JHeimbach LLC

#840

December 31, 2018

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to 21 CFR Part 170, Subpart E, Arla Foods Ingredients Group P/S (Arla), through me as its agent, hereby provides notice of a claim that the addition of *Lactobacillus paracasei* ssp. *paracasei* strain F19 to conventional foods is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Arla has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one copy of the GRAS monograph and one signed copy of the conclusion from each member of the Expert Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the signed statements of the Expert Panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5543 or jh@jheimbach.com.

Sincerely,

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

Encl.

923 Water Street, P.O. Box 66, Port Royal Virginia 22535, USA
tel. (+1) 804-742-5543 cell (+1) 202-320-3063 jh@jheimbach.com

CONCLUSION OF THE EXPERT PANEL: DETERMINATION OF THE GRAS STATUS OF THE USE OF *LACTOBACILLUS PARACASEI* SSP. *PARACASEI* STRAIN F-19 IN CONVENTIONAL FOODS

Prepared for:
Arla Foods Ingredients

December 2018

CONCLUSION OF THE EXPERT PANEL:

We, the members of the Expert Panel, have individually and collectively critically evaluated the publicly available information on *Lactobacillus paracasei* ssp. *paracasei* strain F-19 summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of* Lactobacillus paracasei *ssp.* paracasei *strain F-19 in Conventional Foods* (December 2018), prepared by JHeimbach LLC, and other material deemed appropriate or necessary. Our evaluation included critical evaluation of the identity, genotypic and phenotypic characteristics of the strain, production methods, potential exposure resulting from the intended use of the strain, and published research bearing on the safety of *Lactobacillus paracasei* ssp. *paracasei* strain F-19. Our summary and conclusion resulting from this critical evaluation are presented below.

Summary

- *Lactobacillus paracasei* ssp. *paracasei* strain F-19 is intended to be added to conventional foods at concentrations consistent with current Good Manufacturing Practice to provide at least 10^9 cfu/serving throughout the shelf life of the product. This addition level will usually be between $5x10^9$ and 10^{11} cfu/serving, which provides for the loss of viability of from 80% to 99% of the bacteria added. The strain's function is to serve as a probiotic microorganism.
- *Lactobacillus paracasei* ssp. *paracasei* strain F-19 was isolated from the colon of a healthy adult and deposited in the Belgian Coordinated Collections of Microorganisms, Microbiology Laboratory (BCCM/LMG) under deposit number LMG-P-17806. The taxonomic placement of *L. paracasei* ssp. *paracasei* strain F-19 was determined by ribotyping and confirmed by pulsed-field gel electrophoresis and amplified fragment length polymorphism.
- A complete genome sequence of the strain revealed a circular chromosome of 3.1 Mb, a linear plasmid of 93.7 Kb, three circular plasmids of 8.7 Kb, 6.3 Kb, and 2.2 Kb, and two linear fragments of 2.3 Kb and 3.4 Kb. The genome was analyzed for the presence of antibiotic resistance and virulence genes; none was detected. One gene labeled "ornithine decarboxylase" was found, but it is not accompanied by a transporter required for generation of putrescine.
- *Lactobacillus paracasei* ssp. *paracasei* strain F-19 is produced under certification that systems are in full compliance with ISO 9001:2008, ISO 22000:2005, ISO/TS 2202-1:2009, and Food Safety System Certification 22000. All processing aids meet food-grade specifications, are used consistent with their regulatory status, and are suitable for human consumption.
- *Lactobacillus paracasei* received Qualified Presumption of Safety (QPS) assignment in the initial review in 2007 and this status was renewed each year from 2008 through 2018. The safe history of human exposure to *L. paracasei* ssp. *paracasei* strains is strongly supported by a large body of published research, including 4 oral toxicity studies in mice and rats and more than 50 studies in human infants, children, and adults enrolling more than 7,000 individuals. Participants in these studies received the probiotic at daily levels

up to 10^{12} cfu/day for as long as 9 months with no reported adverse events associated with the probiotic intervention.

- A decision-tree analysis (Pariza et al. 2015) determined that *Lactobacillus paracasei* ssp. *paracasei* strain F-19 is deemed to be safe for use in the manufacture of food for human consumption based on the following responses:
  - Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? **YES**
  - Has the strain genome been sequenced? **YES**
  - Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? **YES**
  - Is the strain genome free of functional and transferable antibiotic resistance gene DNA? **YES**
  - Does the strain produce antimicrobial substances? **NO**
  - Has the strain been genetically modified using rDNA techniques? **NO**
  - Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? **NO** (THE STRAIN WAS ISOLATED FROM THE COLON OF A HEALTHY HUMAN)
  - Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? **NO**

Conclusion

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of microorganisms intended for addition to foods as probiotics. We have individually and collectively critically evaluated the publicly available information on *Lactobacillus paracasei* ssp. *paracasei* strain F-19 summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of* Lactobacillus paracasei *ssp.* paracasei *strain F-19 in Conventional Foods* (December 2018), prepared by JHeimbach LLC, and other material deemed appropriate or necessary.

We have individually and collectively determined that no evidence exists in the available information on *Lactobacillus paracasei* ssp. *paracasei* strain F-19 that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of *Lactobacillus paracasei* ssp. *paracasei* strain F-19.

We unanimously conclude that the intended addition to conventional foods of *Lactobacillus paracasei* ssp. *paracasei* strain F-19, produced consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, at a level consistent with cGMP to provide at least 10^9 cfu per serving of the strain, is safe and is GRAS by scientific procedures.

It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Virginia Commonwealth University School of Medicine
Richmond, Virginia

Signature: _____ Date: _____

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

Signature: _____ Date: _____

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

Signature:  Date: December 28, 2018

Conclusion

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of microorganisms intended for addition to foods as probiotics. We have individually and collectively critically evaluated the publicly available information on *Lactobacillus paracasei* ssp. *paracasei* strain F-19 summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of* Lactobacillus paracasei *ssp.* paracasei *strain F-19 in Conventional Foods* (December 2018), prepared by JHeimbach LLC, and other material deemed appropriate or necessary.

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It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Virginia Commonwealth University School of Medicine
Richmond, Virginia

Signature: [redacted] Date: 21 December 2018

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

Signature: _____ Date: _____

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

Signature: _____ Date: _____

Conclusion

We, the undersigned members of the Expert Panel, are qualified by scientific education and experience to evaluate the safety of microorganisms intended for addition to foods as probiotics. We have individually and collectively critically evaluated the publicly available information on *Lactobacillus paracasei* ssp. *paracasei* strain F-19 summarized in a monograph, *Generally Recognized As Safe (GRAS) Determination for the Intended Use of* Lactobacillus paracasei *ssp.* paracasei *strain F-19 in Conventional Foods* (December 2018), prepared by JHeimbach LLC, and other material deemed appropriate or necessary.

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We unanimously conclude that the intended addition to conventional foods of *Lactobacillus paracasei* ssp. *paracasei* strain F-19, produced consistent with current good manufacturing practice (cGMP) and meeting the food-grade specifications presented in the monograph, at a level consistent with cGMP to provide at least 10^9 cfu per serving of the strain, is safe and is GRAS by scientific procedures.

It is our opinion that other qualified and competent scientists reviewing the same publicly available information would reach the same conclusions.

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Virginia Commonwealth University School of Medicine
Richmond, Virginia

Signature: _____ Date: _____

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

Signature: _____ Date: 19 December 2018

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

Signature: _____ Date: _____

Generally Recognized As Safe (GRAS) Determination for the Intended Use of *Lactobacillus paracasei* ssp. *paracasei* strain F-19 in Conventional Foods

Prepared for:
Arla Foods Ingredients Group P/S
Basking Ridge NJ

Prepared by:
JHeimbach LLC
Port Royal Virginia

December, 2018

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Part 1: Signed Statements and Certification

1.1. GRAS Notice Submission

Arla Foods Ingredients (Arla) submits this GRAS notification through its agent James T. Heimbach, president of JHeimbach LLC, in accordance with the requirements of 21 CFR Part 170, Subpart E.

1.2. Name and Address of Notifier

Arla Foods Ingredients Group P/S
106 Allen Road
Basking Ridge NJ 07920

Notifier Contact
Dr. Kal Ramanujam
Senior Scientific Advisor
Sonderhoj 10-12
8260 DK-Viby J
Denmark
kal.ramanujam@arlafoods.com
+1 (484) 919-5759

Agent Contact
James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
P.O. Box 66
Port Royal VA 22535
jh@jheimbach.com
+1 (804) 742-5543

1.3. Name of Notified Organism

The subject of this Generally Recognized as Safe (GRAS) notification is the probiotic bacterium *L. paracasei* ssp. *paracasei* strain F-19.

1.4. Intended Conditions of Use

L. paracasei ssp. *paracasei* strain F-19 is intended to be added to conventional foods at concentrations consistent with cGMP needed to provide at least 10^9 cfu/serving throughout the shelf life of the product. This addition level will usually be between 5 x 10^9 and 10^{11} cfu/serving, which provides for the loss of viability of from 80% to 99% of the bacteria added. The strain's function is to serve as a probiotic microorganism.

The foods to which *L. paracasei* ssp. *paracasei* strain F-19 is intended to be added are those foods that can sustain viable *L. paracasei* ssp. *paracasei* for the shelf life of the food, including but not limited to dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and functional/nutritional products. Use in USDA-regulated foods is not intended.

1.5 Statutory Basis for GRAS Status

Arla's GRAS determination for the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is based on scientific procedures in accordance with 21 CFR §170.30(b).

1.6. Premarket Exempt Status

The intended use of *L. paracasei* ssp. *paracasei* strain F-19 is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on Arla's determination that it is GRAS.

1.7. Data Availability

The data and information that serve as the basis for the conclusion that *L. paracasei* ssp. *paracasei* strain F-19 is GRAS for its intended use will be made available to the FDA upon request. At FDA's option, a complete copy of the information will be sent to FDA in either paper or electronic format, or the information will be available for review at the home office of JHeimbach LLC, located at 923 Water Street, Port Royal VA 22535, during normal business hours.

1.8. Freedom of Information Act Statement

None of the information in the GRAS notice is exempt from disclosure under the Freedom of Information Act, USC 552.

1.9. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of *L. paracasei* ssp. *paracasei* strain F-19.

1.10 FSIS Statement

Not applicable.

1.11. Name, Position and Signature of Notifier

James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
Agent to Arla Foods Ingredients Group P/S

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

2.1. Name of the GRAS Organism

The notified organism is the probiotic bacterium *L. paracasei* ssp. *paracasei* strain F-19, produced by Chr. Hansen A/S and sold in lyophilized form with a maltodextrin filler under the trade name Probio-Tec F-19 Blend-30 IF; the product contains a minimum of 30 billion ($3.0x10^{10}$ cfu/g powder) throughout its shelf life.

The strain was deposited in the Belgian Coordinated Collections of Microorganisms, Microbiology Laboratory (BCCM/LMG) under deposit number LMG-P-17806.

2.2. Source, Description, Manufacture, and Specifications of the GRAS Organism

2.2.1. Source

The isolation, selection, and characteristics of *Lactobacillus paracasei* ssp. *paracasei* strain F-19 were described in a published paper by Ljungh et al. (2002). At autopsy of hospital patients without gastrointestinal disease, rinsed colons were biopsied and incubated. Over 400 *Lactobacillus* strains were isolated and the 40 strains exhibiting the highest binding of porcine mucin were selected, followed by testing of cell surface hydrophobicity; binding of human fibronectin, collagen, fibrinogen, heparin, and fetuin; and acid tolerance. *L. paracasei* ssp. *paracasei* strain F-19 and 7 other strains with good adhesive and survival ability were chosen for further study, including antibacterial activity and antioxidant activity. The taxonomic placement of *L. paracasei* ssp. *paracasei* strain F-19 was confirmed by ribotyping. (The identification of strain F-19 as *L. paracasei* ssp. *paracasei* was again confirmed by pulsed-field gel electrophoresis [PFGE] and amplified fragment length polymorphism [AFPL] during the EU-PROSAFE project [Vankerckhoven et al. 2008]).

The authors concluded that, "*Lactobacillus F-19* has a documented ability to survive during passage in the GI tract and exerts multiple determinants for colonization and establishment, also expressed during conditions resembling those in the gut. Properties like fiber degradation, immunomodulatory effect and production of antioxidants make *Lactobacillus F-19* a strain which is likely to exert further beneficial effects in the large intestine."

2.2.2. Description

The taxonomic identification of *L. paracasei* ssp. *paracasei* strain F-19 was confirmed in-house at Chr. Hansen by comparison of its 16S rDNA sequence with that of the type strain of *L. paracasei*.

Cells are non-motile rods with rounded ends, single or in pairs or short chains, non-sporing, Gram+, and catalase negative. They are nonaerobic but aerotolerant.

As noted above, the microorganism is sold in lyophilized form with a food-grade maltodextrin filler. The product also is cryoprotected by food-grade sucrose, maltodextrin, and sodium ascorbate.

2.2.2.1. Sugar Fermentation

The strain is homofermentative and ferments ribose, adonitol, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, mannitol, sorbitol, N-acetyl glucosamine, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose, saccharose, trehalose, inuline, melezitose, D-turanose, D-tagatose, and gluconate. It does not ferment glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, β-methyl-xyloside, rhamnose, dulcitol, inositol, α-methyl-D-mannoside, α-methyl-D-glucoside, melibiose, D-raffinose, amidon, glycogen, xylitol, β-gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-keto-gluconate, or 5-keto-gluconate.

In vitro fermentation studies by Hedberg et al. (2008) showed that, in comparison with other tested probiotic bacteria (*L. plantarum* strains 299v and 931, *L. rhamnosus* strains GG and LB21, and *L. reuteri* strain PTA), fermentation by *L. paracasei* ssp. *paracasei* strain F-19 generally proceeds at a significantly slower rate.

2.2.2.2. Genome Analysis

Lactobacillus paracasei ssp. *paracasei* strain F-19 was genome sequenced in the in-house genome sequencing facility at Chr. Hansen using published methods with an average sequencing coverage of 62. The assembly yielded seven contigs—i.e., a circular chromosome of 3.1 Mb, a linear plasmid of 93.7 Kb, three circular plasmids of 8.7 Kb, 6.3 Kb, and 2.2 Kb, and two linear fragments of 2.3 Kb and 3.4 Kb.

The genome sequence of *L. paracasei* ssp. *paracasei* strain TMW 1.1434, which is referred to as being isogenic to F-19, was published by Schott et al. (2016; Accession CP016355 and CP016356) and found to be 3.1 Mb, in line with the genome sequence obtained in-house at Chr. Hansen. Furthermore, Schott et al. (2016) published the sequence of a 106.4 Kb plasmid, but not the three small plasmids. The three linear contigs of 2.3 Kb, 3.4 Kb, and 93.7 Kb. were found to be located on the 106.4 Kb plasmid. The presence of three small plasmids was published by Morelli and Campominosi (2002), who found them to be 9.0 Kb, 6.5 Kb, and 2.2 Kb as determined by gel electrophoresis.

2.2.3. Manufacture

Production of *L. paracasei* ssp. *paracasei* strain F-19 takes place under certification that systems are in full compliance with ISO 9001:2008, ISO 22000:2005, ISO/TS 2202-1:2009, and Food Safety System Certification 22000.

Following is a general description of the manufacturing process for *L. paracasei* ssp. *paracasei* strain F-19; a schematic of the process is shown in Figure 1.

2.2.3.1. Production of Substrate

The individual ingredients are weighed and mixed with tap water, and the medium is subjected to ultra-heat treatment (UHT) to remove foreign contamination. The fermentation media employed by Chr. Hansen are primarily based on food-grade milk powder and yeast extract. Various raw materials are used to optimize the fermentation media. All raw materials are regulated processing aids used consistent with their regulatory status, meet food-grade specifications, and are suitable for human consumption.

2.2.3.2. Inoculation and Fermentation

Pre-inoculation material is used to inoculate the milk yeast medium and the medium is incubated. The medium is then transferred to a pre-fermenter containing milk yeast medium. Fermentation in the pre-fermenter is carried out under anaerobic conditions. Then the content is transferred from the pre-fermenter to the fermenter.

The fermentation is conducted with milk yeast medium under anaerobic conditions.

2.2.3.3. Concentration and Treatment

The bacterial cells are harvested and concentrated by centrifugation using a separator.

The harvested bacterial cells are mixed with cryoprotectants and frozen into pellets in liquid nitrogen. The frozen pellets are lyophilized, resulting in very low water activity, ensuring stability of the culture.

2.2.3.4. Standardization

An authorized electronic worksheet is used to calculate the amount of culture that is needed to produce a specific batch. The cell count of the freeze-dried culture forms the basis of the calculation. When the exact weight of the culture is determined, the weight of excipients can be calculated.

2.2.3.5. Milling and Mixing

The lyophilized bacteria concentrate is milled and sieved. Excipients are added to the concentrate in order to standardize the blends and confer the dry powder with desirable handling properties.

The standardized blend is packed into aluminum foil bags of 5 kg each and stored below -20°C, awaiting approval from Quality Control, and then the product is released and available for shipping.

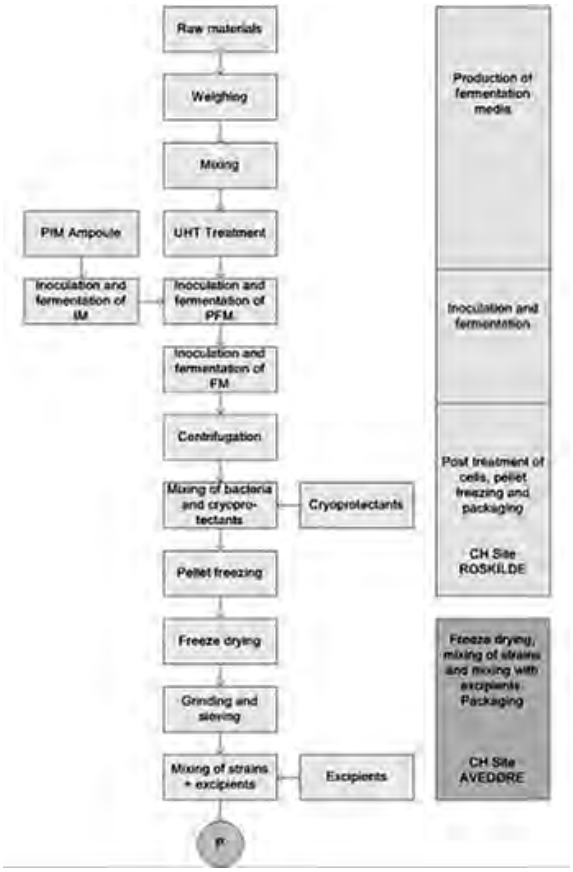


Figure 1. *L. paracasei* ssp. *paracasei* strain F-19 Production Process[1].

