, C., Singh, A., De Castro, C.A., Mercenier, A. and Nutten, S., 2015. Oral administration of *Lactobacillus paracasei* NCC 2461 for the modulation of grass pollen allergic rhinitis: a randomized, placebo-controlled study during the pollen season. Clin Transl Allergy 5:41.

Neto, J.V., de Melo, C.M. and Ribeiro, S.M.L., 2013. Effects of three-month intake of symbiotic on inflammation and body composition in the elderly: a pilot study. Nutrients 5, 1276-1286.

Nes, I.F., Gabrielsen, C., Brede, D.A. and Diep, D.B., 2015. Novel developments in bacteriocins from lactic acid bacteria. Biotechnology of Lactic Acid Bacteria: Novel Applications, 80.

Ouoba, L.I., Lei, V. and Jensen, L.B., 2008. Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: determination and transferability of the resistance genes to other bacteria. Int. J. Food Microbiol 121, 217-224.

Pariza, M.W., Gillies, K.O., Kraak-Ripple, S.F., Leyer, G. and Smith, A.B., 2015. Determining the safety of microbial cultures for consumption by humans and animals. Reg Toxicol and Pharm 73, 164-171.

Perrin, Y., Nutten, S., Audran, R., Berger, B., Bibiloni, R., Wassenberg, J., Barbier, N., Aubert, V., Moulin, J., Singh, A., Magliola, C., Mercenier, A. and Spertini, F., 2014. Comparison of two oral probiotic preparations in a randomized crossover trial highlights a potentially beneficial effect of *Lactobacillus paracasei* NCC2461 in patients with allergic rhinitis. Clin Transl Allergy 4:1.

Plaza-Diaz, J., Gomez-Llorente, C., Campaña-Martin, L., Matencio, E., Ortuño, I., Martinez-Silla, R., Gomez-Gallego, C., Periago, M.J., Ros, G., Chenoll, E., Genovés, S., Casinos, B., Silva, A., Corella, D., Portolés, O., Romero, F., Ramón, D., de la Cruz, A. P., Gil, A. and Fontana, L., 2013. Safety and immunomodulatory effects of three probiotic strains isolated from the feces of breast-fed infants in healthy adults: SETOPROB study. PLoS One 8(10).

Prozorov, A.A. and Danilenko, V.N., 2010. Toxin-antitoxin systems in bacteria: apoptotic tools for metabolic regulators? Microbiology 79(2), 120-140.

PURAC, 2008. GRAS notification claim for antimicrobial agent: GRN 000240. FDA.

PURAC, 2011. GRAS notification claim for food fermentation solutions: GRN 000378. FDA.

Renwick, A.G., 1990. Acceptable daily intake and the regulation of intense sweeteners. Food Addit Contam 7, 463-475.

Riezzo, G., Orlando, A., D'Attoma, B., Guerra, V., Valerio, F., Lavermicocca, P., De Candia, S. and Russo, F., 2012. Randomised clinical trial: efficacy of *Lactobacillus paracasei*-enriched artichokes in the treatment of patients with functional constipation – a double-blind, controlled, crossover study. Aliment Pharmacol Ther 35: 441-450.

Roessler A., Forssten, S.D., Glei, M., Ouwehand, A.C. and Jahreis, G., 2012. The effect of probiotics on faecal microbiota and genotoxic activity of faecal water in patients with atopic dermatitis: a randomized, placebo-controlled study. Clinical Nutrition 31(1), 22-29.

Saavedra, J. M., Abi-Hanna, A., Moore, N. and Yolken, R.H., 2004. Long-term consumption of infant formulas containing live probiotic bacteria: Tolerance and safety. Am J Clin Nutr 79:261-267.

Saito, Y., Fujii, M., Watanabe, T., Maruyama, K., Kowatari, Y., Ogata, H. and Kumagai, T., 2017. Randomized, double-blind, placebo-controlled, parallel-group study of the effects of *Lactobacillus paracasei* K71 intake on salivary release of secretory immunoglobulin A. Bioscience of Microbiota, Food and Health Vol 36 (2): 55-63.

Salminen, M.K., Tynkkynen, S., Rautelin, H., Saxelin, H., Vaara, M., Ruutu, P., Sarna, S., Valtonen, V., and Järvinen, A., 2002. *Lactobacillus* Bacteremia during a rapid increase in probiotic use of *Lactobacillus rhamnosus* GG in Finland. Clin Inf Dis 35:1155-60.

Salminen, S., Von, W.A., Morelli, L., Marteau, P., Brassart, D., De Vos, W.M., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S.E. and Mattila-Sandholm, T., 1998. Demonstration of safety of probiotics – a review. Int. J. Food Microbiol 44, 93-106.

Sanders, M.E., Akkermans, L.M., Haller, D., Hammerman, C., Heimbach, J., Hormannsperger, G., Huys, G., Levy, D.D., Lutgendorff, F., Mack, D., Phothirath, P., Solano-Aguilar, G. and Vaughan, E., 2010. Safety assessment of probiotics for human use. Gut Microbes 1, 164-185.

Sanders, M.E., Gibson, G., Gill, H.S. and Guarner, F., 2007. Probiotics: Their potential to impact Human Health. CAST Issue Paper, 36:1-20.

Snydman, D.R., 2008. The safety of probiotics. Clin Infect Dis 46:104-11.

Trachootham, D., Chupeerach, C., Tuntipopipat, S., Pathomyok, L., Boonnak, K., Praengam, K., Promkam, C. and Santivarangkna, C., 2017. Drinking fermented milk containing *Lactobacillus paracasei* 431 (IMULUS™) improves immune response against H1N1 and cross-reactive H3N2 viruses after influenza vaccination: a pilot randomized triple-blinded placebo controlled trial. J of Funct Foods 33: 1-10.

Trautvetter, U., Ditscheid, B., Kiehntopf, M. and Jahreis, G., 2011. A combination of calcium phosphate and probiotics beneficially influences intestinal lactobacilli and cholesterol metabolism in humans. Clinical Nutrition 31, 230-237.

Trivedi, D., Jena, P.K. and Seshadri, S., 2014. Colicin E2 Experssion in *Lactobacillus brevis* DT24, a vaginal probiotic isolate, against uropathogenic *Escherichia coli.* ISRN urology, 2014.

Ventura, M., Canchaya, C., Kleerebezem, M., De Vos, W.M., Siezen, R.J. and Brüssow, H., 2003. The prophage sequences of *Lactobacillus plantarum* strain WCFS1. Virology, 316 (2), 245-255.

Verdenelli, M.C., Silvi, S., Cecchini, C., Orpianesi, C. and Cresci, A., 2011. Influence of a combination of two potential probiotic strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® on bowel habits of healthy adults. Letters in Applied Microbiol 52, 596-602.

Wagner, W., Vogel, M. and Goebel, W., 1983. Transport of hemolysin across the outer membrane of *Escherichia coli* requires two functions. Journal of Bacteriology, 154(1), 200-210.

Wang, I-J. and Wang, J-Y., 2015. Children with atopic dermatitis show clinical improvement after Lactobacillus exposure. Clin & Experimental Allergy, 45, 779-787.

Wang, I.N., Smith, D.L. and Young, R. 2000. Holins: the protein clocks of bacteriophage infections. Annual Reviews in Microbiology 54(1), 799-825.

Wang, H.H., Manuzon, M., Lehman, M., Wan, K., Luo, H., Wittum, T.E., Yousef, A. and Bakaletz, L. O., 2006. Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol. Lett 254, 226-231.

Wang, X. and Wood, T.K., 2011. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. Applied and environmental microbiology 77(16), 5577-5583.

Wattanarat, O., Makeudom, A., Sastraruji, T., Piwat, S., Tianviwat, S., Teanpaisan, R. and Krisanaprakornkit, S., 2015. Enhancement of salivary human neutrophil peptide 1-3 levels by probiotic supplementation. BMC Oral Health 15:19.

West, N.P., Pyne, D. B., Cripps, A., Christophersen, C.T., Conlon, M.A. and Fricker, P.A., 2012. Gut balance, a symbiotic supplement, increases fecal *Lactobacillus paracasei* but has little effect on immunity in healthy physically active individuals. Gut Microbes, 3:3, 221-227.

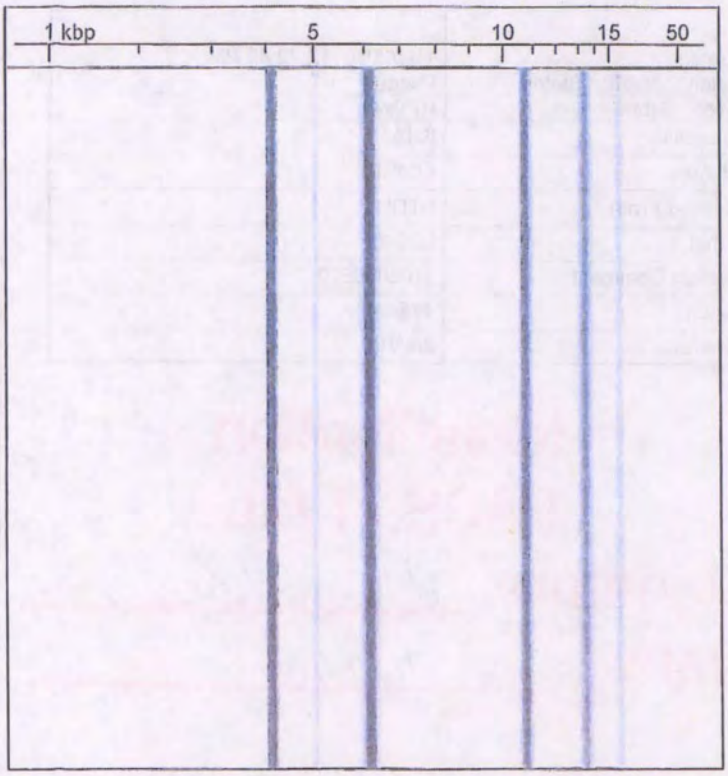
Zhang, H., Sun, J., Liu, X., Hong, C., Zhu, Y., Liu, A., Li, S., Guo, H. *and* Ren, F., 2013. *Lactobacillus paracasei* subsp. *Paracasei* LC01 positively modulates intestinal microflora in healthy young adults. J of Microbiol. Vol. 51, No. 6, pp. 777-782.

Zhang, Y., Zhang, L., Du, M., Yi, H., Guo, C., Tuo, Y., Han, X., Li, J., Zhang, L. and Yang, L., 2011. Antimicrobial activity against Shigell sonnei and probiotic properties of wild lactobacilli from fermented food. Microbiol. Res 167, 27-31.

APPENDIX A

DuPont Qualicon

Created	8/13/2016 7:25:06 PM
Batch Extraction Status	Passed
User - Submitted	RP\skry
Process	KHA
Enzyme	EcoRI
Isolated From	STDUT
Label	LBC-81
Sample Comment	1102818249
Batch	475-881
Operator	skry



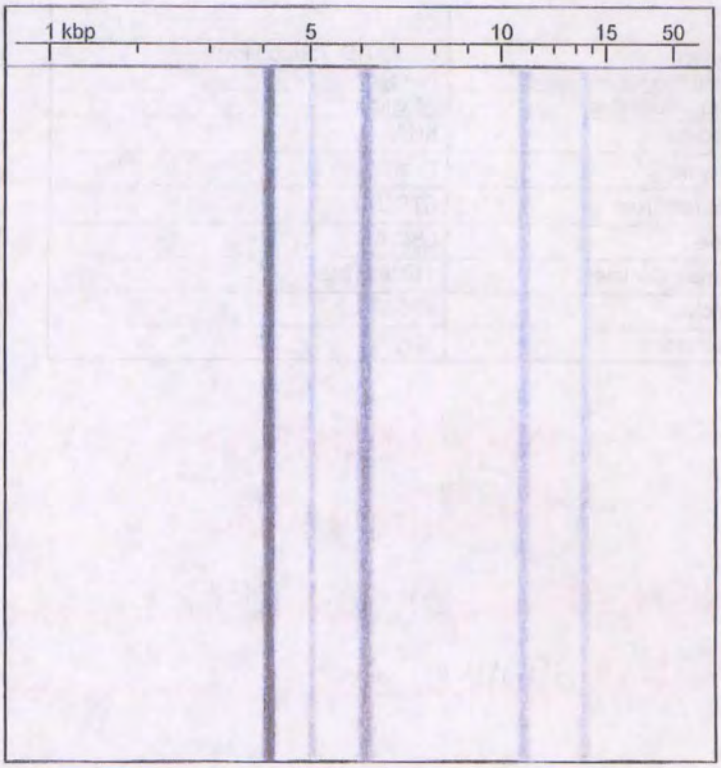
Identification
PASS / FAIL
Signature: (b) (6)
Date: 8/14/16

	Type	Number	Similarity	Label	RiboPrint™ Pattern
1	Custom ID	Madison_EcoRI-12	0.95	Lactobacillus paracasei	
2	RiboGroup	ECORI 475-57-S-5	0.97		