2.2.4. Specifications

Release specifications for *L. paracasei* ssp. *paracasei* strain F-19 are shown in Table 1, along with the results of testing of 5 non-consecutive batches.

[1] UHT = Ultra-High Temperature; PIM = Pre-Inoculation Material; IM = Inoculation Material; PFM = Pre-Fermentation Medium; FM = Fermentation Medium

Table 1. Specifications for *L. paracasei* ssp. *paracasei* Strain F-19.

Parameter	Specification	Test Batches				
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cell count	≥3.0x10^{10}	4.9x10^{10}	4.7x10^{10}	4.1x10^{10}	4.5x10^{10}	5.0x10^{10}
Appearance	Fine powder, white-light beige	Fine powder, white-light beige	Fine powder, white-light beige	Fine powder, white-light beige	Fine powder, white-light beige	Fine powder, white-light beige
Water Activity	≤0.15	0.05	0.05	0.09	0.09	0.06
Purity						
Bacillus cereus (cfu*/g)	<100	<10	<10	<10	<10	<10
Enterobacter-iaceae	Absent/10x10 g	Absent	Absent	Absent	Absent	Absent
Cronobacter sakazakii	Absent/10x10 g	Absent	Absent	Absent	Absent	Absent
Salmonella spp.	Absent/10x10 g	Absent	Absent	Absent	Absent	Absent
S. aureus (cfu/g)	<10	<10	<10	<10	<10	<10
Total aerobic bacteria (cfu/g)	≤2000	<50	<50	<50	<50	<50
Total yeasts & molds (cfu/g)	≤100	<5	<5	<5	<25	<25
*cfu = colony-forming units						

2.2.5. Genetic Stability

Morelli and Campominosi (2002) assessed the stability of the plasmid complement of *L. paracasei* ssp. *paracasei* strain F-19 under the stress of the production process. After examining about 300 colony-forming units for their plasmid profile, the experimenters reported that all of them retained the typical plasmid profile of the wild type, suggesting that industrial production processes do not affect the plasmid content of the strain.

In a follow-up experiment, novobiocin was added to the fermentation medium, producing cured derivatives missing one or two of the largest plasmids; it was not found possible to delete the smallest plasmid. Curing did not affect sugar fermentation patterns, indicating that these traits are not plasmid-linked. The cured strains exhibited resistance to vancomycin, again demonstrating that this resistance is intrinsic and is not plasmidally based. Finally, bile resistance was not affected by plasmid curing. The authors concluded:

> "The plasmid complement of Lactobacillus F-19 does not seem to be altered by the industrial production processes. Also, the plasmid profile was unaltered when compared with the plasmid content determined in the same strain by our laboratory 6 year earlier. Curing is obtained in laboratory conditions only and the two curable plasmids do not seem to be involved in the checked phenotypes."

Genetic stability is monitored during storage and production via DNA fingerprinting and plasmid profiling. The results are compared with the reference material for *L. paracasei* ssp. *paracasei* strain F-19.

2.2.6. Storage Stability

The stability of batch [redacted] *L. paracasei* ssp. *paracasei* strain F-19 was tested when stored at temperatures of -20°C and 5°C for 2 years and at accelerated conditions of 25°C and 60% relative humidity for 6 months. The product was evaluated for appearance, water activity, and cell count at 0, 3, 6, 12, 16, and 24 months. The color of the product remained in compliance with specifications

at all test points; water activity and cell count are shown in Tables 2 and 3; the cell-count data are also shown in Figure 2.

Table 2. Stability: Water Activity.

Months	Water Activity		
	-20°C±6°C	5°C±3°C	25°C, 60% RH
0	0.08	0.08	0.08
3	N/A*	0.09	0.09
6	0.09	0.09	0.06
12	0.07	0.08	N/A
16	0.09	N/A	N/A
24	0.07	0.07	N/A
*N/A = not available			

Table 3. Stability: Cell Count.

Months	Cell Count (cfu/g)		
	-20°C±6°C	5°C±3°C	25°C, 60% RH
0	$4.6x10^{10}$	$4.6x10^{10}$	$4.6x10^{10}$
3	N/A*	$3.7x10^{10}$	$3.0x10^{10}$
6	$5.9x10^{10}$	$5.6x10^{10}$	$4.8x10^{10}$
12	$8.2x10^{10}$	$7.8x10^{10}$	N/A
16	$4.2x10^{10}$	N/A	N/A
24	$3.7x10^{10}$	$3.0x10^{10}$	N/A
*N/A = not available			

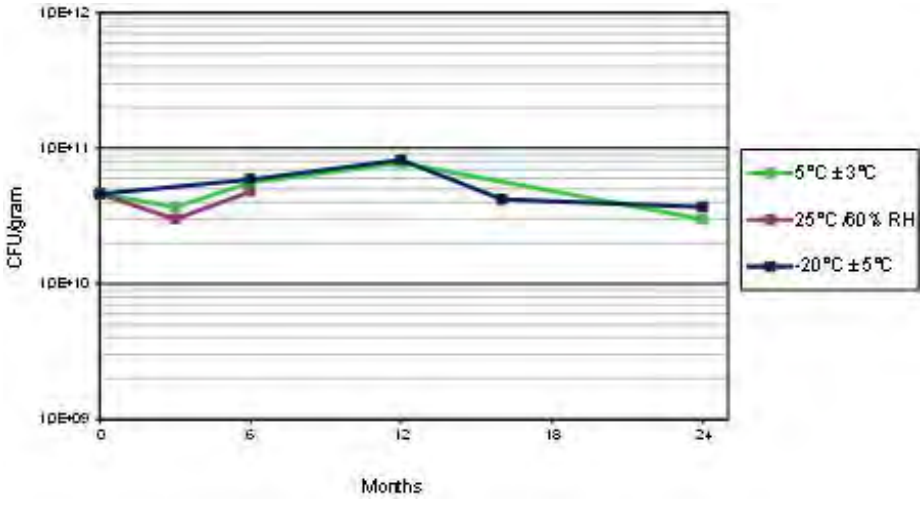


Figure 2. Cell-Count Stability of *L. paracasei* ssp. *paracasei* strain F-19.

The data from the stability study show that *L. paracasei* ssp. *paracasei* strain F-19 is stable with respect to color, water activity, and cell count for up to 2 years at temperatures of -20°C or 5°C and for up to 6 months at 25°C.

Part 3: Intended Use and Dietary Exposure

L. paracasei ssp. *paracasei* strain F-19 is intended to be added to conventional foods at concentrations consistent with cGMP needed to provide at least 10^9 cfu/serving throughout the shelf life of the product. This addition level will usually be between 5 x 10^9 and 10^{11} cfu/serving, which provides for the loss of viability of from 80% to 99% of the bacteria added. The strain's function is to serve as a probiotic microorganism.

The foods to which *L. paracasei* ssp. *paracasei* strain F-19 is intended to be added are those foods that can sustain viable *L. paracasei* ssp. *paracasei* for the shelf life of the food, including but not limited to dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and functional/nutritional products. Use in USDA-regulated foods is not intended.

L. paracasei ssp. *paracasei* strain F-19 is expected to be present in a limited number of foods at between 10^9 and 10^{11} cfu/serving, usually at less than 10^{10} cfu/serving. It will not proliferate in the foods and beverages to which it is added, but instead will decline over the shelf-life of the food. Its likely maximum ingestion is thus less than 10^{11} cfu/day, well within levels that have been shown to be safe.

Part 4: Self-limiting Levels of Use

There is no technological or organoleptic limitation to the concentration of *L. paracasei* ssp. *paracasei* strain F-19 in foods.

Part 5: Experience Based on Common Use in Food

The conclusion that the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is GRAS is based on scientific procedures rather than experience based on common use in food prior to 1958. Since 2001, the strain has been present in consumer products such as Gaio Dofilus, Gaio Yoghurtdryck, and in products of the Cultura brand in Denmark and Sweden, in porridge and baby food by Semper in Sweden, and in follow-on formulas in Sweden and the Dominican Republic. No adverse events associated with *L. paracasei* ssp. *paracasei* strain F-19 have been reported.

Part 6: Narrative

6.1. Genome Annotation

The genome sequence of *L. paracasei* ssp. *paracasei* strain F-19 obtained in-house at Chr. Hansen was used for the genome safety assessment (Chr. Hansen 2018). For the assessment, the genome sequence of *L. paracasei* ssp. *paracasei* strain F-19 was subjected to annotation using published methods. The F-19 genome contained 3,080 coding sequences (CDS) and 74 RNAs with 2,963 CDS and 74 RNAs on the chromosome, 110 CDS on the 106 Kb plasmid, 7 CDS on the 8.7 Kb plasmid, 5 CDS on the 6.3 Kb plasmid, and 2 CDS on the 2.2 Kb plasmid. The number of CDS on the published sequence is slightly lower than the in-house data.

6.1.1. Search Against Antibiotic Resistance Gene Databases

To identify genes with high homology to previously published antibiotic resistance genes, the genome of *L. paracasei* ssp. *paracasei* strain F-19 was analyzed against different published databases of antibiotic resistance genes. The databases focused either on resistance genes of relevance to Gram-positive bacteria and in particular to lactic acid bacteria and bifidobacteria or antibiotic resistance genes identified primarily in pathogenic species as they are most abundantly characterized. The genome of strain F-19 was analyzed and no antibiotic resistance genes were detected.

6.1.2. Search Against the Virulence Factor Database

The genome of *L. paracasei* ssp. *paracasei* strain F-19 was analyzed against a published database of virulence factors containing virulence factors from 30 different pathogens, including Gram positive pathogens such as *Enterococcus, Staphylococcus, Streptococcus,* and *Listeria*.

Most of the hits were associated with Clp and heat shock proteins, surface structures, and transport or secretion systems. None of the hits were assessed to be virulence factors and all hits could be regarded as 'niche factors' (Hill 2012) since they are also found in commensal bacteria.

Two hits were associated with genes that could be linked to adhesion to extracellular matrix or host cell surfaces. Homologs of these genes were observed in many *L. paracasei* strains and, in case they should have a role in adhesion, this could be regarded as a probiotic feature in *L. paracasei* ssp. *paracasei* strain F-19 rather than a safety issue.

One hit had low homology to a gene annotated as "Peptide methionine sulfoxide reductase MsrB (EC 1.8.4.12)." *In vivo*, MsrS proteins are essential for the protection of cells against oxidative damage to proteins and thereby have a role in the virulence of some pathogens; however, a MsrB mutant in *L. reuteri* also shows reduced performance in the murine gut. Thus, MsrB could be regarded as a niche factor important for the probiotic feature of the strain and, since protection against oxidative stress is important for all bacteria, is not a safety issue.

One hit had low homology to a gene annotated as "Hyaluronate lyase precursor (EC 4.2.2.1)." Hyaluronate lyases are reported to play important roles in pathogenesis by providing nutrients to the pathogen to enhance spreading of the pathogen by degrading hyaluronic acid. However, in other pathogens, such as Group A *Streptococci*, hyaluronate lyases are suggested to be anti-virulence factors, as the GAS-capsule mainly consists of hyaluronic acid, suggesting that hyaluronate lyase is not a critical virulence factor. A homolog of the gene is observed in a wide range of *L. casei* and *L. paracasei* strains, supporting the conclusion that it is not a safety issue in *L. paracasei* ssp. *paracasei* strain F-19.

6.1.3. Search of Gene Annotations

The genome sequence of *L. paracasei* ssp. *paracasei* strain F-19 was subjected to annotation using published methods. The gene annotations were searched to identify terms that could be linked to

antibiotic resistance. A total of 61 genes included one or more of these words in its annotation. Many proved to be housekeeping genes or transporters, 18 annotated as "resistance" were associated with heavy metals, and the remainder were annotated with names of antibiotics to which the strain shows no resistance and homologs were observed in many *L. paracasei* strains. All were dismissed as safety concerns.

In a similar fashion, the gene annotations were searched for terms that could be linked to virulence. This search identified 29 genes; 18 were determined to be housekeeping genes of no safety concern. Those containing the word "toxin" in the annotation were found to be bacterial toxin-antitoxin systems, part of the cellular regulatory machinery. Three genes were transporters of unknown specificity, two were adhesion factors, and one gene identified as a hemolysin appeared rather to encode a membrane protein; in any case, the strain is not hemolytic.

Finally, because biogenic amines are produced by decarboxylation of amino acids, the gene annotations were searched for the word "decarboxylase." Only one such gene was found, labeled "ornithine decarboxylase," but it is not accompanied by a transporter required for putrescine, nor does *L. paracasei* ssp. *paracasei* strain F-19 show evidence of biogenic amine production when tested phenotypically.

6.1.4. Conclusion of the Genome Safety Assessment

The genome of *L. paracasei* ssp. *paracasei* strain F-19 was analyzed for the presence of antibiotic resistance genes and no antibiotic resistance genes were detected. No virulence genes were identified in the genome of *L. paracasei* ssp. *paracasei* strain F-19, indicating a very low virulence potential.

In conclusion, there are no indications that *L. paracasei* ssp. *paracasei* strain F-19 is a safety concern as based on the performed genome safety assessment.

6.2. *In Vitro* Studies of *L. paracasei* ssp. *paracasei* Strain F-19

6.2.1. Adhesion

Human mucus was isolated from fecal samples of newborns (n = 28), 2-month-old (n = 11) and 6-month-old (n = 17) infants, and adults (n = 14) to compare mucus binding of 7 strains of probiotic bacteria (Kirjavainen et al. 1998). As compared with *B. animalis* ssp. *lactis* strain Bb-12, *L. crispatus* strain Mu5, *L. crispatus* strain M247, *L. rhamnosus* strain GG, *L. johnsonii* strain LJ-1, and *L. salivarius* strain LM2-118, *L. paracasei* ssp. *paracasei* strain F-19 was of intermediate adhesion ability for all age groups. In newborns, it exhibited greater adhesion than 3 strains and inferior adhesion to 3 other strains; in the other 3 age groups it adhered better than 2 strains and less well than 4 strains. The authors suggested that "the age of the target group may be worthy of consideration when planning a a schedule for probiotic or functional food therapy.".

Juntunen et al. (2001) studied adhesion to human intestinal mucus *in vitro* using mucus derived from fecal samples from 20 infants during and after rotavirus diarrhea and 10 healthy aged-matched infants. Both monoculture inocula of *L. casei* strain Shirota, *L. paracasei* F-19, *L. rhamnosus* GG, *L. acidophilus* LA5, and *B. animalis* ssp. *lactis* Bb12 and combinations of these bacteria were tested. Adherence was highest for *L. rhamnosus* GG (34%) and *B. animalis* ssp. *lactis* Bb12 (31%) and lowest for *L. casei* strain Shirota (1%); *L. paracasei* F-19 was second lowest with 3% adhension. Some probiotic combinations appeared to result in synergistically enhanced adherence. There were no significant differences in adhesion between the samples from infants with rotavirus diarrhea, infants after rotavirus diarrhea, or healthy infants.

6.2.2. Antibiotic Resistance

Minimum inhibitory concentrations (MIC) of 9 antibiotics were determined for *L. paracasei* ssp. *paracasei* strain F-19 according to the ISO 10932 | IDF 223 international standard. These MIC, shown in Table 4, were compared with the cut-off values established for the Lactobacillus casei/paracasei group by the European Food Safety Authority (EFSA; FEEDAP 2012).

Table 4. MIC Values for *L. paracasei* ssp. *paracasei* Strain F-19.

Antibiotic Type	Antibiotic	MIC (µg/ml)	EFSA Cut-Off Value (µg/ml)
Aminoglycoside	Gentamicin	1-2	32
	Kanamycin	32	64
	Streptomycin	16	64
Tetracycline	Tetracycline	2	4
Macrolide	Erythromycin	0.12-0.25	1
Lincosamide	Clindamycin	0.25	1
Chloramphenicol	Chloramphenicol	4-8	4
β-lactam	Ampicillin	1	4
Glycopeptide	Vancomycin	>128	n.r.
n.r. = not required to be tested			

L. paracasei ssp. *paracasei* strain F-19 is sensitive to most of the antibiotics tested with MIC values that are below the EFSA 2012 cut-off values for *Lactobacillus* casei/paracasei group. Although the MIC value for chloramphenicol is one two-fold dilution above the EFSA cut-off value in some replicates, that is considered acceptable due to the technical variation of the phenotypic method.

6.2.3. Production of Biogenic Amines

L. paracasei ssp. *paracasei* strain F-19 was analyzed for production of histamine, tyramine, cadaverine, and putrescine using an in-house procedure based on published methods; no production of the four biogenic amines was detected (Chr. Hansen, 2018).

6.2.4. Production of D-Lactate

L. paracasei ssp. *paracasei* strain F-19 was analyzed for production of L- and D-lactate; the ratio between L- and D-lactic acid was detected and it was determined that over 95% of the lactate produced was the L-enantiomer (Chr. Hansen, 2018).

6.3. Human Studies of *L. paracasei* ssp. *paracasei* Strain F-19

The studies discussed in this section are summarized in Table 5 at the end of the section.

6.3.1. Studies in Infants

West and her colleagues (West et al. 2008; West et al. 2009) reported on a clinical trial of *L. paracasei* ssp. *paracasei* strain F-19 (referred to as LF-19) with follow-ups when the infants reached the age of 8-9 years (West et al. 2013, Chorell et al. 2013, Hasslof et al. 2013, Videhult et al. 2015a, Videhult et al. 2015b).