RiboPrinter® Microbial Characterization System
Sample 475-945-S-3 Report

DuPont Qualicon

Created	11/1/2016 12:22:42 PM
Batch Extraction Status	Passed
User - Submitted	RP\jawe
Process	KHA
Enzyme	EcoRI
Isolated From	STDUT
Label	LBC-81
Sample Comment	1102858520
Batch	475-945
Operator	JAWE



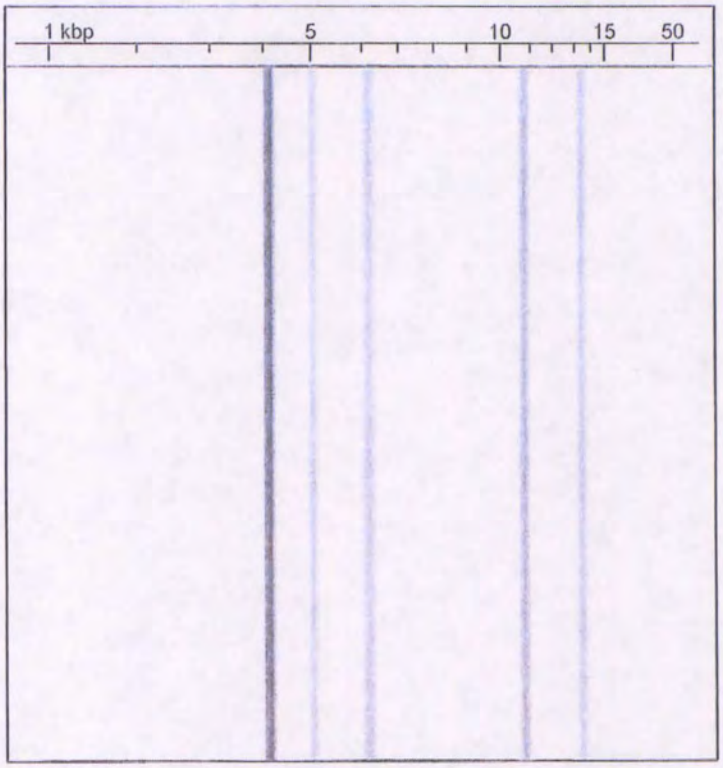
Identification
PASS / FAIL
Signature: (b) (6)
Date: 11/2/16

	Type	Number	Similarity	Label	RiboPrint™ Pattern
1	Custom ID	Madison_EcoRI-12	0.95	Lactobacillus paracasei	
2	RiboGroup	ECORI 475-57-S-5	0.97		

RiboPrinter® Microbial Characterization System
Sample 475-1061-S-8 Report

DuPont Qualicon

Created	2/17/2017 12:07:10 PM
Batch Extraction Status	Passed
User - Submitted	RPI\Administrator
Process	KHA
Enzyme	EcoRI
Isolated From	STDUT
Label	LBC-81
Sample Comment	1102927434
Batch	475-1061
Operator	SJK



Identification
PASS / FAIL
Signature: (b) (6)
Date: 2/2017

	Type	Number	Similarity	Label	RiboPrint™ Pattern
1	Custom ID	Madison_EcoRI-12	0.97	Lactobacillus paracasei	
2	RiboGroup	ECORI 475-57-S-5	0.97		

APPENDIX B

Danisco USA, Inc.
3329 Agriculture Drive
Madison, WI 53716
800-255-6837 Tel
608-395-2603 Fax

FOOD GRADE STATEMENT

Date: September 20, 2017

Product: *Lactobacillus paracasei* Lpc-37™

To Whom It May Concern,

The above listed product is produced in accordance with the U.S. FDA's current Good Manufacturing Practices guidelines (21 CFR 117), and is considered Food Grade and safe for human consumption.

This information is given in respect of DuPont's policy of openness and transparency with its customers.

Sincerely,

(b) (6)

Sarah Pace
Quality & Food Safety Coordinator
DuPont - Nutrition & Health

APPENDIX C

DANISCO
First you add knowledge...

Certificate of Analysis

Date: 12 May 2017
Our ref. no.: 0
Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102693769	Best before date:	20 Jan 2018
Quantity:	0.000	Production date:	21 Jan 2016

Test	Result	Specification	Unit	Reference
Viable Cell Count	6.80E+11	4.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 400 billion CFU/gm of freeze-dried Lb. paracasei.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed to Genus/species level (or sub-species level where applicable) based on DNA

DANISCO
First you add knowledge...

Certificate of Analysis

Date: 12 May 2017
Our ref. no.: 0
Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102693769	Best before date:	20 Jan 2018
Quantity:	0.000	Production date:	21 Jan 2016

Fingerprinting Analysis generated by Automated Ribotyping.

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102740627	Best before date:	30 Mar 2018
Quantity:	0.000	Production date:	30 Mar 2016

Test	Result	Specification	Unit	Reference
Viable Cell Count	5.30E+11	4.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 400 billion CFU/gm of freeze-dried Lb. paracasei.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed to Genus/species level (or sub-species level where applicable) based on DNA

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102740627	Best before date:	30 Mar 2018
Quantity:	0.000	Production date:	30 Mar 2016

Fingerprinting Analysis generated by Automated Ribotyping.

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.
MADISON WI 53718

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102940742	Best before date:	27 Jan 2019
Quantity:	0.000	Production date:	27 Jan 2017

Test	Result	Specification	Unit	Reference
Viable Cell Count	5.75E+11	4.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 400 billion CFU/gm of freeze-dried Lb. paracasei.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed to Genus/species level (or sub-species level where applicable) based on DNA

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.
MADISON WI 53718

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102940742	Best before date:	27 Jan 2019
Quantity:	0.000	Production date:	27 Jan 2017

Fingerprinting Analysis generated by Automated Ribotyping.

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.
MADISON WI 53716

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102848089	Best before date:	09 Sep 2018
Quantity:	0.000	Production date:	09 Sep 2016

Test	Result	Specification	Unit	Reference
Viable Cell Count	8.80E+11	4.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 400 billion CFU/gm of freeze-dried Lb. paracasei.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed to Genus/species level (or sub-species level where applicable) based on DNA

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.
MADISON WI 53716

DANISCO

First you add knowledge...

Certificate of Analysis

Date: 12 May 2017

Our ref. no.: 0

Your ref.

Material:	M85414C	LPC-37 400B - 1 KG	
Batch No.:	1102848089	Best before date:	09 Sep 2018
Quantity:	0.000	Production date:	09 Sep 2016

Fingerprinting Analysis generated by Automated Ribotyping.

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Danisco US - Madison Plant
CULTURE PLANT
3322 Agriculture Dr.
MADISON WI 53716

APPENDIX D

DANISCO
First you add knowledge...

PRODUCT DESCRIPTION - PD 204412-8.0EN

Material no. M85414C

Lpc-37 400B - 1 KG

FloraFIT® Probiotics

Description

Freeze-dried probiotic powder. White to cream-color in appearance.

Directions for use

See Danisco Probiotic Usage & Handling Guide

Composition

Lactobacillus paracasei (Lpc-37)

Microbiological specifications

Cell count	> 4.00E+11 / g
Non-Lactic Count	< 5000 / g
Enterococci	< 100 / g
Coliforms	< 10 / g
E. coli	neg. by test (< 0.3 / g)
Staphylococcus (coag. pos.)	neg. by test (< 10 / g)
Salmonella	neg. (40 g enrichment)
Listeria	neg. (25 g enrichment)

Storage

Shelf life is 24 months when stored in the original, sealed package at or below 4°C. Frozen storage will extend shelf life.

Packaging

High barrier foil laminate bags

Quantity

1 kg

Purity and legal status

Local regulations should always be consulted concerning the status of this product, as legislation regarding its intended use may vary from country to country.

Safety and handling

MSDS is available on request.

Kosher status

Circle K certification

Halal status

IFANCA certification

DANISCO
First you add knowledge...

PRODUCT DESCRIPTION - PD 204412-8.0EN Material no. M85414C

Lpc-37 400B - 1 KG

FloraFIT® Probiotics

Allergens

Below table indicates the presence (as added component) of the following allergens and products thereof:

Yes	No	Allergens	Description of components
	X	wheat	
	X	other cereals containing gluten	
	X	crustacean shellfish	
	X	eggs	
	X	fish	
	X	peanuts	
	X	soybeans	
	X	milk (including lactose)	
	X	nuts	
	X	celery	
	X	mustard	
	X	sesame seeds	
	X	sulphur dioxide and sulphites (> 10 mg/kg)	
	X	lupin	
	X	molluscs	

Local regulation has always to be consulted as allergen labelling requirements may vary from country to country.

Additional information

Country of Origin: USA

GMO status

Lpc-37 400B - 1 KG does not consist of, nor contains, nor is produced from genetically modified organisms according to the definitions of Regulation (EC) 1829/2003 and Regulation (EC) 1830/2003 of the European Parliament and of the Council of 22 September 2003.

APPENDIX E

Decision Tree Analysis for Determining the Safety of Microbial Food Cultures for Consumption

1. Has the strain[i] been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology?[ii] (if YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding.)	YES
2. Has the strain genome been sequenced? (if YES, go to 3. If No, the genome must be sequenced before proceeding to 3.)[iii]	YES
3. Is the strain genome free of genetic elements[iv] encoding virulence factors[v] and/or toxins[v] associated with pathogenicity?[vi] (If YES, go to 4. If No, go to 15.)	YES
4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA?[vii] (If YES, go to 5. If NO, got to 15.)	YES
5. Does the strain produce antimicrobial substances?[viii] (If NO, go to 6. If YES, go to 15.)	NO
6. Has the strain been genetically modified using rDNA techniques? (If YES, go to 7. if NO, go to 8.)	NO
7. Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in food[ix]? (If YES, go to 8. If NO, the expressed product(s) must be shown to be safe before proceeding to 8.)[x]	
8. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial[xi] and chracterizing[xii] component (not simply an 'incidental isolate')? (If YES, go to 9. If NO, go to 13)[xiii]	YES
9. Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food use by an authoritative group of qualified scientific experts?[xiv] (If YES, go to 10. If NO, go to 13.)	YES
10. Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9 continue to support the conclusion that the species to which the strain belongs, is safe for use in food? (If YES, go to 11. If NO, go to 13.)	YES
11. Will the intended use of the strain expand exposure to the species beyond the group(s) that typically consume the species in "traditional" food(s) in which it is typically found (for example, will a strain that was isolated from a fermented food typically consumed by healthy adults be used in food intended for an 'at risk' group)? (if No, go to 12, if YES, go to 13.)	NO
12. Will the intended use of the strain expand intake of the species (for example, increasing the number of foods beyond the traditional foods in which the species typically found or using the strain as a probiotic rather than as a fermented food starter culture, which may significantly increase the single dose and/or chronic exposure)? (If No, go to 14. If YES, go to 13.)	YES

APPENDIX E

13. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies?[xv] (if YES, go to 15. If NO, go to 14.)	NO
14. **The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.**	YES
15. The strain is NOT APPROPRIATE for human or animal consumption.[xvi]	

[i] A strain is a "population of organisms that descends from a single organism or pure culture isolate." P. 392 Prescott, Harley and Klein, 1996, Microbiology, Wiley. We recognize that the genotype and/or phenotype of a strain may change slightly when carried in culture, but such changes are irrelevant to safety considerations because there is no known mechanism or precedent for isolated strains in culture to begin spontaneously expressing pathogenic traits, unless that potential was already present in the genome at the time of isolation.

[ii] Whole Genome Sequencing provides distinct advantages for identification and characterization of microorganisms. In-depth analysis, including functional and comparative genomic studies, is afforded by sequencing the whole genome. This technology can provide a wealth of information that can be used for identification and characterization, including evidence of genetic evolution for adaption of a species to a nutrient-rich environment, such as dairy products or the gastrointestinal tract (Pfeiler, EA, Klaenhammer, TR. 2007. The genomics of lactic acid bacteria. TRENDS in Microbiol, 15(12); 546-553). Less comprehensive molecular analysis, such as RAPD, FISH, and MLST, may also provide adequate information for identification, but the characterization ability is often times limited within a bacterial species (Gosiewski, T, Chmielarczyk, A, Strus, M, Brzychczy-Wloch, M, Heczko, PB, 2012. The application of genetics methods to differentiation of three *Lactobacillus* species of human origin. Ann Microbiol 62:1437-1445).