In a prospective, randomized, double-blind, placebo-controlled study reported by West et al. (2008), 179 healthy 4-month-old term infants (75 boys and 104 girls) were enrolled and assigned to receive cereal supplemented with 0 (n = 90) or 10^8 cfu (n = 89) of *L. paracasei* ssp. *paracasei* strain F-19 daily to age 13 months. All infants were immunized with DTaP (diphtheria and tetanus

toxoid and acellular pertussis), polio, and Hib-conjugate vaccines at 3, 5½, and 12 months of age. Parents recorded feedings, stooling, and any symptoms. Venous blood was collected at 5½, 6½, 12, and 13 months of age for analysis of IgG antibodies to tetanus toxoid and diphtheria toxin. Fecal samples were collected at enrolment and at 6½, 9, and 13 months of age for bacterial testing.

Five infants from the test group and 3 from the controls were withdrawn by their parents for reasons not associated with the intervention. *L. paracasei* ssp. *paracasei* strain F-19 was detected in feces of 90% of the infants receiving the probiotic. The number of days with fever, respiratory illness, or diarrhea did not differ between the groups, but days receiving antibiotics were significantly fewer among infants ingesting strain F-19. Exposure to *L. paracasei* ssp. *paracasei* strain F-19 enhanced anti-diphtheria concentrations. No adverse effects of the probiotic were reported, and the authors concluded that, "feeding LF-19 [West et al.'s term for *L. paracasei* ssp. *paracasei* strain F-19] did not prevent infections, but increased the capacity to raise immune responses to protein antigens."

In additional analyses of the data from this study, West et al. (2009) found that the ratio of IFN-γ to IL4 mRNA was significantly greater in infants receiving *L. paracasei* ssp. *paracasei* strain F-19 and the incidence of eczema was significantly reduced, suggesting "enhancing effects of *Lactobacillus* F-19 on the T-cell mediated immune response." In further analyses, West et al. (2012a), reported little difference between test and control groups in expression levels of IL-2, IFN-γ, IL-4, IL-17A, and IL-10 messenger RNAs, suggesting "modest effects by probiotics on T-cell maturation."

West et al. (2013) conducted a follow-up with 121 of the infants from the trial described previously (West et al. 2008) at 8-9 years of age to assess the prevalence of allergic disease (eczema, allergic rhinitis, asthma, and food allergy) by clinical examination. The available participants included 59 from the probiotic group and 62 from the treatment group. Neither benefits nor adverse effects were reported. The authors concluded that, "There was no long-term effect of LF-19 on any diagnosed allergic disease, airway inflammation or IgE sensitization."

In yet another report on data from the West et al. (2008) study, Chorell et al. (2013) reported that there were no effects on anthropometrics of serum lipids due to ingestion of F-19, but there was a significant increase in putrescine, which the authors suggested might contribute to increased gut integrity.

Hasslof et al. (2013) published still another report on the later experience of infants from the West et al. (2008) study—this time their dental health at 9 years of age. The authors explained that the rationale for the study was that "It has been suggested that the chronic use of probiotics . . . in young children may lead to undesirable side effects in the intestine and the oral cavity." No differences were seen between those who had received *L. paracasei* ssp. *paracasei* strain F-19 and those who had not, either beneficial or adverse, and the authors concluded that, "early intervention with LF-19 [*L. paracasei* ssp. *paracasei* strain F-19] did not affect the frequency of dental caries, [mutans streptococci], or [lactobacilli]. LF-19 did not establish itself as a permanent facet of the oral microbiota in any of the subjects included in this study."

Videhult et al. (2015a) reassessed the effects of *L. paracasei* ssp. *paracasei* strain F-19 on body composition, growth, and metabolic markers of 8-9-year-old children given the probiotic as infants, as reported in West et al. (2008). Of 179 children included in the original study, 120 were available for the follow-up at 8–9 years of age, 58 in the probiotic group and 62 in the placebo group. Anthropometrics, body composition, serum lipids, insulin, glucose, and transaminases were measured. There were no significant differences between groups on any measures, and the authors reported that, "Feeding LF-19 during infancy did not modulate body composition, growth or any of

the assessed metabolic markers at school age." They concluded that "we observed no adverse effect of LF-19 on body composition or metabolism in this follow-up study."

Videhult et al. (2015b) assessed the effects of *L. paracasei* ssp. *paracasei* strain F-19 given to infants, as reported in West et al. (2008), on the metabolic and inflammatory profile of 120 of the infants as 8-9-year-old children. Overweight or obese children had increased plasma C-peptide, plasminogen activator inhibitor-1, leptin, and serum high-sensitivity C-reactive protein compared with normal weight children, but there was no association with *L. paracasei* ssp. *paracasei* strain F-19. The authors concluded that "the probiotic LF-19 had no long-term effect on the inflammatory and metabolic profile."

Zampieri et al. (2013) reported a prospective, randomized, unblinded trial enrolling 32 preterm infants (all delivered before gestational week 32; birth weight 600-1500 g) at Bell's stage 2 necrotizing enterocolitis (NEC). Eighteen patients were randomly assigned to receive $6x10^9$ cfu/ day of *L. paracasei* ssp. *paracasei* strain F-19 along with standard medical treatment, while 14 patients received only standard treatment. During the study, records were kept of ventilator support need and need for nasogastric feeding. Infants receiving *L. paracasei* ssp. *paracasei* strain F-19 had a significantly reduced probability of progression to Bell's stage 3 NEC; the authors suggested that improved intestinal motility might have contributed to this result. The authors reported that, "No collateral effects were observed during the study period; none of our patients presented sepsis due to *L. paracasei* subsp. *paracasei* strain F-19. No patient required preventive exclusion from the trial. No patient [in the probiotic group] presented treatment-related intestinal complications (i.e., diarrhea)." Their conclusion was that "The use of *L. paracasei* subsp. *paracasei* strain F-19 is safe and effective."

Growth of infants receiving formula supplemented with prebiotics or synbiotics was studied in a prospective, randomized, double-blind, two-arm, multicenter study (Szajewska et al. 2017). A total of 182 healthy term infants, 93 males and 89 females, aged 28 days were randomized to receive formula supplemented with fructooligosaccharides (FOS) and galactooligosaccharides (GOS) or with FOS, GOS, and 10^9 cfu of *L. paracasei* ssp. *paracasei* strain F-19 to 6 months of age. Study visits occurred every 28 days to age 4 months, then at 6, 9, and 12 months, for anthropometric measures, assessment of tolerance and health-related outcomes, and description of stool characteristics. The primary outcome measure was growth (body weight, length, and head circumference) during the first year of life. The secondary outcome measures were health-related parameters.

There was no difference in growth between the two groups, but the infants receiving synbiotics exhibited a weak but significant reduction of lower respiratory tract infections. With regard to safety, the authors reported that, "Probiotic supplementation of infant formula with *Lactobacillus* F-19 was well tolerated, and no significant differences between the experimental and control groups were observed in regard to adverse events," and concluded:

> "The lack of a significant difference between the formula-fed groups for growth and for the occurrence of serious adverse events supports the safety of using *Lactobacillus* F-19-supplemented synbiotic formula in healthy term infants."

6.3.2. Studies in Children

Sixty-one apparently healthy and fully weaned children (sex not reported; mean age = 13.1±5.3 years) were enrolled in a prospective, randomized, double-blind, placebo-controlled study (Sullivan et al. 2002) of the effect of *L. paracasei* ssp. *paracasei* strain F-19 on their colonic microbiota. Both the test group (n = 30) and the placebo group (n = 31) consumed gelatin capsules containing 0 or 10^{10} cfu of *L. paracasei* ssp. *paracasei* for 3 weeks. Children's parents kept a daily record of

stooling characteristics and gastrointestinal symptoms. Fecal samples were collected at baseline and at weeks 2 and 5 for bacterial analysis.

Children receiving *L. paracasei* ssp. *paracasei* strain F-19 showed a significant increase in fecal levels of the strain, but not of other lactobacilli or bifidobacteria. The authors reported that, "The products were generally well tolerated . . . Fecal characteristics and occurrences of gastrointestinal symptoms were similar in the treatment and placebo groups."

As part of a multicenter project named PROBDEMO (Crittenden et al. 2002) 61 apparently healthy male and female children aged 1-1.5 years enrolled in a prospective, randomized, double-blind, placebo-controlled study received gelatin capsules providing 0 or $2x10^{10}$ cfu of *L. paracasei* ssp. *paracasei* strain F-19 for 3 weeks. Fecal samples were taken for assessment of F-19 and other bacteria. Ingestion of the F-19 strain resulted in its presence in fecal samples, but did not significantly alter the balance of the major population groups within the intestinal microbiota. The study design included observations monitoring potential side-effects of probiotic consumption, including intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. No such adverse effects were observed and the probiotic was well tolerated by all individuals. The authors concluded that, "The fact that no side-effects were detected in subjects . . . suggests that *Lactobacillus* F-19 is a safe microbial food supplement."

6.3.3. Studies in Adults

Sullivan et al. (2001) reported a prospective, randomized, double-blind, placebo-controlled trial of the effects of treating elderly *Helicobacter pylori* patients with *L. paracasei* ssp. *paracasei* strain F-19 and inulin. Thirty-five patients (sex not reported; age range 58-89 years with a median age of 76 years) were randomized into treatment (n = 17) and placebo (n = 18) groups; the groups received cultured buttermilk with 0 or 10^{11} cfu of F-19 and 0 or 6 g inulin for 12 weeks. Fecal samples were collected at baseline and at weeks 4, 12, and 20 for bacterial analysis. The numbers of *L. paracasei* ssp. *paracasei* strain F-19 and other lactobacilli increased significantly in the test group. The authors concluded that the probiotic "survives the passage through the gastrointestinal tract." There was no discussion of any adverse events.

Thirty elderly *Helicobacter pylori* patients (sex not reported; mean age = 76.8±6.5 years) were enrolled in a prospective, randomized, double-blind, placebo-controlled study (Sullivan et al. 2002) of the effect of *L. paracasei* ssp. *paracasei* strain F-19 on their colonic microbiota. Both the test group (n = 13) and the placebo group (n = 17) consumed fermented milk twice daily for 12 weeks; the test group's milk provided 10^{11} cfu of *L. paracasei* ssp. *paracasei* daily. Patients kept a daily record of stooling characteristics and gastrointestinal symptoms, serum samples were examined for anti *H. pylori* IgG, and breath was analyzed for urea. Fecal samples were collected at baseline and at weeks 4, 12, and 20 for bacterial analysis.

Patients receiving *L. paracasei* ssp. *paracasei* strain F-19 showed a significant increase in fecal levels of the strain and other lactobacilli, but no effect was reported on *H. pylori* infection. The authors reported that, "The products were generally well tolerated . . . Fecal characteristics and occurrences of gastrointestinal symptoms were similar in the treatment and placebo groups."

As part of a multicenter project named PROBDEMO (Crittenden et al. 2002), 5 apparently healthy adults (sex not reported) consumed fermented milk providing $4x10^{10}$ cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. longum* per day for 12 days in an open-label study. Fecal samples were taken at baseline and at 12 days, and biopsy samples were taken from the colonic mucosa after 12 days, for enumeration of F-19 and other lactobacilli. In the fecal samples, lactobacilli were present at a level of $2.0±1.3x10^{6}$ cfu/g both before and after treatment, with the F-19 strain absent prior to treatment but constituting 65% of total lactobacilli after treatment. In the colonic mucosal biopsy, strain F-19 constituted 10% of the $0.9±1.7x10^{5}$ cfu/g lactobacilli present.

Similar levels of *L. paracasei* ssp. *paracasei* F-19 were found at all locations within the colon, ascending, transverse, and descending; "demonstrating," according to the authors,"that it can colonize the length of the colon."

In another study within the PROBDEMO project, 9 apparently healthy adults (sex not reported), 4 with milk hypersensitivity, consumed non-fermented milk providing 0 or $4x10^8$ cfu of *L. paracasei* ssp. *paracasei* strain F-19 for one week in a prospective, single-blind, placebo-controlled, crossover study (Crittenden et al. 2002). Fecal samples were taken for assessment of F-19 and other bacteria. As in the first study, the F-19 strain was found in fecal samples after a week of ingestion but did not significantly disturb the balance of the major population groups within the intestinal microbiota in individuals with or without milk hypersensitivity.

In another prospective, randomized, double-blind, placebo-controlled study within the PROBDEMO project, 30 elderly patients (>65 years) seropositive to *H. pylori* consumed fermented milk providing 0 or $1.5x10^{11}$ cfu of *L. paracasei* ssp. *paracasei* strain F-19 daily for 12 weeks. Fecal samples were taken for assessment of F-19 and other bacteria. Consumption of *L. paracasei* ssp. *paracasei* strain F-19 did not significantly disturb the balance of the major population groups within the intestinal microbiota in *H. pylori*-infected patients.

All PROBDEMO trials included observations monitoring potential side-effects of probiotic consumption (Crittenden et al. 2002). These included intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. The trials included healthy subjects, adults with verified milk-hypersensitivity, and elderly people infected with *H. pylori*. The authors concluded that, "No adverse effects of probiotic administration were observed in any of the pilot studies. The probiotic was well tolerated by all individuals, including the elderly *H. pylori* patients who consumed *L. paracasei* ssp. *paracasei* strain F-19 daily for 3 months without adverse effects. The fact that no side-effects were detected in subjects ranging in age from 1 to 85 years, and in healthy individuals and subjects suffering mild illnesses (milk-hypersensitivity and *H. pylori* infection) suggests that *Lactobacillus* F-19 is a safe microbial food supplement."

Rayes and her colleagues published a series of similar studies (2002a, 2002b, 2002c, 2005, 2007, 2012) investigating the ability of *L. paracasei* ssp. *paracasei* strain F-19 to reduce the incidence of post-operative bacterial infection in patients recovering from major abdominal surgery or organ transplantation.

In a prospective, randomized, double-blind, placebo-controlled trial of patients receiving liver transplants, Rayes et al. (2002a) divided 95 patients (49 males, 46 females with mean age = 49.0±2.5 years) into 3 groups—one group received standard enteral nutrition formula, a second group received the formula with added fiber and $2x10^9$ cfu of *L. paracasei* ssp. *paracasei* strain F-19, and a third group received the formula with added fiber plus heat-killed bacteria. All infections and isolated bacteria were recorded as well as days in the ICU and the hospital and any side effects. Non-infectious complications such as biliary fistulas, anastomotic leaks, and impaired kidney function were monitored, and blood was taken on post-operative days 1, 5, and 10 for hematology, clinical chemistries, and immunology. Body temperature was measured three times daily and surveillance cultures from urine, blood, bile, and intra-abdominal drainages were done twice a week.

The patients who received living lactobacilli plus fiber developed significantly fewer bacterial infections (13%) than the patients on standard formula (48%) or inactivated bacteria and fiber (34%). Both standard enteral formula and strain F-19 were well tolerated. Abdominal side effects (distension, cramps, or diarrhea) were seen in 8 of 32 patients in the standard-formula group, 6 of 31 patients in the live *Lactobacillus* group, and 11 of 32 patients in the inactivated-bacteria group.

Rayes et al. (2002b, abstract) enrolled 172 patients following major abdominal surgery or liver transplantation in a prospective, randomized, double-blind, placebo-controlled trial in which they received either conventional enteral nutrition, enteral nutrition with fiber and $2x10^9$ cfu of *L. paracasei* ssp. *paracasei* strain F-19, or enteral nutrition with fiber and heat inactivated lactobacilli. The groups receiving live *Lactobacillus* cultures experienced significantly reduced incidences of bacterial infection (among resection patients, 4% vs. 13% in the prebiotic group and 31% in the conventional-therapy group; among transplant recipients, 13% in the probiotic group vs. 34% in the prebiotic group and 48% in the conventional therapy group). The authors reported that "Fibre and lactobacilli were well tolerated in most cases."

Ninety patients (48 males and 42 females, mean age = 61.0±14.1 years) recovering from major abdominal surgery were enrolled in a prospective, randomized, double-blind, placebo-controlled trial (Rayes et al. 2002c). Three randomly assigned groups of n = 30 received conventional enteral nutrition, enteral nutrition with fiber and $2x10^9$ cfu of *L. paracasei* ssp. *paracasei* strain F-19, or enteral nutrition with fiber and heat inactivated bacteria. All infections and isolated bacteria were recorded as well as days in the ICU and the hospital and any side effects. Non-infectious complications such as biliary fistulas, anastomotic leaks, and impaired kidney function were monitored, and blood was taken on post-operative days 1, 5, and 10 for hematology, clinical chemistries, and immunology. Body temperature was measured three times daily and surveillance cultures from urine, blood, bile, and intra-abdominal drainages were done regularly.

All patients completed the study, receiving the full course of their assigned nutrition. The incidence of infections was significantly lower in groups receiving fiber and either live or killed lactobacilli (10% each) than in the group receiving standard nutritional formula (30%). Patients receiving live F-19 strain needed antibiotics for a significantly shorter time than did the patients in the other groups. The length of hospital stay and the incidence of non-infectious complications did not differ significantly. Fibers and lactobacilli were well tolerated; side effects did not differ between groups and there was no diarrhea. The authors concluded that "application of fiber and lactobacilli is feasible even after major abdominal surgery and caused few side effects."

In a prospective, randomized, double-blind, placebo-controlled trial (Rayes et al. 2005), 66 liver transplant recipients (38 males, 28 females, mean age = 51.5±2.5 years), 33 per group, received enteral nutrition with prebiotics and with or without probiotics for 14 days beginning the day prior to surgery. The daily oral probiotic administration included $2x10^{10}$ cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus* strain 5-33:3, *Leuconostoc mesenteroides* strain 77:1, and *L. plantarum* strain 2362. The primary endpoint was the occurrence of post-operative bacterial infection. All infections and isolated bacteria were recorded as well as days in the ICU and the hospital and any side effects. Non-infectious complications such as biliary fistulas, anastomotic leaks, and impaired kidney function were monitored, and blood was taken on post-operative days 1, 4, and 8 for hematology, clinical chemistries, and immunology. Body temperature was measured twice daily and surveillance cultures from urine, blood, bile, and intra-abdominal drainages were done three times a week.