[iii] The genomic sequence provides the tools to mine the genome for a number of functions, uncovering information spanning from safety to host-cell interactions (Callanan M. 2005. Mining the Probiotic Genome: Advanced Strategies, Enhanced Benefits, Perceived Obstacles. Current Pharmaceutical Design, 11: 25-36). From a regulatory perspective, the ability to show percentage/regions of similarity and differentiation between a new strain of interest in comparison with a *type strain*, or an accepted strain with history of safe use, is beneficial (U.S. FDA; July 2011. Draft Guidance for Industry: Dietary Supplements: New Dietary Ingredient Notifications and Related Issues). The genome sequence is analogous to a chemical specification for a food ingredient, that is, it defines precisely what is being evaluated and permits a genetic assessment of pathogenic and toxigenic potential. Isolates from a type-strain culture collection, or a strain collection held by a commercial culture manufacturer, may be considered to have the same safety characteristics as, and to be substantially equivalent to, the original source pure culture, so in these cases the requirement for genome sequencing may be satisfied by sequencing the genome of the original source pure culture.

[iv] The term "genetic elements" refers to gene sequences encoded in the chromosome or extra-chromosomal DNA.

[v] Known genetic element sequences for virulence factors and protein toxins are searchable, e.g. the MvirDb database of microbial virulence factors (http://mvirdb.llnl.gov) [ref Nucl. Acids Res. (2007) 35 (suppl 1): D391-D394. Doi: 10.1093/nar/gkl791].

APPENDIX E

[vi] In considering the issue of "pathogenicity" and the potential to produce an infection, it is important to distinguish between *true pathogens* (i.e., microbes that possess virulence factors and are therefore capable of crossing or evading non-compromised host barriers) versus *opportunistic pathogens* (i.e., microbes that do not possess the required virulence factors to produce an infection in a non-compromised host). Typically, this can be accomplished via genome analysis for known virulence factors coupled with a comprehensive search of the peer-reviewed scientific literature for infectious potential.

[vii] A functional antibiotic resistance gene results in an antibiotic resistance phenotype.

[viii] In this context, the term 'antimicrobial substances' refers to antibiotics that are used in medical or veterinary applications, for example, substances that are positive in the JECFA test (*FAO Food and Nutrition Paper: 25th Session of the Joint FAO/WHO Expert Committee on Food Additives,* Appendix A, pp. 317-318, FAO/WHO, Geneva, Switzerland).

[ix] The use of the terms "food" and "feed" includes supplements, which are in most jurisdictions considered to be a subset of the general categories.

[x] Demonstration of the safety of the expressed product may be accomplished by testing, e. g. toxicological testing as required by various regulatory bodies such as the US FDA Redbook 2000 (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformtion/IngredientsAdditivesGRASPackaging/ucm2006826.htm) or by establishing a substantial equivalence of the test article to a substance with a safe history of use in food, or, in the case of animal feed additives, establishing a substantial equivalence of the test article to a substance with a history of safety use in target animal feeds.

[xi] Food fermentations, e.g. Cheddar cheese or yogurt, commonly result in "substantial" microbial food culture populations of 106 – 108 colony forming units per gram of the food. Significance should be judged relative to the fermented food, i.e. numbers of different organisms in a microbial population may change during the course of the life of the fermented food, e.g. Lactobacilli counts in Cheddar cheese are routinely low in the initial stages of cheese maturation, but begin to increase in numbers while the Lactococci, responsible for initial acid production, count decreases as the cheese ripens and ph decreases. [Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. N.A. Fitzsimons, T.M. Cogan, S. Condon, T. Beresford. Journal of Applied Microbiology 90(4): 600-608, 2001; Kosikowski F.F., and V.V. Mistry. Cheese and Fermented Milk Foods. 1997. 3rd Ed. F.V. Kosikowski, L.L.C. Westportm CT).

[xii] A species is a "characterizing" component of a food if it has a measurable impact on flavor, texture, stability or preservation properties that are characteristic of the food, e.g. typical color and flavor of "blue" cheeses derived from *Penicillium roqueforti*; or surface texture, flavor of Limburger cheese resulting from *Brevibacterium linens* growth on the surface. The color and flavor of "blue" cheese and the aroma, flavor and texture of Limburger cheese are characteristic of the food and the microbial cultures that are responsible for these traits are characterizing components.

[xiii] A strain that was isolated from a type-strain or a commercial culture, with a history of safet use in food fermentations, is deemed to have satisfied this requirement and may proceed to 9.

[xiv] For example, the Qualified Presumption of Safety list (http://www.efsa.europa.eu/en/topics/topic/qps.htm) prepared and periodically updated by the

APPENDIX E

European Food Safety Authority is the output from a systematic safety review of the included microorganisms by qualified experts.

[xv] Experimental evidence of safety is required. Such evidence may include, but is not necessarily limited to, studies in appropriate animal models, and clinical trials in humans.

[xvi] In some cases, the strain may be shown to be appropriate by test and re-application of the decision tree, e.g., where an undesirable genetic element has been removed from a strain's genome.

APPENDIX F

Michael W. Pariza Consulting LLC
7102 Valhalla Trail
Madison, WI 53719
(608) 271-5169
mwpariza@gmail.com

Michael W. Pariza, Member

July 17, 2017

Amy B. Smith, Ph.D.
Senior Manager, Regulatory & Scientific Affairs, NA
DuPont Nutrition & Health
Danisco USA, Inc.
3329 Agriculture Drive
Madison, WI 53716

RE: GRAS opinion on the intended uses of DuPont's *Lactobacillus paracasei* Lpc-37

Dear Dr. Smith,

I am writing regarding your request for an evaluation of safety of DuPont's *Lactobacillus paracasei* Lpc-37 (Lpc-37) for use in yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and in supplement format including sachets, tablets and capsules. In conducting this evaluation, I considered the biology of *Lactobacilli* and *L. paracasei*, relevant information available in the peer-reviewed scientific literature, and information that you provided on Lpc-37.

By way of background, *Lactobacillus spp.* are Gram-positive, non-spore forming, homofermentative, facultative anaerobes that occur naturally in food and the digestive tract of humans and other animals. They are major contributors to the safety and organoleptic properties of fermented meats, vegetables (e.g. sauerkraut, olives) and dairy products (yogurt, cheeses). Accordingly, *Lactobacilli* have a history of safe use in food fermentations that spans millennia.

Lactobacillus paracasei has a long history of safe use in starter cultures for fermented milks and cheeses. The genomes of many strains of *L. paracasei*, including Lpc-37 have been sequenced and found to contain no evidence of pathogenic or toxigenic traits. Rather, evidence indicates that the *L. paracasei* genome evolved through acquisition of foreign genomic islands that likely conferred a fitness benefit in plant-associated niches, followed by the loss of unnecessary ancestral traits so as to enhance the organism's fitness in a protein and lactose-rich milk environment (Broadbent, Jeff R., et al. "Analysis of the Lactobacillus casei supragenome and its influence in species evolution and lifestyle adaptation." BMC genomics 13.1:533, 2012).