Inclusion of probiotics in the enteral nutrition formula significantly reduced the incidence of post-operative bacterial infection and reduced the severity of infections that occurred. Hematology, immunology, and biochemistry parameters did not differ significantly between groups. The authors reported that "Enteral nutrition, containing the synbiotic combination, was well tolerated in all patients," with fewer probiotic patients exhibiting diarrhea or abdominal cramps. "All side effects disappeared under temporary reduction in the amount of enteral nutrition." The authors concluded that inclusion of probiotics "is an effective means to prevent post-operative bacterial infections in high-risk surgical patients, and since it causes no resistant strains and has no serious side effects, it could be widely used."

Rayes et al. (2007) reported a fifth prospective, randomized, double-blind, placebo-controlled trial of the effect of synbiotics on the incidence of post-surgical bacterial infections. Eighty patients (45 males, 35 females, mean age = 58.5±12.5 years) recovering from pylorus-preserving pancreatoduodenectomy received enteral nutrition with prebiotics and with or without probiotics for 14 days beginning the day prior to surgery. The daily oral probiotic administration included $2x10^{10}$ cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus* strain 5-33:3, *Leuconostoc mesenteroides* strain 77:1, and *L. plantarum* strain 2362. The primary endpoint was the occurrence of post-operative bacterial infection. All infections and isolated bacteria were recorded as well as days in the ICU and the hospital and any side effects. Non-infectious complications such as biliary fistulas, anastomotic leaks, and impaired kidney function were monitored, and blood was taken on post-operative days 1, 4, and 8 for hematology, clinical chemistries, and C-reactive protein. Body temperature was measured twice daily and surveillance cultures from urine, blood, bile, and intra-abdominal drainages were done in case of suspected infection.

Inclusion of probiotics in the enteral nutrition formula significantly reduced the incidence and duration of post-operative bacterial infection to 12.5% vs. 40% in the fiber-only formula group. The incidences of diarrhea and abdominal cramps were the same in both groups of patients. The authors reported that, "Enteral nutrition, containing the synbiotic combination, was well tolerated in all patients… All side effects disappeared under temporary reduction in the amount of enteral nutrition," and concluded, "Early enteral nutrition with synbiotics was able to significantly reduce postoperative bacterial infections in patients following [pancreatoduodenectomy] with only single-shot antibiotic prophylaxis. In contrast to antibiotics, it is relatively cheap and does not cause resistant strains or serious side effects."

The same combination of probiotics ($2x10^{10}$ cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus* strain 5-33:3, *Leuconostoc mesenteroides* strain 77:1, and *L. plantarum* strain 2362) was tested for its effect on liver function after hepatic resection (Rayes et al. 2012). Nineteen right-hepatectomy patients (14 males and 5 females, mean age = 60.1±13.9 years) were enrolled in a prospective, randomized, double-blind, placebo-controlled pilot study in which they received enteral nutrition formula containing prebiotic or containing both pre- and probiotic for 10 days post-operatively. The primary study end point was restoration of liver function, tracked via laboratory parameters. The incidence of surgical complications, bacterial infections, and any side effects was monitored. Body temperature was measured twice daily and blood was drawn for tests of hematology, clinical chemistries, and C-reactive protein.

Results of liver function tests were ambiguous, and the authors suggested that patient numbers were too small and the clinical courses to heterogeneous to draw conclusions regarding the effectiveness of synbiotic intervention in restoration of liver function. The authors were able to conclude that, "The synbiotic combination was well-tolerated in all patients. Mild side-effects (abdominal distension and cramps) occurred in three patients of each group but disappeared under symptomatic therapy. No severe side effects were recorded."

A prospective, randomized, double-blind, placebo-controlled study (Sullivan et al. 2003) was designed to test the ability of a probiotic mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain NCFB 1748, and *B. animalis ssp. lactis* strain Bb12 to prevent antibiotic-associated ecological disturbances of the intestinal microbiota. Twenty-four apparently healthy adults (7 men and 17 women aged 21-48 years) ingested 4 clindamycin capsules daily for 7 days; 12 of them also consumed a yogurt product providing $2x10^{8}$ cfu of each strain while the other 12 consumed plain yogurt for 14 days. Stool samples were collected at baseline and on days 2, 5, 7, 10, 14, and 21 for microbiological analysis.

Consumption of the probiotic prevented ecological disturbances in the numbers of lactobacilli and *Bacteroides fragilis* group. One participant in the probiotic group developed diarrhea and one reported looser stools. The first person's stool was cytotoxin positive and was cured by metronidazole; the loose stools resolved spontaneously. No other adverse events were reported.

Riordan et al. (2003) reported an open label study in which 11 cirrhosis patients (age and sex not reported) and 5 healthy controls consumed sachets providing beta glucan, inulin, pectin, resistant starch, and $8x10^{10}$ cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *L. plantarum* strain 2362, *Pediococcus pentosaceus* strain 5-33:3, and *Lactococcus raffinolactis* strain 32-77:1 for 7 days. Toll-like receptor (TLR) 2 expression, serum TNF-α levels, and production of TNF-α were measured at baseline and at 7 and 28 days after cessation of supplementation. Supplementation with the synbiotic regimen resulted in significant up-regulation of peripheral blood mononuclear cell expression of TLR2. Serum TNF-α levels were further increased while TNF-α production was reduced in most patients. The authors reported that, "Administration of the synbiotic supplement was well tolerated without any reported adverse events or change in general clinical state."

Liu et al. (2004) reported on a prospective, randomized, double-blind, placebo-controlled trial in which 55 patients (53 M, 2 F) aged 56±11 years with minimal hepatic encephalopathy were randomized to receive a synbiotic preparation containing fermentable fiber and 10^{10} cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus, Leuconostoc mesenteroides*, and *L. plantarum* (n = 20), fermentable fiber alone (n = 20), or a non-fermentable placebo (n = 15) for 30 days. Patients were re-assessed for minimal hepatic encephalopathy on day 30, while fecal samples were taken at baseline, on day 30, and 14 days later for bacterial analysis.

Either the synbiotic or fiber-alone produced significant improvement in minimal hepatic encephalopathy and reduction in potentially pathogenic *E. coli* and *Staphylococcal* species. The authors reported that, "The synbiotic, fermentable fiber and placebo preparations were well-tolerated by all patients, with no reports of adverse side effects. In particular, no patient reported diarrhea or abdominal pain or became noncompliant for other reasons."

Sullivan et al. (2004) studied the ability of *L. paracasei* ssp. *paracasei* strain F-19 to prevent the emergence of antibiotic-resistant microorganisms in patients receiving penicillin, ciprofloxacin, or norfloxacin. Twenty hospital patients (sex not reported) aged 18-89 years (mean = 59 years) receiving penicillin and 16 patients aged 28-86 years (mean = 61 years) receiving ciprofloxacin or norfloxacin were enrolled in a prospective, randomized, double-blind, placebo-controlled study in which half received $2x10^{10}$ cfu of *L. paracasei* ssp. *paracasei* strain F-19 daily for 14 days and half received placebo. Fecal samples were taken at baseline and on days 10 and 30 and isolates of enterococci, enterobacteria, and bacteroides were screened for antibiotic resistance.

The authors reported that, "No major differences were observed between the probiotic- and placebo-supplemented groups." They also reported that, "No adverse events were reported. Eight patients treated with penicillin reported looser stools, four in the active and four in the placebo group. In the quinolone-treated patients there were two individuals who experienced looser stools, one from each of the placebo and active groups."

Sixty otherwise apparently healthy women aged 18-40 years (mean = 32.5±12/8 years) with bacterial vaginosis were enrolled in a prospective, randomized, double-blind, placebo-controlled study (Delia et al. 2006) and treated with *Lactobacillus acidophilus* vaginal suppositories along with oral *L. paracasei* ssp. *paracasei* strain F-19 or placebo for 3 months. Both treatments were successful in reducing vaginal pH and subjective symptomatology scores. No adverse effects of the treatment were reported and the authors concluded, "The associàtion of oral administration is useful to balance the vaginal environment with the ìntestinal microflora with improvement of long-term results."

Fourteen apparently healthy adults (5 males and 9 females aged 36-74 years) scheduled for routine colonoscopy were enrolled in an open-label study of the intestinal survival and persistence of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain NCFB 1748, and *B. animalis* ssp. *lactis* strain Bb-12 (Matto et al. 2006). The probiotics were provided in yogurt at daily levels of $2x10^{10}$ cfu for the 2 *Lactobacillus* strains and 10^{11} cfu for the bifidobacteria for 10 days. Fecal samples were collected at the beginning and end of the ingestion period and at several time points up to 18-19 days after cessation of intake. Biopsy samples were taken and the end of ingestion, or 8-9 days later, or 18-19 days later. All samples were analyzed for bacterial content.

The F-19 and Bb-12 strains survived well and were detected in the feces of 100% and 79%, respectively, of the individuals consuming them while only 21% of them shed strain NCFB 1748. However, the probiotic bacteria washed out quickly and were rarely detected in fecal samples after a few weeks. Nor were the probiotics often isolated from colonic biopsies, suggesting that they do not exhibit strong adhesion properties. No adverse effects were reported by the authors.

To assess the effect of oral supplementation with synbiotics, glutamine, and peptide on intestinal permeability and the clinical outcome of critically ill trauma patients, Spindler-Vesel et al. (2007) enrolled 113 such patients (88 males and 25 females, mean age = 41.0±18.9 years) in a prospective, randomized, double-blind, placebo-controlled trial. Patients were randomized into 4 groups to receive glutamine (n = 32), soluble fiber (n = 29), peptide (n = 26), or fiber plus 10^{10} cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *L. plantarum* strain 2362, *Pediococcus pentosaceus* strain 5-33:3, and *Lactococcus raffinolactis* strain 32-77:1 (n = 26) for 7 days. Multiple organ failure was assessed daily and intestinal permeability was measured on days 2, 4, and 7.

No differences between groups were reported for multiple organ failure. However, out of a total of 51 bacterial infections, only 5 were observed in the synbiotic group, a significantly lower incidence than the other 3 groups. Only the synbiotic group showed evidence of decreased intestinal permeability. No adverse events associated with the synbiotic were reported. The authors concluded that, "Patients supplemented with synbiotics did better than the others, with lower intestinal permeability and fewer infections."

One hundred patients (38 male, 62 female, aged 18-68 years with mean = 39±26 years) suffering from irritable bowel syndrome (IBS) were enrolled in a prospective open-label study of the effectiveness of *L. paracasei* ssp. *paracasei* strain F-19 in treating the condition (Lombardo et al. 2009). Fifty two had diarrhea and 48 had constipation and all patients were given $2.4x10^{10}$ cfu per day of *L. paracasei* ssp. *paracasei* strain F-19 for 14 days. The probiotic treatment significantly reduced abdominal distension and pain and cured both diarrhea and constipation in more than 80% of those afflicted. The authors reported that tolerability of the probiotic was excellent, "No significant side effects that could definitely be attributed to the treatment were observed. Only one case of slight nausea was reported, but this did not require withdrawal from treatment."

In an open-label study, Sullivan et al. (2009) studied the effect of a probiotic mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain NCFB 1748, and *B. animalis ssp. lactis* strain Bb12 on 15 chronic fatigue syndrome patients, 5 men and 10 women aged 30-56 years. Patients were observed for 2 weeks to establish a baseline of fecal bacteria and calprotectin levels, then they received $4x10^{10}$ cfu/day of the probiotic for 4 weeks, followed by a 4-week washout. Fatigue symptoms and fecal bacteria and calprotectin were measured at baseline, the end of the intervention, and the end of the washout period.

There were no significant changes in fatigue and physical activity scores and no major changes occurred in the gastrointestinal microflora; nevertheless, 6 of the 15 patients reported that they had improved. The authors did not report any adverse events. The authors concluded that the challenge is to "identify the responders to the therapy with probiotics as this pilot study demonstrates that

some individuals do respond with less fatigue, less bodily symptoms and better neurocognitive functions."

Simren et al. (2010) reported a prospective, randomized, double-blind, placebo-controlled study of the effect of a probiotic mixture in patients with irritable bowel syndrome (IBS). A total of 74 IBS patients (22 males and 52 females with mean age = 43.0±15.5 years) were randomized to receive acidified milk with or without $2x10^{10}$ cfu of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain La5, and *B. animalis* ssp. *lactis* strain Bb-12 for 8 weeks. The patients completed self-questionnaires weekly, blood was tested for clinical chemistries and hematology at baseline and following the intervention, and fecal samples were collected at the end of the intervention for enumeration of *L. paracasei* ssp. *paracasei* strain F-19 as a check on compliance.

IBS symptoms improved significantly in both groups over the study period, but with no significant difference between them. No clinically significant effects on biochemistry or hematology were noted and no adverse events were reported by the patients. The authors stated that, "The yoghurt was well tolerated."

In a prospective, randomized, double-blind, placebo-controlled multicenter study of patients with irritable bowel syndrome (IBS), Sondergaard et al. (2011) enrolled 13 males and 39 females aged 29-67 years (mean age = 51.3 years) fulfilling Rome II criteria to consume acidified milk providing 0 or $2.5x10^{10}$ cfu of a combination of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain La5, and *B. animalis* ssp. *lactis* strain Bb-12 for 8 weeks. The patients completed weekly questionnaires and fecal samples were collected at the end of the intervention for enumeration of *L. paracasei* ssp. *paracasei* strain F-19 as a check on compliance.

While IBS symptoms significantly improved over the study period in both groups, there was no significant difference between treatment with acidified milk alone or acidified milk containing the probiotics. The authors reported that, "The investigational products were well tolerated and no serious adverse events occurred."

Fifty patients (18 males and 32 females, mean age = 65.2±8.1 years) with symptomatic uncomplicated diverticular disease received a high-fiber diet and were randomized to the diet alone (n = 16), $2.4x10^{10}$ cfu/day of *L. paracasei* ssp. *paracasei* strain F-19 (n = 18), or $4.8x10^{10}$ cfu/day of the probiotic (N = 16; Annibale et al. 2011) in a prospective, randomized, placebo-controlled, unblinded 6-month study. The probiotic was ingested for the first 14 days of each month. At enrolment and after 6 months, patients were examined for abdominal and dyspeptic symptoms and blood was taken for complete blood count, erythrocyte sedimentation rate, C-reactive protein, and protein electrophoresis.

Ingestion of the probiotic at either dose significantly reduced intestinal bloating and prolonged abdominal pain compared to the control group, with no difference between dosages. The authors concluded that, "Overall, the cyclic long-term (6 months) supplementation with *L. paracasei* sub. *paracasei* strain F-19 was found to be safe, since only in one patient was a side-effect (diarrhoea) found to lead to withdrawal from treatment."

Pedersen et al. (2011) reported a prospective, randomized, double-blind, placebo-controlled study in which 61 patients (16 men and 45 women age 18-79 years; mean age = 42.5 years) with irritable bowel syndrome (IBS) consumed acidified milk with (n = 30) or without (n = 31) $2x10^{10}$ cfu of each of the probiotics *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain LA-5, and *B. animalis* ssp. *lactis* strain Bb-12 for 8 weeks. Blood was drawn before and after the intervention and analyzed for serum glucose, lactate, glutamine, proline, creatinine/creatine, and aspartic acid. While significant changes were reported in serum metabolites in both groups, these effects appeared to be due to the acidified milk since there were no significant differences between those receiving milk with or without the probiotics. The authors did not report any adverse events, and

concluded that "the delivering vector for probiotics, which was acidified milk in this study, can be very important for the study outcome."

Begtrup et al. (2013) investigated the long-term effect of daily ingestion of $5.2x10^{10}$ cfu of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus* strain La5, and *B. animalis* ssp. *lactis* strain Bb-12 in the management of irritable bowel syndrome (IBS). A total of 131 IBS patients (34 males, 97 females; mean age = 30.52±9.42 years) were enrolled in a prospective, randomized, double-blind, placebo-controlled study in which they received capsules containing either the probiotic mixture (n = 67) or placebo (n = 64) for 6 months with a 6-month follow-up. Patients visited a study nurse at 3, 6, and 12 months for questioning about compliance, symptoms, and adverse effects.

The authors concluded that, "During a 6-month treatment period, we were not able to detect a positive effect of probiotic when compared with placebo." They also concluded that, "No serious adverse effects were reported."

In a three-arm prospective, randomized, double-blind, placebo-controlled study of 58 obese postmenopausal women aged 59.5±5.9 years, Brahe et al. (2015) investigated the effect of daily ingestion of $9.4x10^{10}$ cfu of *L. paracasei* ssp. *paracasei* strain F-19 or of 10 g flaxseed mucilage on gut microbiota and metabolic risk markers. Nineteen women each were randomized to receive either probiotic or mucilage for 6 weeks while 20 women received maltodextrin placebo. Bacterial viability was confirmed at the end of the intervention. The participants were interviewed about all types of potential adverse effects at each visit by the use of broad, open-ended questions, and asked about changes in stool characteristics. Additionally, body composition was assessed; blood samples were taken for analysis of glucose, insulin, total cholesterol, LDL- and HDL-cholesterol, triacylglycerol, leucocytes, C-reactive protein, lipopolysaccharide-binding protein, TNF-α, IL-6, and angiopoietin-like protein 4; and DNA in fecal samples was analyzed for identification of shed bacteria.

Intake of *L. paracasei* ssp. *paracasei* strain F-19 did not modulate metabolic markers compared with placebo. The authors reported on probiotic-related adverse events as follows:

> "More adverse events were reported in the flaxseed group compared with the placebo group, while there was no difference in the occurrence of adverse events between the placebo and the *L. paracasei* F-19 group. . . There was no difference between the adverse events reported following the probiotic and placebo intervention that included both more frequent and less-frequent defecation. No serious adverse events were registered during the study" (Brahe et al. 2015).

Compare et al. (2015) reported a prospective, randomized, double-blind, placebo-controlled, multicenter study of the effect of *L. paracasei* ssp. *paracasei* strain F-19 on bowel symptom onset in gastroesophageal reflux disease (GERD) patients on long-term proton-pump inhibitors. The study had 4 arms: $2.4x10^{10}$ cfu of F-19 for 3 days per week for 6 months, $2.4x10^{10}$ cfu of F-19 for 3 days/week for 3 months then placebo for 3 months, placebo for 3 months then $2.4x10^{10}$ cfu of F-19 for 3 days/week for 3 months, and placebo for 6 months. The 100 enrolled patients (56 male, 44 female; mean age = 39±10.4 years) were assigned 25 per arm. Patients completed questionnaires each month to assess bowel habits and symptoms.