Lpc-37, manufactured by DuPont (legacy Danisco), was isolated from a dairy source. It is produced under cGMP using only good grade ingredients. The specifications for DuPont's Lpc-37 product are appropriate for a microorganism that is used in human food and as a probiotic, and the proposed use levels are appropriate for a species that normally resides in the human colon and is associated with beneficial health effects.

From these considerations, I conclude that DuPont's *Lactobacillus paracasei* Lpc-37 product, manufactured consistent with cGMP and As Safe (GRAS) for use in yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods and in supplement format including sachets, tablets and capsules. It is my professional opinion that other qualified experts would also concur in this conclusion.

Please note that this is a professional opinion directed at safety considerations only and not an endorsement, warranty or recommendation regarding the possible use of the subject product by you or others.

Sincerely,

(b) (6)

Michael W. Pariza
Member, Michael W. Pariza Consulting, LLC
Professor Emeritus, Food Science
Director Emeritus, Food Research Institute
University of Wisconsin-Madison

STUDY TITLE: Lactobacillus paracasei Lpc-37: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure

TEST GUIDELINES: U.S. FDA, Redbook 2000: IV.C.2: Acute Oral Toxicity Tests (1993)

U.S. EPA Health Effects Test Guidelines
OPPTS 870.1100 (2002)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 425) (2008)

AUTHOR: Pushkor Mukerji, B.A.

STUDY COMPLETED ON: March 17, 2015

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
P.O. Box 30
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-20999-834

WORK REQUEST NUMBER: 20999

SERVICE CODE NUMBER: 834

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. FDA (21 CFR part 58) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the items documented below. None of the items listed impact the validity of the study.

1. Neither the characterization of the test substance nor the analysis of dosing formulation were performed under full compliance with GLPs. The procedures were conducted by trained personnel using established methods; therefore the accuracy of the data was considered sufficient for the purposes of this study.

2. The dosing formulation used in the study was analyzed for concentration, but not stability or homogeneity. Analyses of stability and homogeneity were not considered necessary to meet study objectives because the formulation was prepared on the day of dosing and stirred prior to and throughout the dosing procedure.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: (b) (6) 17 March 2015

Pushkor Mukerji, B.A.
Staff Toxicologist
E.I. du Pont de Nemours and Company
Date

Sponsor: _____ _____

Sponsor Representative Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 20999
Service Code Number: 834

Key inspections for the above referenced study were completed by the Quality Assurance Unit of DuPont Haskell and the findings were submitted on the following dates:

Audit Dates	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:		
August 29, 2014	August 29, 2014	August 29, 2014
Conduct:		
September 5, 2014	September 5, 2014	September 5, 2014
Report/Records:		
November 24-25, 2014	November 25, 2014	December 3, 2014
March 13, 2015	March 13, 2015	March 13, 2015

Reported by: (b) (6) 16 MAR 2015
Annet L. Reigel
Staff Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Anatomic Pathology Evaluation Reported by: (b) (6) 13 Mar 2015

Ashley E. Wood, A.S., HT(ASCP)CM
Laboratory Technician
Date

Anatomic Pathology Evaluation Reviewed by: (b) (6) 16 March 2015

Steven R. Frame, D.V.M., Ph.D., Diplomate ACVP
Research Manager
Date

Reviewed by (b) (6) 16 March 2015

Melissa N. Fallers, B.A.
Associate Scientist
Date

Issued by Study Director: (b) (6) 17 March 2015

Pushkor Mukerji, B.A.
Staff Toxicologist
Date

TABLE OF CONTENTS

STUDY INFORMATION

Substance Tested: Lactobacillus paracasei Lpc-37

Haskell Number: 31323

Composition: 100% (6.55E+11 CFU/g) Lactobacillus paracasei Lpc-37

Purity: Not applicable

Physical Characteristics: Solid

Study Initiated/Completed: August 26, 2014 / (see report cover page)

Experimental Start/Termination: September 5, 2014 / March 16, 2015

In-Life Initiated/Completed: September 5, 2014 / September 19, 2014

SUMMARY

A single dose of Lactobacillus paracasei Lpc-37 was administered by oral gavage to fasted female rats at a dose of 5000 mg/kg. The five rats were dosed on the same day. All rats were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The rats were necropsied to detect grossly observable evidence of organ or tissue damage.

There were no incidents of mortality, clinical abnormalities, or overall (test day 1-15) body weight losses. Gross findings were limited to a large spleen identified in one animal, which was considered nonspecific.

Under the conditions of this study, Lactobacillus paracasei Lpc-37 was not considered acutely toxic via the oral route of exposure in female rats at a dose level of 5000 mg/kg (equivalent to $3.35_{x}10^{12}$ cfu/kg by analysis, and corresponding to a range of $7.19_{x}10^{11}$ to $8.07_{x}10^{11}$ cfu/animal). In the absence of test substance related mortality, an LD_{50} was not calculated.

According to the guidance provided by the U.S. EPA for classification and label statements regarding hazards due to pesticides (Label Review Manual, Chapter 7: Precautionary Statements, revised July 2014), Lactobacillus paracasei Lpc-37 is classified in Toxicity Category IV.

In accordance with the provisions of Directive 67/548/EEC amended by COMMISSION DIRECTIVE 2001/59/EC of 6 August 2001, Annex VI, classification is not required.

In accordance with the provisions of Regulation (EC) Number 1272/2008 amended by Commission Regulation (EU) Number 286/2011 of 10 March 2011 (ATP002) on the Classification, Labeling, and Packaging of Substances and Mixtures, classification is not required.

According to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition 2013, classification is not required.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of Lactobacillus paracasei Lpc-37 when administered by oral gavage to female rats. Per the test guidelines, the starting dose level of 5000 mg/kg was chosen based on the lack of toxicity historically observed for probiotic test substances.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 2011). All studies conducted by or for DuPont Haskell Global Centers for Health & Environmental Sciences (DuPont Haskell) adhere to the following principles:

- The sponsor and/or the study director ensure that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA 2013) Guidelines on Euthanasia.
- Animals were provided with species-appropriate environmental enrichment.
- The procedures in the protocol have been reviewed by the Haskell Animal Welfare Committee and comply with acceptable standards for animal welfare and humane care.

DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. FDA, Redbook 2000: IV.C.2: Acute Oral Toxicity Tests (1993)

- U.S. EPA, OPPTS 870.1100: Acute Oral Toxicity, *Health Effects Test Guidelines* (2002)
- OECD, Section 4 (Part 425): Acute Oral Toxicity: Up-and-Down Procedure, *Guideline for the Testing of Chemicals* (2008)

B. Test Substance

(Appendix A)

The test substance, Lactobacillus paracasei Lpc-37, was supplied by the sponsor as an off-white solid (powder), and was assigned Haskell number 31323. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test System

Female (nulliparous and non-pregnant) Crl:CD(SD) rats were received from Charles River Laboratories International, Inc., Raleigh, North Carolina, U.S.A.

The Crl:CD(SD) rat was selected based on consistently acceptable health status and on extensive experience with the strain at DuPont Haskell.

D. Animal Husbandry

1. Housing

Animals were housed individually in solid-bottom caging with bedding and appropriate species-specific enrichment.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 20-26°C (68-79°F) and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and water were available *ad libitum* except as noted in section F. Dosing.

4. Identification

Each rat was assigned an identification number, which was written on each rat's tail with a water-insoluble marker.

5. Acclimation

The rats were weighed and observed for general health during the 7-day quarantine period.

6. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Formulation Samples

Duplicate samples (1.5 mL) were collected on the day of study start, and frozen at <-60°C. One sample was shipped on dry ice to the sponsor's analytical laboratory for concentration analysis (Principal Investigator and Test Site are listed below). Analysis was conducted by enumerating colony forming units. Detailed methods are in Appendix A.

The back-up samples were saved at <-60°C and then discarded because no additional analysis was necessary.

Connie Sindelar
DuPont Nutrition & Health
Danisco USA Inc.
3329 Agriculture Dr.
Madison, WI 53716
(608) 395 2612
connie.sindelar@dupont.com

F. Dosing

A single oral dose of Lactobacillus paracasei Lpc-37, suspended in phosphate buffered saline, was administered oral gavage to fasted female rats at a dose level of 5000 mg/kg. The five rats were dosed on the same day.

The rats were approximately 10 weeks old on the day of dosing. The rats were fasted approximately 16 hours prior to dosing, with food being returned to the rats approximately

3 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing. The rats were dosed at a volume of 20 mL per kg of body weight. The weight of each animal was within the ±20% of the mean weight for the group of animals. The dosing formulations were stirred prior to and throughout the dosing procedure.

G. Observations, Body Weights, and Anatomic Pathology

Daily animal health observations were conducted throughout the study for mortality and signs of illness, injury, or abnormal behavior. Animals were weighed on test days -1, 1, 8, and 15, and were observed for clinical signs at the beginning of fasting, just before dosing (test day 1), once during the first 30 minutes after dosing and 2 more times on the day of dosing, and once each day thereafter. On test day 15, the rats were euthanized and necropsied to detect grossly observable evidence of organ or tissue damage. The rats were euthanized by exsanguination while under isoflurane anesthesia.

The complete GI tract (esophagus to rectum) from each animal was excised and preserved in formalin. Because no further examination was required, the organs were discarded at the conclusion of the study.

RESULTS AND DISCUSSION

Analytical Evaluation

A. Dose Formulation Analysis

(Appendix A)

The concentration of the dosing formulation was considered acceptable. The concentration of Lactobacillus paracasei Lpc-37 in the sample was measured at 1.67×10^{11} cfu/mL (colony forming units per mL), corresponding to an administered dose of 3.35×10^{12} cfu/kg bodyweight. The administered dose is calculated for each animal below.

animal	body weight (g) on test day 1	cfu/animal ($\times 10^{11}$)
3881	214.6	7.19
3882	238.1	7.98
3883	234.4	7.85
3884	238.8	8.00
3885	240.8	8.07

In-life Toxicology

A. Dose Progression and Mortality

No deaths occurred.

The LD_{50} is greater than 5000 mg/kg.

B. Clinical Signs and Body Weights

(Appendix B through Appendix D)

There were no clinical abnormalities or overall (test day 1-15) body weight losses.

Anatomic Pathology Evaluation

A. Gross Observations

(Appendix E)

Gross findings were limited to a large spleen identified at scheduled sacrifice in one female rat administered 5000 mg/kg. This single incidence is nonspecific.

CONCLUSIONS

Under the conditions of this study, Lactobacillus paracasei Lpc-37 was not considered acutely toxic via the oral route of exposure in female rats at a dose level of 5000 mg/kg (equivalent to 3.35×10^{12} cfu/kg by analysis, and corresponding to a range of 7.19×10^{11} to 8.07×10^{11} cfu/animal). In the absence of test substance related mortality, an LD_{50} was not calculated.

According to the guidance provided by the U.S. EPA for classification and label statements regarding hazards due to pesticides (Label Review Manual, Chapter 7: Precautionary Statements, revised July 2014), Lactobacillus paracasei Lpc-37 is classified in Toxicity Category IV.

In accordance with the provisions of Directive 67/548/EEC amended by COMMISSION DIRECTIVE 2001/59/EC of 6 August 2001, Annex VI, classification is not required.

In accordance with the provisions of Regulation (EC) Number 1272/2008 amended by Commission Regulation (EU) Number 286/2011 of 10 March 2011 (ATP002) on the Classification, Labeling, and Packaging of Substances and Mixtures, classification is not required.

According to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Fifth revised edition 2013, classification is not required.

RECORDS AND SAMPLE STORAGE

Raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware or Iron Mountain Records Management, Wilmington, Delaware.

APPENDICES

Appendix A
Certificate of Analysis, Analytical Results, and Analytical Methods

DANISCO
First you add knowledge...

Certificate of Analysis

Date: 08 Aug 2014
Our ref. no.: 0
Your ref.

Material: MSAMPLPC37-400B LPC-37 400B 100 GM STD SAMPLE
Batch No.: 1102201698 Best before date: 03 Jun 2015
Production date: 02 Dec 2013

Test	Result	Specification	Unit	Reference
Viable Cell Count	6.55E+11	> 4.00E+11	/g	ISO 7889/IDF 117
Enterococcus	< 100	< 100	/g	CMMEF, 4TH EDITION
Non Lactics	< 5000	< 5000	/g	ISO 13559
Coliforms	< 10.0	< 10.0	/g	AOAC
E. coli, neg. by test (<0.3/g)	Negative	Negative		AOAC
Staph. aureus, neg. by test (<10/g)	Negative	Negative		AOAC
Salmonella, negative in 40 g	Negative	Negative		AOAC
Listeria, negative in 25 g	Negative	Negative		AOAC

Comments

Exceeds 400 billion CFU/gm of freeze-dried Lb. paracasei.

The above product has been analyzed by Danisco and/or its contract testing laboratory. Analytical results on a representative sample from this batch show that this product meets the above criteria.