Treatment with *L. paracasei* ssp. *paracasei* strain F-19 significantly reduced flatulence and bloating but not abdominal pain. There was no discussion of any adverse effects of the probiotic intervention.

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Studies in Infants						
Szajewska et al. 2017	Growth of infants receiving formula supplemented with prebiotics or synbiotics	Prospective, randomized, double-blind, parallel-group, multicenter	182 healthy term infants, 93M 89F, aged 28 days	10^9 cfu/day	5 months	There was no difference in growth between the two groups. The authors reported that, "Probiotic supplementation of infant formula with *Lacto-bacillus* F-19 was well tolerated, and no significant differences between the experimental and control groups were observed in regard to adverse events," and concluded: "The lack of a significant difference between the formula-fed groups for growth and for the occurrence of serious adverse events supports the safety of using *Lactobacillus* F-19-supplemented synbiotic formula in healthy term infants."
West et al. 2008 West et al. 2009 West et al. 2012a West et al. 2013 Chorell et al. 2013 Hasslof et al. 2013 Videhult et al. 2015a Videhult et al. 2015b	Effects of *L. paracasei* ssp. *paracasei* strain F-19 in infants	Prospective, randomized, double-blind, placebo-controlled with follow-up at age 8-9 years	179 healthy term infants, 75M 104F, aged 4 months	10^8 cfu/day	9 months	5 infants from the test group and 3 from the con-trols were withdrawn by their parents for reasons not associated with the intervention. Days receiving antibiotics were fewer among infants ingesting F-19. No adverse effects of the probiotic were reported. In additional analyses of the data from this study, neither benefits nor adverse effects were reported. In follow-ups with infants when they reached the age of 8-9 years, the authors concluded that "we observed no adverse effect of LF-19 on body composition or metabolism in this follow-up study," and that "the probiotic LF-19 had no long-term effect on the inflammatory and metabolic profile."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Zampieri et al. 2013	Prevention of progression of NEC from Bell's stage 2	Prospective, randomized, unblinded	32 preterm infants (all delivered before gestational week 32; birth weight 600-1500 g) at Bell's stage 2 NEC	$6x10^9$ cfu/day	21 days	The authors reported that, "No collateral effects were observed during the study period; none of our patients presented sepsis due to *L. paracasei* subsp. *paracasei* strain F-19. No patient required preventive exclusion from the trial. No patient [in the probiotic group] presented treatment-related intestinal complications (i.e., diarrhea)." Their conclusion was that "The use of *L. paracasei* subsp. *paracasei* strain F-19 is safe and effective."
Studies in Children						
Crittenden et al. 2002	Assess the suitability of strain F-19 as a probiotic	Prospective, randomized, double-blind, placebo-controlled	61 apparently healthy young children aged 1-1.5 years	$2x10^{10}$ cfu/day	3 weeks	The study design included observations monitoring potential side-effects of probiotic consumption, including intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. No such adverse effects were observed and the probiotic was well tolerated by all individuals. The authors concluded that, "The fact that no side-effects were detected in subjects . . . suggests that *Lactobacillus* F-19 is a safe microbial food supplement."
Sullivan et al. 2002	Effect of *L. paracasei* ssp. *paracasei* strain F-19 on the colonic microbiota	Prospective, randomized, double-blind, placebo-controlled	61 apparently healthy and fully weaned children (mean age = 13.1±5.3 years)	10^{10} cfu/day	3 weeks	Children receiving *L. paracasei* ssp. *paracasei* strain F-19 showed a significant increase in fecal levels of the strain, but not of other lactobacilli or bifidobacteria. The authors reported that, "The products were generally well tolerated . . . Fecal characteristics and occurrences of gastrointestinal symptoms were similar in the treatment and placebo groups."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Studies in Adults						
Annibale et al. 2011	Treatment of symptomatic uncomplicated diverticular disease	Prospective, randomized, placebo-controlled, unblinded	50 patients (18M, 32F) with symptomatic uncomplicated diverticular disease, mean age = 65.2±8.1 years)	$4.8x10^{10}$ cfu/day	14 days/ month for 6 months	Ingestion of the probiotic significantly reduced intestinal bloating and prolonged abdominal pain compared to the control group. The authors concluded that, "Overall, the cyclic long-term (6 months) supplementation with *L. paracasei* sub. *paracasei* strain F-19 was found to be safe, since only in one patient was a side-effect (diarrhoea) found to lead to withdrawal from treatment."
Begtrup et al. 2013	Long-term effect of daily ingestion of probiotics in management of IBS	Prospective, randomized, double-blind, placebo-controlled	131 IBS patients (34M, 97F); mean age = 30.52±9.42 years	$5.2x10^{10}$ cfu/ day of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis* ssp. *lactis*		The authors concluded that, "During a 6-month treatment period, we were not able to detect a positive effect of probiotic when compared with placebo." They also concluded that, "No serious adverse effects were reported."
Brahe et al. 2015	Effect on gut microbiota and metabolic risk markers in obese women	Three-arm prospective, randomized, double-blind, placebo-controlled	58 obese postmenopausal women aged 59.5±5.9 years	$9.4x10^{10}$ cfu/ day	6 weeks	The authors stated that "there was no difference in the occurrence of adverse events between the placebo and the *L. paracasei* F-19 group. . . There was no difference between the adverse events reported following the probiotic and placebo intervention that included both more frequent and less-frequent defecation. No serious adverse events were registered during the study."
Compare et al. 2015	Effect on symptom onset in gastro-esophageal reflux disease	Prospective, randomized, double-blind, placebo-controlled, multi-center	100 GERD patients (56M, 44 F); mean age = 39±10.4 years	$2.4x10^{10}$ cfu/ day	3 days/ week for 6 months	Treatment with *L. paracasei* ssp. *paracasei* strain F-19 significantly reduced flatulence and bloating but not abdominal pain. There was no discussion of any adverse effects of the probiotic intervention.

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Crittenden et al. 2002	Assess the suitability of strain F-19 as a probiotic	Open-label	5 apparently healthy adults	$2x10^{10}$ cfu/day	12 days	Side effects monitored included intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. The authors concluded that, "No adverse effects of probiotic administration were observed in any of the pilot studies. The probiotic was well tolerated by all individuals, including the elderly *H. pylori* patients who consumed *L. paracasei* ssp. *paracasei* strain F-19 daily for 3 months without adverse effects. The fact that no side-effects were detected in subjects ranging in age from 1 to 85 years, and in healthy individuals and subjects suffering mild illnesses (milk-hypersensitivity and *H. pylori* infection) suggests that *Lactobacillus* F-19 is a safe microbial food supplement."
Crittenden et al. 2002	Assess the suitability of strain F-19 as a probiotic	Prospective, single-blind, placebo-controlled, crossover	9 apparently healthy adults, 4 with milk hypersensitivity	$2x10^{10}$ cfu/day	1 week	Side effects monitored included intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. The authors concluded that, "No adverse effects of probiotic administration were observed. The probiotic was well tolerated by all individuals. The fact that no side-effects were detected in subjects ranging in age from 1 to 85 years, and in healthy individuals and subjects suffering mild illnesses (milk-hypersensitivity and *H. pylori* infection) suggests that *Lactobacillus* F-19 is a safe microbial food supplement."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Crittenden et al. 2002	Assess the suitability of strain F-19 as a probiotic	Prospective, randomized, double-blind, placebo-controlled	30 elderly (>65 years) patients seropositive to *H. pylori*	$7.5x10^{10}$ cfu/ day	12 weeks	Side effects monitored included intestinal discomfort, increased flatulence, and changes in stool consistency and frequency. The authors concluded that, "No adverse effects of probiotic administration were observed. The probiotic was well tolerated by all individuals, including the elderly *H. pylori* patients who consumed *L. paracasei* ssp. *paracasei* strain F-19 daily for 3 months without adverse effects. The fact that no side-effects were detected in subjects ranging in age from 1 to 85 years, and in healthy individuals and subjects suffering mild illnesses (milk-hypersensitivity and *H. pylori* infection) suggests that *Lactobacillus* F-19 is a safe microbial food supplement."
Delia et al. 2006	Treatment of bacterial vaginosis	Prospective, randomized, double-blind, placebo-controlled	60 otherwise apparently healthy women aged 18-40 years (mean = 32.5±12/8 years) with bacterial vaginosis	Dose not reported	3 months	No adverse effects of the treatment were reported.
Liu et al. 2004	Treatment of patients with minimal hepatic en-cephalopathy	Prospective, randomized, double-blind, placebo-controlled	55 patients aged 56±11 years with minimal hepatic encephalopathy	10^{10} cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus, Leuconostoc mesenteroides*, and *L. plantarum*	30 days	The authors reported that, "The synbiotic, fermentable fiber and placebo preparations were well-tolerated by all patients, with no reports of adverse side effects. In particular, no patient reported diarrhea or abdominal pain or became noncompliant for other reasons."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Lombardo et al. 2009	Treatment of IBS	Open-label	100 patients (38M, 62F) aged 18-68 years with mean = 39±26 years) with IBS	$2.4x10^{10}$ cfu/ day	14 days	The authors reported that tolerability of the probiotic was excellent; "No significant side effects that could definitely be attributed to the treatment were observed. Only one case of slight nausea was reported, but this did not require withdrawal from treatment."
Matto et al. 2006	Study the intestinal survival and persistence of probiotics	Open-label	14 apparently healthy adults (5M, 9F) aged 36-74 years scheduled for routine colonoscopy	$2x10^{10}$ cfu/day	10 days	The F-19 survived well and were detected in the feces of 100% of the individuals consuming them. However, they washed out quickly and were rarely detected in fecal samples after a few days or weeks. Nor were the probiotics often isolated from colonic biopsies, suggesting that they do not exhibit strong adhesion properties. No adverse effects were reported by the authors.
Pedersen et al. 2011	Treatment of IBS	Prospective, randomized, double-blind, placebo-controlled	61 IBS patients (16M, 45W) aged 18-79 years; mean age = 42.5 years)	$2x10^{10}$ cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis* ssp. *lactis*	8 weeks	There were no significant differences between those receiving milk with or without the probiotics. The authors did not report any adverse events.
Rayes et al. 2002a	Reduce post-operative bacterial infection in patients recovering from major abdominal surgery	Prospective, randomized, double-blind, placebo-controlled	95 patients (49M, 46F) with mean age = 49.0±2.5 years recovering from abdominal surgery	$2x10^{9}$ cfu/day	10 days	Both standard enteral formula and strain F-19 were well tolerated. Abdominal side effects (distension, cramps, or diarrhea) were seen in 8 of 32 patients in the standard-formula group, 6 of 31 patients in the live *Lactobacillus* group, and 11 of 32 patients in the inactivated-bacteria group.

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Rayes et al. 2002b	Reduce post-operative bacterial infection in patients recovering from major abdominal surgery	Prospective, randomized, double-blind, placebo-controlled	172 patients following major abdominal surgery or liver transplantation	$2x10^9$ cfu/day	Not reported	The groups receiving live *Lactobacillus* cultures experienced significantly reduced incidences of bacterial infection (among resection patients, 4% vs. 13% in the prebiotic group and 31% in the conventional-therapy group; among transplant recipients, 13% in the probiotic group vs. 34% in the prebiotic group and 48% in the conventional therapy group). The authors reported that "Fibre and lactobacilli were well tolerated in most cases."
Rayes et al. 2002c	Reduce post-operative bacterial infection in patients recovering from major abdominal surgery	Prospective, randomized, double-blind, placebo-controlled	90 patients (48M, 42F), mean age = 61.0±14.1 years, recovering from major abdominal surgery	$2x10^9$ cfu/day	10 days	All patients completed the study, receiving the full course of their assigned nutrition. The incidence of infections was significantly lower in groups receiving fiber and either live or killed lactobacilli (10% each) than in the group receiving standard nutritional formula (30%). Patients receiving live F-19 strain needed antibiotics for a significantly shorter time than did the patients in the other groups. The length of hospital stay and the incidence of non-infectious complications did not differ significantly. Fibers and lactobacilli were well tolerated; side effects did not differ between groups and there was no diarrhea. The authors concluded that "application of fiber and lactobacilli is feasible even after major abdominal surgery and caused few side effects."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Rayes et al. 2005	Reduce post-operative bacterial infection in patients recovering from liver transplantation	Prospective, randomized, double-blind, placebo-controlled	66 liver transplant recipients (38M, 28F), mean age = 51.5±2.5 years)	$2x10^{10}$ cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, and *L. plantarum*	14 days	Inclusion of probiotics in the enteral nutrition formula significantly reduced the incidence of post-operative bacterial infection and reduced the severity of infections that did occur. Hematology, immunology, and biochemistry parameters did not differ significantly between groups. The authors reported that "Enteral nutrition, containing the synbiotic combination, was well tolerated in all patients," with fewer probiotic patients exhibiting diarrhea or abdominal cramps. "All side effects disappeared under temporary reduction in the amount of enteral nutrition." The authors concluded that inclusion of probiotics "is an effective means to prevent post-operative bacterial infections in high-risk surgical patients, and since it causes no resistant strains and has no serious side effects, it could be widely used."
Rayes et al. 2007	Reduce post-operative bacterial infection in patients recovering from major abdominal surgery	Prospective, randomized, double-blind, placebo-controlled	80 patients (45M, 35F, mean age = 58.5±12.5 years) recovering from pylorus-preserving pancreato-duodenectomy	$2x10^{10}$ cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, and *L. plantarum*	8 days	Inclusion of probiotics in the enteral nutrition formula significantly reduced the incidence and duration of post-operative bacterial infection to 12.5% vs 40% in the fiber-only formula group. The incidences of diarrhea and abdominal cramps were the same in both groups of patients. The authors reported that, "Enteral nutrition, containing the synbiotic combination, was well tolerated in all patients… All side effects disappeared under temporary reduction in the amount of enteral nutrition," and concluded, "Early enteral nutrition with synbiotics was able to significantly reduce postoperative bacterial infections in patients following [pancreatoduodenectomy] with only single-shot antibiotic prophylaxis. In contrast to antibiotics, it is relatively cheap and does not cause resistant strains or serious side effects."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Rayes et al. 2012	Reduce post-operative bacterial infection in patients recovering from major abdominal surgery	Prospective, randomized, double-blind, placebo-controlled	19 right-hepatectomy patients (14M, 5F), mean age = 60.1±13.9 years	$2x10^{10}$ cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, and *L. plantarum*	10 days	The authors concluded that, "The synbiotic combination was well-tolerated in all patients. Mild side-effects (abdominal distension and cramps) occurred in three patients of each group but disappeared under symptomatic therapy. No severe side effects were recorded."
Riordan et al. 2003	Effect of F-19 on TNF-α level in cirrhosis patients	Open-label	11 cirrhosis patients and 5 healthy controls	$8x10^{10}$ cfu/day each of *L. paracasei* ssp. *paracasei* strain F-19, *L. plantarum*, *Pediococcus pentosaceus*, and *Lactococcus raffinolactis*	7 days	The authors reported that, "Administration of the synbiotic supplement was well tolerated without any reported adverse events or change in general clinical state."
Simren et al. 2010	Treatment of IBS	Prospective, randomized, double-blind, placebo-controlled	74 IBS patients (22M, 52F) with mean age = 43.0±15.5 years)	$2x10^{10}$ cfu/day of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis* ssp. *lactis*	8 weeks	IBS symptoms improved significantly in both groups over the study period, but with no significant difference between them. No clinically significant effects on biochemistry or hematology were noted and no adverse events were reported by the patients. The authors stated that, "The yoghurt was well tolerated."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Sondergaard et al. 2011	Treatment of IBS	Prospective, randomized, double-blind, placebo-controlled multi-center	52 patients (13M, 39F) aged 29-67 years (mean age = 51.3 years) fulfilling Rome II criteria	$2.5x10^{10}$ cfu/ day of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis* ssp. *lactis*	8 weeks	There was no significant difference between treatment with acidified milk alone or acidified milk containing the probiotics. The authors reported that, "The investigational products were well tolerated and no serious adverse events occurred."
Spindler-Vesel et al. 2007	Effect on intestinal permeability and the clinical outcome of critically ill trauma patients	Prospective, randomized, double-blind, placebo-controlled	113 trauma patients (88M, 25F), mean age = 41.0±18.9 years)	10^{10} cfu each of *L. paracasei* ssp. *paracasei* strain F-19, *L. plantarum*, *Pediococcus pentosaceus*, and *Lactococcus raffinolactis*	7 days	Out of 51 bacterial infections, only 5 were observed in the synbiotic group, a significantly lower incidence than the other 3 groups. Only the synbiotic group showed evidence of decreased intestinal permeability. No adverse events associated with the synbiotic were reported. The authors concluded that, "Patients supplemented with synbiotics did better than the others, with lower intestinal permeability and fewer infections."
Sullivan et al. 2001	Treatment of elderly *Helicobacter pylori* patients	Prospective, randomized, double-blind, placebo-controlled	35 *H. pylori* patients (sex not reported; age range 58-89 years with a median age of 76 years)	10^{11} cfu/day	12 weeks	. The authors concluded that the probiotic "survives the passage through the gastrointestinal tract." There was no discussion of any adverse events.
Sullivan et al. 2002	Treatment of elderly *Helicobacter pylori* patients	Prospective, randomized, double-blind, placebo-controlled	30 elderly *H. pylori* patients (sex not reported; mean age = 76.8±6.5 years)	10^{11} cfu/day	12 weeks	The authors reported that, "The products were generally well tolerated . . . Fecal characteristics and occurrences of gastrointestinal symptoms were similar in the treatment and placebo groups."

Table 5. Studies of *L. paracasei* ssp. *paracasei* Strain F-19.

Reference	Objective	Study Design	Subjects	*L. paracasei* ssp. *paracasei* Strain F-19 Dose	Duration	Safety-Related Results
Sullivan et al. 2003	Prevention of antibiotic-associated ecological disturbances of the intestinal microbiota	Prospective, randomized, double-blind, placebo-controlled	24 apparently healthy adults (7M, 17W) aged 21-48 years receiving antibiotic therapy	$2x10^8$ cfu of each of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis ssp. lactis*	14 days	Consumption of the probiotic prevented ecological disturbances in the numbers of lactobacilli and *Bacteroides fragilis* group. One participant in the probiotic group developed diarrhea and one reported looser stools. The first person's stool was cytotoxin positive, cured by metronidazole; the loose stools resolved spontaneously. No other adverse events were reported.
Sullivan et al. 2004	Prevention of the emergence of antibiotic-resistant micro-organisms in patients receiving penicillin, ciprofloxacin, or norfloxacin	Prospective, randomized, double-blind, placebo-controlled	20 hospital patients aged 18-89 years (mean = 59 years) receiving penicillin and 16 patients aged 28-86 years (mean = 61 years) receiving ciprofloxacin or norfloxacin	$2x10^{10}$ cfu/day	14 days	The authors reported that, "No adverse events were reported. Eight patients treated with penicillin reported looser stools, four in the active and four in the placebo group. In the quinolone-treated patients there were two individuals who experienced looser stools, one from each of the placebo and active groups."
Sullivan et al. 2009	Treatment of chronic fatigue syndrome	Open-label	15 chronic fatigue syndrome patients, (5M, 10W) aged 30-56 years	$4x10^{10}$ cfu/day of a mixture of *L. paracasei* ssp. *paracasei* strain F-19, *L. acidophilus*, and *B. animalis ssp. lactis*	4 weeks	There were no significant changes in fatigue and physical activity scores and no major changes occurred in the gastrointestinal microflora; nevertheless, 6 of the 15 patients reported that they had improved. The authors did not report any adverse events.

6.5. Studies of Other Strains of *L. paracasei* ssp. *paracasei*

Human studies of other strains of the species are summarized in Table 6. These fifty studies include 28 different strains of *L. paracasei* ssp. *paracasei*: CUL08, LPC-S01, LPC-37, W8, ST11, CBA L74, LP-33, DG, CNCM I-2116, B21060, NCC 2461, BRAP01, 317, CNCM I-1518, HF A000232, 431, IMC502, HN019, N24, N1115, CNCM I-4034, 8700, IMPC 2.1, SD1, K71, IMC 502, LPC09, and LMGP22043. No adverse events clearly attributable to the probiotic were reported in any study.

The studies summarized in Table 6 do not include the extensive literature on *L. casei* strain Shirota, the subject of GRN 000429, although the GRAS submission observes:

> "The taxonomy of the genus *Lactobacillus* is currently in a state of flux, and nowhere is taxonomic placement less certain than in the complex of *L. casei* strains. According to the most recent opinion (as of this writing) of the Judicial Commission of the International Committee on Systematics of Bacteria in 2008 (JCICSB 2008), *L. casei* strain Shirota would now be regarded as a strain of *L. paracasei* rather than *L. casei*" (p8).

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Allen et al. 2014	Evaluate efficacy in prevention of eczema.	Prospective, randomized, double-blind, placebo-controlled, parallel-group	454 pregnant women and their infants	*L. paracasei* ssp. *paracasei* strain CUL08, 1.25×10^9 cfu (& *L. salivarius, B. animalis* ssp. *lactis,* and *B. bifidum*), mother ~4 weeks, infant to age 6 months	Authors' conclusion: "Probiotic administration was not associated with adverse effects in either mothers or their infants."
Balzaretti et al. 2015	Assess use of *L. paracasei* ssp. *paracasei* strain LPC-S01as a probiotic.	Prospective, randomized, double-blind, placebo-controlled, cross-over	11 apparently healthy adults	*L. paracasei* ssp. *paracasei* strain LPC-S01, $2.4x10^{10}$ cfu, 7 days	"Capsules, which contained about 24 billion CFU of *L. paracasei*, were well tolerated by all participants and no adverse events were reported." Authors' conclusion: "*L. paracasei* LPC-S01 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 (24 billion CFU) to healthy people without adverse events."
Barker et al. 2017	Evaluate use against *C. difficile* infection.	Prospective, randomized, double-blind, placebo-controlled, pilot trial	33 patients with mild *C. difficile* infection	*L. paracasei* ssp. *paracasei* strain LPC-37 (& *B. lactis* and *L. acidophilus*), 28 days	"There was no significant difference in the rate of CDI recurrence or functional improvement over time between treatment groups."
Bjerg et al 2015a,b	Effect of probiotic on blood TAG levels.	Prospective, randomized, double-blind, placebo-controlled, two-arm parallel	64 apparently healthy young adults	*L. paracasei* ssp. *paracasei* strain W8, 10^{10} cfu, 4 weeks	"Four weeks supplementation with L. casei W8 did not affect the overall composition of the gut microbiota."
Chouraqui et al. 2008	Effect on risk of diarrhea in infants.	Prospective, randomized, double-blind, placebo-controlled, parallel group	284 healthy term infants	*L. paracasei* ssp. *paracasei* strain ST11 (& *B. longum*), 14 weeks	Infants in all groups had similar weight gain, length, head circumference, digestive tolerance, and adverse events at 2, 4, 8, 12, 16, and 52 weeks of age. The authors concluded that: "Infants fed formulas containing probiotics or synbiotics show a similar rate in weight gain compared with those fed a control formula and tolerate these formulas well."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Corsello et al. 2017	Effect on risk of common infectious diseases in children.	Prospective, randomized, double-blind, placebo-controlled, multi-center	146 health children (84M, 62F) aged 12-48 months (mean = 33±9 months)	*L. paracasei* ssp. *paracasei* strain CBA L74, $5.9x10^{11}$ cfu, 3 months	"No changes were observed for body weight and height in the two study groups, indicating that the consumption of the study products was safe, at least in the short term. No adverse events related to the consumption of the active or placebo products were recorded." The authors concluded that: "The dietary supplementation was well accepted by the children and safe, as demonstrated by the low dropout rate together with the high level of adherence, and the absence of ad-verse events observed during the study period."
Costa et al. 2014	Study effect of probiotics in patients with allergic rhinitis.	Prospective, randomized, double-blind, placebo-controlled	425 patients with allergic rhinitis to grass pollen treated with loratadine	*L. paracasei* ssp. *paracasei* strain LP-33, 5 weeks	Improved quality of life; no adverse events reported.
Farrario et al. 2014	Ability of probiotic to modulate fecal Clostridiales bacteria and butyrate.	Prospective, randomized, double-blind, placebo-controlled, crossover design	Apparently healthy adults age 23-55 years	*L. paracasei* ssp. *paracasei* strain DG $2.4x10^{10}$ cfu, 4 weeks	No adverse events were reported.
Gore et al. 2012	Treatment and secondary prevention of early infant eczema.	Prospective, randomized, double-blind, placebo-controlled with 3-year follow-up	208 infants aged 3-6 months with eczema	*L. paracasei* ssp. *paracasei* strain CNCM I-2116, 3 months	No adverse events were reported.
Grossi et al. 2010	Treatment of acute diarrhea.	Prospective, randomized, double-blind, placebo-controlled	174 adult patients with acute diarrhea	*L. paracasei* ssp. *paracasei* strain B 21060, 10 days	"The 2 treatments showed a very good tolerability profile, with negligible and similar adverse event rates and similar concomitant medication usage rates."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Gueniche et al. 2014	Effect of probiotic on skin reactivity.	Prospective, randomized, double-blind, placebo-controlled	64 women with reactive/ sensitive skin	*L. paracasei* ssp. *paracasei* strain NCC 2461, 60 days	No adverse events were reported.
Hemalatha et al. 2017	Effect of probiotic on SCFA in children.	Prospective, randomized, double-blind, placebo-controlled	379 apparently healthy children aged 2-5 years	*L. paracasei* ssp. *paracasei* strain LPC-37, $2x10^9$ cfu, 9 months	No adverse events were reported.
Ho et al. 2014	Effect of probiotic on cytotoxicity of human NK cells.	Prospective multi-arm open-label study	100 apparently healthy adults with average age = 55 years	*L. paracasei* ssp. *paracasei* strain BRAP01, $2x10^{10}$ CFU, 1 day	No adverse events were reported.
Hutt et al. 2011	Evaluate the safety of probiotics.	Prospective open-label study	9 apparently healthy adults	*L. paracasei* ssp. *paracasei* strain 317 (vs. other pro-biotics), 10^{10} cfu, 5 days	Data on gut health, blood parameters, and liver and kidney function were collected. The authors reported that "The administration of high doses of different *Lactobacillus* strains did not result in any severe adverse effects in GIT and/or abnormal values of blood indices."
Jespersen et al. 2015	Effect of probiotic on immune response to influenza vaccination.	Prospective, randomized, double-blind, placebo-controlled, parallel-group, multi-center	1104 apparently healthy adults (453M, 651F) aged 18-60 years (mean = 31.4 years)	*L. paracasei* ssp. *paracasei* strain 431, 10^9 cfu, 42 days	The authors reported that: "A total number of 2212 AEs in 914 subjects were reported during the study. Of these, 41 events in 34 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 AEs 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 AEs were defined as serious; none of these were assessed to be related to the study product or vaccine."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Lahner et al 2012	Use of probiotic in treatment of diverticular disease.	Prospective, randomized, double-blind, placebo-controlled, multi-center	52 patients (17M, 35F) aged 40-80 years with diverticular disease (mean age = 66.3±9.6 years)	*L.* paracasei ssp. *paracasei* strain B21060, $5x10^9$ cfu, 6 months	The authors reported that: "None of the patients developed altered biochemical inflammatory parameters, acute diverticulitis or other diverticular disease complications throughout the 6-mo study period. In both groups no adverse event was registered over the 6-mo treatment period."
Lee et al. 2017	Effect of probiotics on immune function.	Prospective, randomized, placebo-controlled, open-label	152 healthy adults (45M, 107F) aged >60 years (mean age = 65.7±0.4 years)	*L.* paracasei ssp. *paracasei* strain 431, $1.2x10^9$ cfu, 12 weeks (& *B. animalis* ssp. *lactis*)	The authors reported that: "No adverse events were reported from the participants."
Lenoir-Wijnkoop et al. 2014	Prevention of antibiotic-associated diarrhea.	Prospective, randomized, double-blind, placebo-controlled	135 elderly hospitalized patients (mean age = 74 years)	*L. paracasei* ssp. *paracasei* strain CNCM I-1518, approx.. 2 weeks	The authors reported that: "There were no adverse events in the probiotic group."
Lin et al. 2014	Treatment of children with perennial allergic rhinitis.	Prospective, randomized, double-blind, placebo-controlled	60 children (47M, 13F) aged 6-13 years with allergic rhinitis	*L. paracasei* ssp. *paracasei* strain HF.A00232, $5x10^9$ cfu, 12 weeks	The authors reported that: "No serious adverse events were recorded in either group. The vital signs and physical examination of all systems revealed no differences between these two groups."
Lundelin et al. 2017	Long-term safety of perinatal probiotic intervention.	Prospective, randomized, double-blind, placebo-controlled with long-term follow-up	303 mother-infant pairs	*L. paracasei* ssp. *paracasei* strain ST11	The authors reported that: "We found no differences in growth or non-communicable disease prevalence between children receiving perinatally probiotics or placebo" and concluded: "Perinatal probiotic administration is safe in long-term follow-up."
Maretti and Cavallini 2017	Treatment of idiopathic oligoas-thenoterato-spermia.	Prospective, randomized, double-blind, placebo-controlled	41 patients with idiopathic oligoastheno-teratospermia	*L. paracasei* ssp. *paracasei* strain B21060, $5x10^9$ cfu, 6 months	The authors concluded that: "There were no side effects in either group. These data showed that [the probiotic] constitutes a safe therapy."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Martarelli et al. 2011	Effect of probiotics on oxidant and antioxidant parameters in plasma of athletes.	Prospective, randomized, open-label	24 healthy athletes	*L. paracasei* ssp. *paracasei* strain IMC 502, 10^9 cfu, 4 weeks	No adverse events were reported.
Nembrini et al. 2015	Modulation of allergic rhinitis.	Prospective, randomized, double-blind, placebo-controlled	131 allergic rhinitis patients (64M, 67F) aged 18-65 years	*L. paracasei* ssp. *paracasei* strain NCC 2461, $5x10^9$ cfu, 8 weeks	The authors reported that: "Subjects receiving NCC 2461 reported fewer episodes of nasopharyngitis, however the frequency of minor adverse events was not a trial outcome and was therefore not considered in the statistical analysis." They concluded that: "Probiotic administration was considered safe as no serious adverse event was recorded during the trial."
Ouwehand et al. 2014	Reduce incidence & severity of diarrhea in hospital.	Prospective, randomized, double-blind, multiple-dose	503 patients in hospital	*L. paracasei* ssp. *paracasei* strain HN019, $1.7x10^{10}$ cfu, approx. 10 days	No adverse events were reported.
Passariello et al. 2012	Treatment of acute diarrhea in children.	Prospective, randomized, double-blind, placebo-controlled	107 children (58M,49F) aged 17-24 months with acute diarrhea (mean age = 20.5 months)	*L. paracasei* ssp. *paracasei* strain B21060, $5x10^9$ cfu, 5 days	The authors reported that: "The rate of patients requiring hospitalisation because of worsening of symptoms was slightly but not significantly higher in placebo group. No adverse event was observed in the two groups."
Perrin et al. 2014	Modulation of allergic rhinitis.	Prospective, randomized, double-blind, cross-over	31 allergic rhinitis patients (21M, 10F) aged 20-35 years (mean age = 26.8± 3.7 years)	*L. paracasei* ssp. *paracasei* strain NCC2461, 10^{10} cfu, 4 weeks	The authors reported that: "There was no noticeable clinical issue and no formulation-related adverse event during the study." They concluded that: "This suggests that both probiotic treatments are safe and do not affect the immune system steady state."
Pino et al. 2017	Test viability after passing GI tract.	Prospective open-label study	Apparently healthy adult volunteers	*L. paracasei* ssp. *paracasei* strain N24, 15 days	No adverse events were reported.

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Plaza-Diaz et al. 2013	Safety and immune-modulatory effects of probiotics.	Prospective, randomized, double-blind, placebo-controlled, multi-center	100 healthy adults (46M, 54F) with mean age = 28.6 years	*L. paracasei* ssp. *paracasei* strain CNCM I-4034, $9x10^9$ cfu, 30 days	The authors reported: "All symptom scores were less than 2, and there was no significant difference between the control group and the probiotic-treated group. The median score of the daily recorded GI symptoms of acid regurgitation, nausea, vomiting, abdominal distension, and eructation did not change during the probiotic supplementation (intervention) and subsequent follow-up period. Additionally, the stool consistency and defecation frequency did not change during the supplementation period and the subsequent follow-up period in the probiotic and placebo groups. Therefore, no serious adverse events occurred during the supplementation period in any of the groups, which shows that the differences between the probiotic and placebo groups were not significant for any of the reported symptoms. Likewise, no difference between placebo and probiotic groups occurred in any of the haematological parameters." They concluded: "These results demonstrate that the consumption of these three bacterial strains was safe."
Pu et al. 2017	Probiotics to protect older people from respiratory infections.	Prospective, randomized, placebo-controlled, open-label	205 healthy adults (59M, 146F) with mean age = 58.5±8.3 years	*L. paracasei* ssp. *paracasei* strain N1115, 10^{10} cfu, 12 weeks	The authors reported that: "No significant difference in the distribution of subjects leaving the study was observed. Furthermore, no adverse events associated with the consumption of the tested yogurt were reported."
Rask et al. 2013	Effect of probiotics on cell-mediated immunity.	Prospective, randomized, open-label	57 apparently healthy adults (20M, 37F) aged 18-55 (20M, 37F)	*L. paracasei* ssp. *paracasei* strain 8700:2, 10^{10} cfu, 5 weeks	The authors reported that: "Only mild adverse gastrointestinal side effects were reported following intake of study products and there was no apparent relation between symptoms and treatment group."
Rautava et al. 2012	Maternal probiotics to reduce risk of eczema in the infant.	Prospective, randomized, double-blind, placebo-controlled	241 mother-infant pairs; mother with allergic disease	*L. paracasei* ssp. *paracasei* strain ST11, 4 months	The authors reported that: "No adverse effects were related to the use of probiotics," and concluded that: "Prevention regimen with specific probiotics administered to the pregnant and breast-feeding mother, that is, prenatally and postnatally, is safe and effective in reducing the risk of eczema in infants with allergic mothers positive for skin prick test."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Reygagne et al. 2017	Effect of probiotics on severe dandruff.	Prospective, randomized, double-blind, placebo-controlled	60 men aged 18-60 years with severe dandruff	*L. paracasei* ssp. *paracasei* strain ST11, 10^9 cfu, 56 days	The authors stated that "No adverse events were reported" and concluded that "Regular intake of ST11 over 56 days is safe."
Riezzo et al. 2012	Effect of probiotics on functional constipation.	Prospective, randomized, double-blind, placebo-controlled, cross-over	20 functional constipation patients (3M, 17F), mean age = 38.8± 14.4 years	*L. paracasei* ssp. *paracasei* strain IMPC 2.1, $2x10^{10}$ cfu, 15 days	The authors reported that: "No adverse events were noted during the study period" and concluded that use of probiotics is "a useful and safe tool for managing constipation."
Ritthagol et al. 2014	Probiotics on mutans streptococci in cleft lip patients.	Prospective, randomized, double-blind, placebo-controlled	30 patients aged 19.2± 3.7 years with cleft lip and palate	*L. paracasei* ssp. *paracasei* strain SD1, 4 weeks	No adverse events were reported.
Rizzardini et al. 2012	Immune benefits of probiotics.	Prospective, randomized, double-blind, placebo-controlled	211 healthy adults (93M, 118 F) aged 33.2±13.1 years	*L. paracasei* ssp. *paracasei* strain 431, 10^9 cfu, 6 weeks	No adverse events were reported.
Roessler et al. 2012	Effects of probiotics in patients with atopic dermatitis.	Prospective, randomized, double-blind, placebo-controlled, crossover	15 apparently healthy adults & 15 atopic dermatitis patients	*L. paracasei* ssp. *paracasei* strain LPC-37, 8 weeks	No adverse events were reported.
Saito et al. 2017	Safety of extreme intake of strain K71.	Open-label safety study	10 apparently healthy adults (5/sex) aged 20-64 years (mean age = 41.6± 14.0 years)	*L. paracasei* ssp. *paracasei* strain K71, 10^{12} cfu, 4 weeks	The authors reported: "During this study, the following adverse events were observed: fullness feeling (n=1), loose stool (n=1), diarrhea (n=1), and abdominal pain loose stool (n=1). Each of these adverse events was mild and judged by the principal investigator as being unrelated to test food consumption. There were several slight but significant changes (from pretrial values) in parameters in tests for hematology and blood biochemistry, whereas the principal investigator judged that none of these deviations was of any clinical significance." They concluded that "doses of 10^{12} bacteria daily for 4 weeks were shown to be safe."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Simakachom et al. 2011	Tolerance and safety of synbiotic formula in critically ill children.	Prospective, randomized, double-blind, placebo-controlled	94 patients under mechanical ventilation aged 1-3 years	*L. paracasei* ssp. *paracasei* strain NCC 2461, 14 days	The authors reported that "Abdominal distention, vomiting, and stool frequency were not affected by the supplementation with pre- and probiotics" and concluded that : "The enteral formula supplemented with synbiotics was well tolerated by children in intensive care units; it was safe."
Teanpaisan and Piwat 2014	Effect of probiotics on mutans streptococci.	Prospective, randomized, double-blind, placebo-controlled	40 apparently healthy young adults	*L. paracasei* ssp. *paracasei* strain SD1, 4 weeks	No adverse events were reported.
Trautvetter et al. 2012	Effect of probiotics on colonic lactobacilli & cholesterol metabolism.	Prospective, randomized, double-blind, placebo-controlled, crossover	32 apparently healthy adults	*L. paracasei* ssp. *paracasei* strain LPC-37, 10^{10} cfu, 4 weeks	No adverse events were reported.
Valerio et al. 2011	Effect of probiotics on human biochemistry.	Prospective, randomized, double-blind, placebo-controlled, crossover	20 healthy adults (3M, 17F) aged 37.8±13.9 years	*L. paracasei* ssp. *paracasei* strain LMGP22043, $2x10^{10}$ cfu, 15 days	No adverse events were reported.
Valsecchi et al. 2014	Effect of probiotics on secretory IgA during antibiotic therapy in children.	Prospective, randomized, double-blind, placebo-controlled	77 pediatric patients with recurrent airway infections receiving antibiotic therapy	*L. paracasei* ssp. *paracasei* strain CFL-431 (& *B. lactis* & *S. thermophilus*), 7 days	No adverse events were reported.
Verdenelli et al. 2011	Influence of probiotics on adult bowel habits.	Prospective, randomized, double-blind, placebo-controlled	50 apparently healthy adults (23M, 27F) aged 23-65 years	*L. paracasei* ssp. *paracasei* strain IMC 502, $2x10^{9}$ cfu, 12 weeks	The authors reported that "No significant differences were detected with respect to constipation and flatulence by both groups. The probiotic-enriched foods consumption was very well tolerated, and no side effects were experienced."