Best if used before the date listed above when stored at or below 4°C.

AOAC references above reflect the current edition of AOAC.

Culture identity is confirmed to Genus/species level (or sub-species level where applicable) based on DNA

Danisco US - Madison Plant
Maintenance Dept.
3322 Agriculture Dr.
MADISON WI 53716

DANISCO
First you add knowledge...

Certificate of Analysis

Date: 08 Aug 2014
Our ref. no.: 0
Your ref.

Material:	MSAMPLPC37-400B	LPC-37 400B 100 GM STD SAMPLE	
Batch No.:	1102201698	Best before date:	03 Jun 2015
		Production date:	02 Dec 2013

Fingerprinting Analysis generated by Automated Ribotyping.

This certificate is generated automatically

(b) (6)

Phil Ihrke

Quality Control Department

Danisco US - Madison Plant
Maintenance Dept.
3322 Agriculture Dr.
MADISON WI 53716

Concentration of Probiotic in the Dosing Formulation Sample
Enumeration Results

Sample Name/Description	Probiotic composition of sample	Replicate			Dilution Factor	CFU/mL
		1	2	3		
LPC37 Stand Alone	LPC37	86	81	84	5.00E-10	1.67E+11

The results of the analysis were considered acceptable.

(b) (6)

11/7/14

Connie Sindelar Date
DuPont Nutrition & Health
Danisco USA Inc.
3329 Agriculture Dr.
Madison, WI 53716

ANALYTICAL PROCEDURE

DANISCO.

ENUMERATION OF FREEZE-DRIED *LACTOBACILLUS PARACASEI* POWDER

Procedure

1. Aseptically weigh 11 grams of the dried powder into a sterile Stomacher® bag.

2. Aseptically add 99 mL of sterile, room temperature, Difco™ MRS broth to the 11 grams of dried powder in the Stomacher® bag.

3. Turn Stomacher® on and allow to blend for two minutes.

4. Hold the sample at room temperature for 30 minutes to rehydrate the freeze-dried powder.

5. Return the sample to the Stomacher® and blend for an additional two minutes.

6. Make serial dilutions in 99 mL 0.1% peptone dilution blanks by adding 1 mL of the primary 10^{-1} dilution (from the Stomacher® bag) to 99 mL of diluent with a 1 mL pipette so as to obtain a 10^{-3} dilution. Rinse the pipette three times. Repeat this operation until the desired dilution series is obtained. Shake dilution bottles as directed in the current edition of *Standard Methods for the Examination of Dairy Products*.

7. Proceeding in triplicate, transfer 1 mL of each appropriate dilution to labeled, sterile Petri plates with sterile 1 mL pipettes.

8. Take a bottle of sterile Difco™ MRS agar that has been melted (100 C for 30 minutes) and tempered to 45 C in a 45 C water bath, and sanitize the bottle by dipping it into a 200 ppm chlorine solution (made fresh daily), or by flaming the lip of the bottle.

9. Pour approximately 15 mL of the MRS agar into each plate. Swirl the plates to mix, and let solidify at room temperature on a cool, level surface.

10. Incubate the plates at 38 C under anaerobic conditions (BD GasPak™ EZ Container Systems with indicator in an anaerobic jar) for 72 hours.

Calculations

Count colonies on the MRS agar plates and record as viable cell count per gram, taking into account the dilution factor of the plates counted. Only plates having between 25 and 250 colonies should be counted.

References

- *Standard Methods for the Examination of Dairy Products*, 17th Edition, 2004
- ISO 7889: 2003

02-L-12

DuPont Nutrition & Health
supplements@danisco.com • www.danisco.com/dietarysupplements

Appendix B
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g	-	grams
mg/kg	-	Milligrams per kiligoram
N	-	Number of values used in calculation
SD	-	Standard Devation

Individual Body Weights

Sex: Female Bodyweight (g)

5000 mg/kg 5000 mg/kg	Day(s) Relative to Start Date			
	-1	1	8	15
3881	223.9	214.6	244.5	256.8
3882	246.0	238.1	270.8	285.5
3883	242.2	234.4	267.0	272.5
3884	242.4	238.8	264.7	272.7
3885	253.0	240.8	269.5	282.7
Mean	241.5	233.3	263.3	274.0
SD	10.8	10.7	10.8	11.3
N	5	5	5	5

Appendix C
Individual Body Weight Gains

INDIVIDUAL BODY WEIGHT GAINS

EXPLANATORY NOTES

ABBREVIATIONS:

g	-	grams
mg/kg	-	Milligrams per kilogram
N	-	Number of values used in calculation
SD	-	Standard Deviation

Individual Body Weight Gains

Sex: Female Body Weight Gain (g)

5000 mg/kg 5000 mg/kg	Day(s) Relative to Start Date		
	1 → 8	8 → 15	1 → 15
3881	29.9	12.3	42.2
3882	32.7	14.7	47.4
3883	32.6	5.5	38.1
3884	25.9	8.0	33.9
3885	28.7	13.2	41.9
Mean	30.0	10.7	40.7
SD	2.9	3.8	5.0
N	5	5	5

Appendix D
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

A	-	time slot for observations
Ts1	-	post-dose observation 1 (within 30 minutes of dosing)
Ts2	-	post-dose observation 2
Ts3	-	post-dose observation 3
X	-	present
mg/kg	-	milligrams per kilogram

Individual Clinical Observations and Mortality Records

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	-1 A	1 A	1 Ts1	1 Ts2	1 Ts3	2 A	3 A	4 A	5 A	6 A	7 A	8 A	9 A	10 A
1	f	3881	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.
		3882	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.
		3883	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.
		3884	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.
		3885	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present

Group 1 - 5000 mg/kg 5000 mg/kg

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	11 A	12 A	13 A	14 A	15 A
1	f	3881	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X
		3882	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X
		3883	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X
		3884	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X
		3885	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X

Severity Codes: X = Present

Group 1 - 5000 mg/kg 5000 mg/kg

Appendix E
Individual Animal Gross Observations

Individual Animal Gross Observations

Group: 1 Dose: 5000 mg/kg Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3881	SCHEDULED SACRIFICE	15	(3)	No Visible Lesions
3882	SCHEDULED SACRIFICE	15	(3)	No Visible Lesions
3883	SCHEDULED SACRIFICE	15	(3)	No Visible Lesions
3884	SCHEDULED SACRIFICE	15	(3)	No Visible Lesions
3885	SCHEDULED SACRIFICE	15	(3)	SPLEEN; Large GROSS OBSERVATIONS; Present Any remaining protocol required tissues, which have been examined, have no visible lesions