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Vicariotto 2014	Effect of probiotics on urinary tract infections.	Open-label	33 premeno-pausal women with acute cystitis	*L. paracasei* ssp. *paracasei* strain LPC09, 60 days	No adverse events were reported.
Vlieger et al. 2009	Tolerance and safety of probiotics.	Prospective, randomized, double-blind, placebo-controlled	126 healthy term neonates <7 days of age	*L. paracasei* ssp. *paracasei* strain CRL-431, 10^7 cfu, 3 months (& *B. animalis* ssp. *lactis*)	The authors stated that: "No serious adverse events were reported that could be related to the study formula. Parents were asked if they had noticed any symptoms that could have been caused by the study feed. Fewer infants in the probiotics group had developed a rash in the first 3 months. No differences were seen in other adverse effects between the two groups in both the first and second trimester." They concluded: "Normal growth occurred in all infants and no statistically significant differences were detected between the probiotics group and the control group for gain in weight, length and head circumfer-ence. Infants in the probiotics group produced softer and more frequent stools during the first 3 months of life. No differences were found in crying and sleeping hours, number of parent-diagnosed infections, anti-biotic use, visits to the general practitioner and number of adverse events. The use of a prebiotic-containing starter formula supplemented with *L. paracasei* ssp. *paracasei* and *B. animalis* ssp. *lactis* in early infancy is safe, well tolerated and has no adverse effects on growth and infant behaviour."
Wang et al. 2004	Effect of probiotics on allergic rhinitis.	Prospective, randomized, double-blind, placebo-controlled	80 allergic rhinitis patients (41M, 39F) with mean age = 15.4±1.8 years	*L. paracasei* ssp. *paracasei* strain 33, $2x10^9$ cfu, 30 days	The authors reported that no patients left the study due to adverse events, and "Subjects reported no severe adverse effects such as fever, abdominal pain, or diarrhea. " They concluded that: "The results suggest that ingestion of LP-33-fortified fermented milk for 30 days can effectively and safely improve the quality of life of patients with allergic rhinitis."
Wassenberg et al. 2011	Effect of probiotics on allergic rhinitis.	Prospective, randomized, double-blind, placebo-controlled, cross-over	31 patients with allergic rhinitis	*L. paracasei* ssp. *paracasei* strain ST11, 4 weeks	No adverse events were reported.

Table 6. Human Studies in *L. paracasei* ssp. *paracasei* Strains Other Than F-19.

Reference	Objective	Study Design	Subjects	Strain, Dose, and Duration	Safety-Related Results
Wattanarat et al. 2015	Effect of probiotics on salivary HNP1-3 levels in children.	Prospective, randomized, double-blind, placebo-controlled, parallel-group	60 apparently healthy children (26M, 34F) aged 13-15 years	*L. paracasei* ssp. *paracasei* strain SD1, $3.75x10^9$ cfu, 6 months	The authors reported that: "No adverse side effects from probiotics or milk powder intake in this cohort were reported."
West et al. 2012	Effect of probiotics on immune system.	Prospective, randomized, double-blind, placebo-controlled, parallel-group	22 healthy males with mean age = 32.9±4.5 years	*L. paracasei* ssp. *paracasei* strain 431, $4.6x10^8$ cfu, 21 days (& *B. lactis, L. acidophilus & L. rhamnosus*)	The authors reported that: "There were five episodes of mild GI symptoms that included flatulence and stomach rumbles in both groups during supplementation. Both supplements were otherwise well tolerated."
Zhang et al. 2013b	Effect of probiotics on colonic microbiota	Prospective, randomized, double-blind, placebo-controlled, cross-over	52 apparently healthy adults	*L. paracasei* ssp. *paracasei* strain LC01, 10^{10} cfu, 4 weeks	No adverse events were reported.

6.6. Toxicity Studies of *L. paracasei* ssp. *paracasei*

Oral toxicity studies were conducted on 4 strains of *L. paracasei* ssp. *paracasei*: strain Lpc-37, LC-01, NTU 101, and GM080 (Morovic et al. 2017; Zhang et al. 2013a; Tseng et al. 2015, and Jia et al. 2011). The first was an acute study, the next two were subacute, and the fourth was subchronic.

Morovic et al. (2017) investigated the safety of HOWARU® Restore, a blend of *Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium animalis* subsp. *lactis* Bl-04 and *B. lactis* Bi-07, as well as the individual strains. Acute oral toxicity testing was done in 10-week-old female Crl:CD(SD) rats that received single gavage doses of 5000 mg/kg bw, equivalent to $2.64x10^{12}$ cfu/kg bw for HOWARU® Restore and $3.35x10^{12}$ CFU/kg bw for *L. paracasei* Lpc-37 tested individually. Rats were observed for 14 days and weighed on days -1, 1, 8, and 15 prior to euthanasia. Necropsy was performed 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 reported in any animal administered either HOWARU® Restore or *L. paracasei* Lpc-37 and no gross findings detected at necropsy suggestive of acute toxicity.

In an assessment of the safety of *L. paracasei* ssp. *paracasei* strain LC-01 (Zhang et al. 2013a), 40 7-9-week-old female Balb/C mice were assigned to 4 groups (10 mice/group) to receive daily gavage of 0, 10^8, 10^9, or 10^{10} cfu of strain LC-01 for 28 days. Mice were observed daily; feed intake and body weight were recorded weekly. On day 29, blood was collected, organs and tissues were observed for gross abnormalities, and liver, spleen, and kidney were excised. Blood was analyzed for red blood cell and platelet counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase activity, total protein, albumin, glucose, cholesterol, and bacterial translocation.

No noticeable activity or behavioral changes were reported in the mice during the in-life portion of the study, and no treatment-related illness or death were reported. There were no significant differences in feed intake, bodyweight gain, or organ weights between the control and treatment groups, and the hematological and biochemical parameters did not show statistically significant differences between control and strain-treated groups. The authors stated that, “Our findings suggest that LC-01 do not have adverse effect on murine haematology or blood biochemistry.”

Additionally, no bacteremia was reported in any of the groups, and there were no significant differences in the incidence of translocation to the liver, spleen, or kidney between the control and treated groups at any of the tested doses. The authors concluded that, “Therefore, it can be concluded that LC-01 is likely to be non-pathogenic and safe for human consumption.”

After completing *in vitro* and *in vivo* genotoxicity studies (reverse bacterial mutation, micronucleus assay, and mammalian chromosomal aberration test) of *L. paracasei* ssp. *paracasei* strain NTU 101, finding no evidence of mutagenicity, Tseng et al. (2015) performed a subacute oral toxicity study in Wistar rats. Eighty rats, 40 of each sex, weighing 170-200 g were assigned to 4 groups (10 rats/sex/group) to receive 0, 300, 1500, or 5000 mg/kg bw/day of the bacterial powder for 28 days; these doses provided 0, $1.8x10^9$, $4.5x10^9$, and $1.5x10^{10}$ cfu of strain NTU 101/kg bw/day. Animals were observed and weighed daily and feed intake was measured semiweekly. On the day of euthanasia, blood was taken from the abdominal aorta and analyzed for hematology (hemoglobin, hematocrit, lymphocytes, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, mean platelet volume, platelet distribution width, platelet large-cell ratio, platelet count, red blood cell count, red blood cell distribution width, and white blood cell count) and clinical chemistry parameters (alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, cholesterol, chloride, creatinine, glucose,

potassium, magnesium, sodium, inorganic phosphorus, and triacylglycerol). All organs and tissues were examined macroscopically (and microscopically for rats in the high-dose and control groups) and weights were obtained for the liver, kidney, heart, spleen, lung, adrenal gland, epididymis, testis, uterus, and ovary.

During the study period, no clinical signs or deaths were reported in either the control or the treatment groups. The animals showed no significant differences in feed consumption, body weight, hematological parameters, absolute or relative organ weights, or histology between the control and treatment groups. Although there were some significant differences between treated and control rats in potassium, aspartate aminotransferase, and triacylglycerol levels, all values were within the normal range. The authors concluded that:

> "This study demonstrates that Vigiis 101 has no mutagenic/genotoxic effects based on the results of the Ames test, the *in vitro* chromosomal aberration test, or the *in vivo* micronucleus assay; there was no evidence of toxicity in the 28-day oral toxicity assay at 5000 mg/kg/day in rats. Taken together, these results support the safety of Vigiis 101 made from *L. paracasei* subsp. *paracasei* NTU 101."

The safety of a fourth strain, *L. paracasei* ssp. *paracasei* strain GM080, was assessed in a sub-chronic study (Jia et al. 2011) in Sprague-Dawley rats weighing 66.6±6.5 g (males) and 64.2±7.1 g (females). Eighty rats were divided into 4 groups of 10 rats/sex/group to receive by gavage 0, 1.25, 2.5, or 5.0 g strain GM080/kg bw/day for 90 days; the doses were equivalent to 0, $2.5x10^9$, $5x10^9$, and 10^{10} cfu/kg bw/day. Rats were individually caged and observed twice daily; feed intake and weight were measured weekly. Blood was tested at day 46 and day 91 for hematology (red blood cell count, hemoglobin, platelet count, white blood cell count, and differential blood cell count) and clinical chemistry (alanine aminotransferase, aspartate aminotransferase, total protein, albumin, glucose, blood urea nitrogen, creatinine, cholesterol, triglyceride and alkaline phosphatase). After euthanasia, selected organs (heart, kidneys, liver, spleen, testes and thymus) were excised and weighed and macro- and microscopic histological examinations were performed on organs and tissues from rats in the control and high-dose groups (cecum, colon, duodenum, esophagus, femur with bone marrow, ileum, jejunum, lacrimal gland, lung, lymph node, mammary gland, nasal turbinates, pancreas, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin, spinal cord, sternum with bone marrow, trachea, urinary bladder and vagina).

No mortality, adverse clinical reactions, or differences in feed intake or bodyweight gain were reported. There were some sporadic, statistically significant changes in some hematology and clinical chemistry parameters, but none were consistent and there was no dose-response relationship. There were no statistically significant differences in absolute or relative organ between treatment groups and control groups and no macroscopic pathology findings. Slightly sporadic focal necrosis in liver was found in one animal in the control group; focal necrosis was reported in the heart in some rats (1/20 animal in the control group and 3/20 animals in the high-dose group).

The authors concluded that, "the results of the present study demonstrate that *L. paracasei* GM080 are non-toxic up to a level of 5.0 g/kg body weight, when given orally. The no-observed-adverse-effect-level (NOAEL) for *L. paracasei* GM080 was 5.0 g/kg body weight (approximately equivalent to $1x10^{10}$ cfu/kg bw) in male and female rats, the highest dose tested."

In summary, all toxicity testing of 4 different strains of *L. paracasei* ssp. *paracasei* has found no evidence that would suggest any adverse reactions in mice or rats.

6.6. Decision-Tree Analysis

The decision tree of Pariza et al. (2015) was utilized to assess the evidence regarding the safety of the intended use of *L. paracasei* ssp. *paracasei* strain F-19. Significant questions follow:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? ***YES***
2. Has the strain genome been sequenced? ***YES***
3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? ***YES***
4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? ***YES***
5. Does the strain produce antimicrobial substances? ***NO***
6. Has the strain been genetically modified using rDNA techniques? ***NO***
7. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? ***NO***—Isolated from a healthy human colon
8. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? ***NO***

The outcome of this decision-tree analysis is that "the strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption" (Pariza et al., 2015).

6.7. Evaluations by Authoritative Bodies

Noting that a wide variety of microbial species is used in food, some with a long history of apparent safe use, and facing the need to set priorities for risk assessment, the European Food Safety Authority (EFSA) proposed a system referred to as "Qualified Presumption of Safety" (QPS; EFSA 2007a, 2007b). This system proposed basing the safety assessment of a defined taxonomic group (e.g., a genus or a species) on 4 pillars: established identity, body of knowledge, possible pathogenicity, and end use. If the taxonomic group did not raise safety concerns or, if safety concerns existed but could be defined and excluded, the grouping could be granted QPS status. Thereafter, "any strain of microorganism the identity of which could be unambiguously established and assigned to a QPS group would be freed from the need for further safety assessment other than satisfying any qualifications specified" (EFSA 2007a, p1).

EFSA's Scientific Committee was asked to recommend organisms regarded as suitable for QPS status. The list of such organisms proposed by the Committee included *L. paracasei*. In listing *L. paracasei* and other species of *Lactobacillus* as suitable for QPS status, the Committee stated, "Where QPS status is proposed, the Scientific Committee is satisfied that the body of knowledge available is sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and capable of exclusion" (EFSA 2007a, p8) and that the recommendations are "based on a thorough review of the available scientific literature and the knowledge and experience of the scientists involved" (EFSA 2007a, p8).

EFSA has issued annual updates of the QPS status of bacterial strains from 2008 through 2016; no need for either review or change of the QPS status of *L. paracasei* was reported (EFSA 2008b, 2009, 2010, 2011, 2012b, 2013, 2014, 2015a, 2015b, 2016, 2017, 2018).

The Danish Veterinary and Food Administration, having previously approved *L. paracasei* ssp. *paracasei* strain F-19 for use in dietary supplements and several types of foods, was petitioned

to extend this approval to use in infant formula, transition foods, and other food types. This approval was granted in a letter dated 1 December 2009 (DVFA 2009).

6.8. Safety Assessment and GRAS Determination

This section presents an assessment that demonstrates that the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is safe and is GRAS based on scientific procedures.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of consumers to *L. paracasei* ssp. *paracasei* strain F-19 under its intended conditions of use is not harmful. In the second step, the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is determined to be GRAS by demonstrating that the safety of this product under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

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

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

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

The criteria outlined above for a scientific-procedures GRAS determination are applied below in an analysis of whether the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is safe and is GRAS.

6.8.1. Evidence of Safety

The body of evidence supporting the safety of oral administration of *Lactobacillus* strains in general, and *L. paracasei* ssp. *paracasei* strain F-19 in particular, is large and convincing. Numerous commentators and authoritative bodies such as EFSA have noted the safe history of human ingestion of *L. paracasei* ssp. *paracasei* strains over many years. *L. paracasei* ssp. *paracasei* strain F-19 produces no deleterious metabolites and is not destructive of mucin. Any effects that this probiotic microorganism has on intestinal permeability appear to be beneficial in strengthening barrier function. While it is theoretically possible for biogenic amines to be produced as a result of fermentation of dairy products by lactobacilli, this phenomenon has not been reported. When *Lactobacillus* strains are ingested as probiotics, they produce lactic acid, lowering the intestinal pH and reducing the opportunity for production of harmful biogenic amines by putrefactive bacteria.

Lactobacilli are not regarded as pathogens, although some strains are capable of opportunistic infection in extremely favorable circumstances invariably involving severe underlying disease states and most often also involving a facilitated pathway such as surgical intervention or the presence of central lines. Documented cases of *Lactobacillus* bacteremia are so rare, in comparison to the widespread use of *Lactobacillus* strains in the environment, in food production, and in probiotic applications, that the participants in the 2007 EU-PROSAFE project (Vankerckhoven et al. 2008) suggested that "they are more medical exceptions, or even curiosities, than a genuine public health issue."

Consumption of live lactic acid bacteria included in lactic-acid-fermented foods has been a regular part of the food intake of humans for hundreds of years and individuals consuming lactic-acid-fermented products of dairy origin also consume large amounts of *L. paracasei* ssp. *paracasei*.

The safe history of human exposure to *L. paracasei* ssp. *paracasei* strains is strongly supported by a large body of published research. In addition to *in vitro* work, the published literature includes 4 oral toxicity studies in mice and rats and more than 50 studies in human infants, children, and adults enrolling more than 7,000 individuals. Participants in these studies received the probiotic at daily levels up to 10^{12} cfu/day for as long as 9 months with no reported adverse events associated with the probiotic intervention.

The strain *L. paracasei* ssp. *paracasei* strain F-19 originates from human intestinal mucosa (Ljungh et al. 2002) and it is sold in food products and as a dietary supplement in Europe. Since 2001, products containing *L. paracasei* ssp. *paracasei* strain F-19 have been widely consumed without any reported adverse events.

The genome of *L. paracasei* ssp. *paracasei* strain F-19 was sequenced and genes were annotated and compared with databases of antibiotic resistance genes and virulence factors; additionally, terms were searched for possible associations with antibiotic resistance or virulence. No findings were suggestive of potential risk to consumers. The absence of genes encoding antibiotic resistance was confirmed by phenotypic testing for such resistance in which no resistance was found other than intrinsic resistance to vancomycin found in many *Lactobacillus* strains. Additional phenotypic testing demonstrated that the strain does not produce biogenic amines and only trace levels of D-lactate.

The available evidence demonstrates that there is no reason to suspect harm to individuals from the intended use of *L. paracasei* ssp. *paracasei* strain F-19.

6.8.2. Conclusion of the Expert Panel

The intended use of *L. paracasei* ssp. *paracasei* strain F-19 has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was shown by establishing the identity and probiotic characteristics of the strain, demonstrating its freedom from pathogenic or other risk factors using the decision-tree analysis, and concluding that the expected exposure to *L. paracasei* ssp. *paracasei* strain F-19 is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

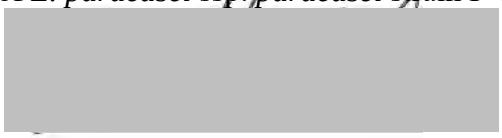
Determination of the safety and GRAS status of the intended use of *L. paracasei* ssp. *paracasei* strain F-19 has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Berthold V. Koletzko, M.D., Ph.D., and Michael W. Pariza, Ph.D., who reviewed a monograph prepared by James T. Heimbach, Ph.D., and other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients, including probiotic bacteria. They independently critically reviewed and evaluated the publicly available information and the potential human exposure to *L. paracasei* ssp.

paracasei strain F-19 anticipated to result from its intended use, and individually and collectively determined that no evidence exists in the available information on *L. paracasei* ssp. *paracasei* strain F-19 that demonstrates, or suggests reasonable grounds to suspect, a hazard to consumers under the intended conditions of use of *L. paracasei* ssp. *paracasei* strain F-19.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and information would reach the same conclusion regarding the safety of the strain under its intended conditions of use. Therefore, the intended use of *L. paracasei* ssp. *paracasei* strain F-19 is GRAS by scientific procedures.

6.9. Statement Regarding Information Inconsistent with GRAS

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with our conclusion of GRAS status of the intended use of *L. paracasei* ssp. *paracasei* strain F-19.



Part 7: List of Supporting Data and Information

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Molly A. Harry
Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Food Ingredients
U.S. Food and Drug Administration

Dear Dr. Harry:

In your e-mail to me dated May 31, 2019, you indicated that FDA reviewers of GRN 000840 had the questions listed below, along with our responses. Production of the notified probiotic is managed by Chr. Hansen, and so the information regarding allergens and monitoring of the fermentation process is provided by this supplier.

1. **In Part 2 of the notice (on pages 4 and 5), you state that *L. paracasei* ssp. *paracasei* strain F-19 is sold in lyophilized form with maltodextrin filler. Please clarify whether maltodextrin used in the formulation is from an allergenic source.**

A Chr. Hansen *Raw Material Specification* for maltodextrin is attached. According to this statement, the only ingredient in the product is maize starch. It also indicates that, "This raw material does not trigger allergen labelling according to EU regulation 2007/68/EC or US Food Allergen Labelling and Consumer Protection Act of 2004 and 21CFR§130.9 when used as an ingredient in food products."

2. **In Section 2.2.3.2, as part of the production process, you described the inoculation and fermentation process. Please confirm that the fermentation process is monitored for contamination.**

To be addressed in a further response.

3. **In Part 3 of the notice (on page 10), you describe the intended uses and levels of use of the subject organism. However, there is no estimate of dietary exposure to the U.S population. Please provide an exposure estimate based on consumption of the foods your ingredient will be used in. Please also state whether the exposure to your ingredient would be substitutional for other subspecies e.g., *Lactobacillus casei* subspecies *paracasei* Lpc-37 (GRN 000736).**

The notice indicated that, given that the probiotic will be present in a limited number of foods (primarily dairy products, fruit drinks, and RTE cereals) at concentrations less than 10^{10} cfu/serving, with the concentration declining over the shelf-life of the food, its likely maximum ingestion is less than 10^{11} cfu/day. Since mean food consumption is about 20

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

food servings/day*, and this estimate allows for ten or more servings of foods or drinks containing the probiotic, it is felt that this is an extremely conservative estimate.
(*Millen AE, D Midthune, FE Thompson, V Kipnis, AF Subar. 2006. The National Cancer Institute diet history questionnaire: validation of pyramid food servings. *Am J Epidemiol* 163:279-288.)

GRN 840 for *L. paracasei* ssp. *paracasei* strain F-19 does not include a prohibition on the use of the probiotic in foods containing another probiotic; I am not aware of any GRAS notice that does. Probiotics suitable for addition to foods are fairly expensive, and adding them under conditions providing for tight control of addition concentrations is complex; it thus seems unlikely that food manufacturers would choose to add multiple strains that were not purchasable as a premixed blend.

4. **Please confirm that an updated literature search was performed on *L. paracasei* ssp. *paracasei* strain F-19, including for opportunistic infection reports, and whether any additional studies relevant to safety were identified.**

The literature review was not updated prior to the initial submission of the GRAS notice, which took place less than two months after submission of GRN000810. However, FDA declined to file the submission due to its mention of intended use of the probiotic in dietary supplements as well as foods, and the recent literature was reviewed before the notice was resubmitted on December 31 (during the shutdown). No additional relevant studies were found.

5. **Please state whether the final product contains allergens from the fermentation media and how this is confirmed.**

A copy of Chr. Hansen's "*Allergen Information for Probio-Tec® F19® Blend-30 IF*" is attached. It reports that no ingredients sourced from potential allergens are used.

Also attached is a statement, "*Allergen Management in Chr. Hansen*," which describes the company's procedures for controlling allergens, including "segregation of all food allergens during storage and handling," "risk assessment and control of all processes where allergens are handled," "cross contamination control via validated/verified allergen cleaning programs," and "full traceability on all raw materials, rework and finished products."

The statement also addresses allergen communication, including "declaration of allergens, and confirmation of allergen management from all suppliers," "allergen risk assessment of all raw materials and finished products," "allergen profiles on all finished products," and "Product Allergen Information sheets on all finished products."

In GRN 000810, *L. paracasei* ssp. *paracasei* strain F-19 is intended for use as an ingredient in non-exempt powdered infant formula. In your response to the above questions, also clarify if the final product contains allergens; and state how this was confirmed.

I believe that the answers to the above questions, especially Question 5, fully respond to the concern over use of the probiotic in non-exempt powdered infant formula as described in GRN 000810.

Sincerely,

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

Statement

July 19, 2018
Valid two years from date of issue

To whom it may concern

Allergen Management in Chr. Hansen

Food safety has the highest priority in Chr. Hansen; as such allergen management is one of our core programs to secure the safety of our products.

We *control* all allergens listed in EU Labeling Regulation 1169/2011 and the US Food Allergen Labeling and Consumer Protection Act of 2004. Chr. Hansen also *communicates* the allergen status of our products in accordance with these two regulations.

Allergen *control* is managed via our Good Manufacturing Practice (GMP) and HACCP programs that are FSSC 22000 certified at all our production sites. The programs include (but are not limited to):

- Segregation of all food allergens during storage and handling
- Risk assessment and control of all processes where allergens are handled
- Cross contamination control via validated/verified allergen cleaning programs
- Full traceability on all raw materials, rework and finished products

Allergen *communication* is managed via our Quality Management and HACCP programs that are ISO 22000 certified in our head office, R&D, and Support functions. The programs include (but are not limited to):

- Declaration of allergens, and confirmation of allergen management from all suppliers
- Allergen risk assessment of all raw materials and finished products
- Allergen profiles on all finished products
- Product Allergen Information sheets on all finished products

More information about Chr. Hansen's 'Quality, GMP and Food Safety principles' is available at our global homepage www.chr-hansen.com. Please refer to our site on policies and positions and open the subfolder on 'Quality & Product Safety'.

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 www.chr-hansen.com

Statement

Allergens and other sensitizing substances, for example on the LEDA and ALBA lists

Chr. Hansen only control the allergens listed in the EU Labeling Regulation 1169/2011 and the US Food Allergen Labeling and Consumer Protection Act of 2004. Cross contamination from other allergens or sensitizing substances mentioned in for example the LEDA and ALBA lists is covered by our standard GMP, but with no specific cleaning programs for these allergens or substances. We can inform upon request if other allergens or sensitizing substances mentioned in for example the LEDA and ALBA lists have been used as ingredients in our finished products.

If you have any further questions, please contact your local sales representative.

Yours sincerely
Chr. Hansen A/S

Michael Dahm-Hansen
Head of Quality Management

Caroline Herody
Director Global Regulatory Affairs

Electronically generated, therefore not signed

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 www.chr-hansen.com

CHR. HANSEN

RAW MATERIAL SPECIFICATION
FOR INTERNAL HH USE

Our GIN: **680851** Document ID: RMS-680851-09

Our material name: **MALTODEXTRIN DE 12**

Issued by:

Thomas Hansen

Digitally signed by Thomas Hansen
DN: cn=Thomas Hansen, o=Chr. Hansen, ou=Supplier Quality Management, email=dkthoh@chr-hansen.dk, c=DK
Date: 2018.06.27 14:39:08 +02'00'

Approved by:

The products from Chr. Hansen are used as ingredients in food, feed and medicinal products. The quality of our raw materials is crucial to ensure the safety and quality of Chr. Hansen's final products. Therefore suppliers must be approved according to Chr. Hansen's vendor management system prior to delivery. Changes to the information given, such as production site, allergen statement, environment, health and safety or HACCP declarations, must be communicated immediately to Chr. Hansen for approval. If a vendor changes his product in such a way that going forward it will no longer comply with this raw material specification, Chr. Hansen must be notified 3 months in advance.

General description

This raw material is produced from maize starch by enzymatic hydrolysis, purification and spray-drying. It must be food grade and comply with the current editions of 1881/2006/EC (as ammended) for Infant formulae and follow-on formulae, FCC, Ph Eur and USP. The raw material is intended for the manufacturing of ingredients to be used in general food, infant formula, medicinal products and feed.

	Acceptance criteria	Reference methods
Identity		
CAS number(s):	9050-36-6	
Chemical name(s):	Maltodextrin	
Identification	Complies	Ph Eur, USP, FCC, Ph Eur 2.2.48, USP 1120
Chemical / physical		
Appearance	White or almost white, crystalline powder or granules	
Assay (as DE) **	11 to 15 DE	Ph Eur
pH **	4,0 to 7,0	Ph Eur, USP
Water activity	< 0,15	USP 1112
Loss on drying **	≤ 5,0 %	Ph Eur, USP
Sulphated ash **	≤ 0,5 %	Ph Eur, USP, FCC
Arsenic **	≤ 0,1 mg/kg	ICP, AAS
Cadmium **	≤ 0,01 mg/kg	ICP, AAS
Mercury **	≤ 0,1 mg/kg	AAS
Lead **	≤ 0,02 mg/kg	ICP, AAS
Total solids	≥ 90,0 %	FCC
Protein (N*6,25) **	≤ 0,1 %	USP
Sulphur dioxide **	≤ 10 mg/kg	Ph Eur, FCC
Particle size distribution	Residue on 40 µm ≥ 60 %, residue on 250 µm ≤ 10 %	Ph Eur 2.9.38

Our GIN:	**680851**	Document ID: RMS-680851-09
Our material name:	**MALTODEXTRIN DE 12**	

Microbiology

Total aerobic microbial count *	≤ 1000 cfu/g	Ph Eur 2.6.12, USP 61, ISO 7218, NMKL 86
Total yeast and mould count *	≤ 100 cfu / g	Ph Eur 2.6.12, USP 61, ISO 7218, NMKL 98
Enterobacteriaceae *	Absent in 100 g	ISO 21528, NMKL 144
Escherichia coli *	Absent in 1 g	Ph Eur 2.6.13, USP 62, NMKL 96
Salmonella *	Absent in 25 g	ISO 6579, NMKL 71
Enterobacter sakazakii *	Absent in 300 g	ISO 22964
Staphylococcus aureus *	Absent in 1 g	Ph Eur 2.6.13, USP 62, ISO 6888, NMKL 66
Bacillus cereus *	< 10 cfu / g	ISO 7932, NMKL 67
Clostridium perfringens	Absent in 1 g	ISO 7937, NMKL 95

Packaging, delivery and storage

Total shelf life	Minimum two years from the date of manufacture and at recommended storage conditions
Remaining shelf life at delivery	Minimum 75% of total shelf life
Net weight	Specified on the label, ≤ 25 kg, consistent for each unit and delivery
Recommended storage conditions	Ambient temperature in original packaging

Certificates and statements (submitted to Chr. Hansen)

Certificate of origin	Legal name and address of the manufacturing company must be specified
Internal testing	Results for tests with '* in this specification must be analysed in the QC setup at the frequency specified in the program
HACCP product information	Covering material origin, manufacturing process and critical control points
Allergen declaration	According to Chr. Hansen form or equivalent
Kosher	Considered Kosher pareve exclusive passover by OK Kosher Certification
Halal	Considered Halal by IFANCA
Environmental and occupational safety	According to Chr. Hansen form and with safety data sheet

Legislation

Allergens	This raw material does not trigger allergen labelling according to EU-regulation 2007/68/EC or US Food Allergen Labelling and Consumer Protection Act of 2004 and 21CFR§130.9 when used as ingredient in food products
GMO	This raw material does not trigger GMO labelling according to EU regulations 2003/1829/EC and 2003/1830/EC when used for manufacturing of food or feed products
Packaging materials	Materials in direct contact with the raw material must comply with relevant EU and US regulations
Good distribution practice	This raw material must be distributed to Chr. Hansen in accordance with EU regulation 2004/852/EC
REACH	For deliveries to Chr. Hansen sites physically located in EU, the raw material must be either registered or pre-registered unless it is 1) exempted according to annex IV or V, or 2) exempted according to the 1 tonne threshold (article 12(1)(a)) - in accordance with EU regulation 2006/1907/EC

Probio-Tec® F19® Blend-30 IF

Allergen Information

Material No: 703222
Version: 2 AL EN 06-22-2015

List of common allergens in accordance with the US Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and EU Regulation 2011/1169/EC with later amendments.	Present as an ingredient in the product	Ingredient species or type
Cereals containing gluten* and products thereof	No	
Crustaceans and products thereof	No	
Eggs and products thereof	No	Not applicable
Fish and products thereof	No	
Peanuts and products thereof	No	Not applicable
Soybeans and products thereof	No	Not applicable
Milk and products thereof (including lactose)	No	Not applicable
Nuts* and products thereof	No	
List of allergens in accordance with EU Regulation 2011/1169/EC only		
Celery and products thereof	No	Not applicable
Mustard and products thereof	No	Not applicable
Sesame seeds and products thereof	No	Not applicable
Lupine and products thereof	No	Not applicable
Mollusks and products thereof	No	Not applicable
Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2	No	

Yes = Allergen labeling required
No = Allergen labeling not required

* Please consult the EU Regulation 2011/1169 Annex II for a legal definition of common allergens, see European Union law at: http://eur-lex.europa.eu/

The product is produced in a facility that produces dairy containing products.

CHR HANSEN

Improving food & health

Statement

Human Health, Health & Nutrition Business Unit
June 12, 2019
Valid two years from date of issue

To Arla Foods

Regarding monitoring for contamination in the fermentation process

Thank you for your inquiry into Chr. Hansen's products.

Chr. Hansen is pleased to confirm that we have a Quality Management system and Food Safety program in place to ensure control, monitoring and assessment of food safety risks in all our production processes including risks of microbiological, chemical and physical contaminations.

Please do not hesitate to contact your local Chr. Hansen representative in case of further questions.

Yours sincerely

Søren Holm
HH QA Release Manager

Chr. Hansen A/S -10-12 Bøge Allé - DK-2970 Hørsholm, Denmark - Phone: +45 45 74 74 74 - Fax: +45 45 74 88 88 - www.chr-hansen.com

From:	Jim Heimbach
To:	Harry, Molly
Subject:	RE: GRN 000840
Date:	Friday, August 23, 2019 1:30:03 PM
Attachments:	image001.png

Dear Dr. Harry—

Thanks for the note. By functional/nutritional products we had in mind such things as nutrition or meal-replacement bars, nutritional beverages, sport drinks, and protein and nutritional powders. We're not wedded to the terminology and would be perfectly happy to follow your suggestion and simply indicate "other food categories." The primary criterion remains addition only to foods that can sustain viable bacteria for the shelf life of the food.

Regards,
Jim

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

From: Harry, Molly [mailto:Molly.Harry@fda.hhs.gov]
Sent: Friday, August 23, 2019 12:43 PM
To: Jim Heimbach
Subject: RE: GRN 000840

Dear Dr. Heimbach,

I have a quick question for you. The food categories in GRN 000840 include "functional and nutritional food products." These are not established food categories and we are uncertain what they mean. Could you provide clarifications on the categories that the food products belong to or could you rephrase these as "other food categories?"

Let me know if you have any questions.

Sincerely,

Molly A. Harry
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Food Ingredients
U.S. Food and Drug Administration
Tel: 240-402-1075
Molly.Harry@fda.hhs.gov


FDA
U.S. FOOD & DRUG
ADMINISTRATION