#856

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OFFICE OF
FOOD ADDITIVE SAFETY

April 11, 2019

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 Office of Food Additive Safety:

In accordance with the guidance issued by the Food and Drug Administration (FDA) under 21 81 Fed. Reg. 54960 (Aug. 17, 2016), Chr. Hansen hereby provides notice of a claim that the use of *Bifidobacterium animalis* ssp. *lactis* BB-12® in conventional foods as described in the enclosed notification is Generally Recognized As Safe (GRAS) based on scientific procedures.

The enclosed triplicate copies of the submission each include form FDA 3667, a comprehensive GRAS assessment, and appendix documents in support of the assessment, for your consideration.

Please feel free to contact me directly to discuss any aspects of this submission.

Sincerely,

Sarah F. Kraak-Ripple
Regulatory Affairs Manager
Human Health – North America
Chr. Hansen, Inc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
000856	4/16/2019
ESTIMATED DAILY INTAKE	**INTENDED USE FOR INTERNET**
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

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

Organization *(if applicable)*: Chr. Hansen, Inc.

Mailing Address *(number and street)*: 9015 West Maple Street

City	State or Province	Zip Code/Postal Code	Country
Milwaukee	Wisconsin	53214	United States of America

Telephone Number	Fax Number	E-Mail Address
414-614-9348	414-607-5959	ussakr@chr-hansen.com

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

Name of Contact Person	Position or Title

Organization *(if applicable)*:

Mailing Address *(number and street)*:

City	State or Province	Zip Code/Postal Code	Country

Telephone Number	Fax Number	E-Mail Address

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

[redacted]

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

☐ Electronic Submission Gateway ☐ Electronic files on physical media

☒ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes 1

Total number of pages 92

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

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

Intended applications include but are not limited to the following: milk and dairy products and other fermented milk products; dairy alternatives fermented milk and yogurt products; beverages; shelf-stable products such as bars and confectionery; and breakfast cereals at levels up to 5x10E11 cfu/serving to ensure proper levels at the end of shelf-life.

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Sarah F. Kraak-Ripple

(name of notifier)

has concluded that the intended use(s) of Bifidobacterium animalis ssp. lactis 8B-12®

(name of notified substance)

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

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

9015 West Maple Street, Milwaukee, WI 53214

(address of notifier or other location)

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

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Sarah F. Kraak-Ripple	04/11/2019

SECTION G – LIST OF ATTACHMENTS

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

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

Generally Recognized as Safe (GRAS) Determination for the Intended Use of *Bifidobacterium animalis* ssp. *lactis* BB-12®

Prepared by
Chr. Hansen A/S
Denmark

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Part 1

Part 1. Signed Statement and Certification

1.1. Name and Address of Notifier

Chr. Hansen A/S
Boege Allé 10-12
2970 Hoersholm, Denmark

1.2. Name of Notified Substance

Bifidobacterium animalis ssp *lactis* BB-12® (*B. lactis* BB-12®).

B. lactis BB-12® originates from Chr. Hansen's collection of diary cultures. The strain was specially selected by Chr. Hansen for the production of probiotic dairy products.

1.3. Intended Condition of Use

B. lactis BB-12® is intended to be used as an ingredient in conventional foods at levels consistent with current good manufacturing practices (cGMPs). It is intended to be consumed by the general population. Intended applications include but are not limited to the following: milk and dairy products such as yogurt and other fermented milk products; dairy alternatives (plant-based (oat, soy, almond, coconut, pea, etc.) fermented milk and yogurt products); beverages such as juice and protein shakes; shelf-stable products such as bars (granola, protein, meal replacement bars), confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings); and breakfast cereals (RTE and hot).

To allow for loss of viability over time and to ensure at least $5x10^{10}$ CFU/serving in conventional food products throughout shelf life, the initial addition level of *B. lactis* BB-12® may be as high as $5x10^{11}$ CFU/serving.

1.4. Basis for GRAS Status

B. lactis BB-12® has been determined to be GRAS through scientific procedures in accordance with 21 CFR § 170.30 (a) and (b).

B. lactis BB-12® strain was the subject of GRAS Notice No. GRN 49, for the use in infant formula and other food for infants and children. The FDA response letter dated November 28, 2005, stated that the Agency had "no questions at this time".

1.5. Premarket Approval Status

B. lactis BB-12® is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on a conclusion that the notified substance is GRAS under the condition of intended use.

1.6. Availability of Information

The information and data that serve as the basis for this GRAS conclusion will be sent to FDA upon request, or will be available for review and copying at reasonable times at Chr. Hansen's office in the USA at the following address:

Chr. Hansen, Inc.
9015 W. Maple St
Milwaukee, WI 53214
Telephone: (414) 607-5700
Fax: (414) 607-5959

1.7. Freedom of Information Act Statement

No information contained in this GRAS notice is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

1.8. Certification

To the best of our knowledge, this conclusion of GRAS status is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of *B. lactis* BB-12®.

1.9. FSIS Statement

Not Applicable

1.10. Name, Position, and Signature of Notifier

Sarah F. Kraak-Ripple
Regulatory Affairs Manager
Human Health – North America
Chr. Hansen, Inc.

Date: April 11, 2019

Part 2

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

2.1 Identity of the Organism

2.1.1 Source and Description of GRAS Organisms

B. lactis BB-12® was deposited in Chr. Hansen's Culture Collection in 1983 and was specifically selected for the production of probiotic dairy products. At the time of isolation, the probiotic strain *B. lactis* BB-12® was considered to belong to the species *Bifidobacterium bifidum*. Modern molecular classification techniques reclassified this probiotic strain as *Bifidobacterium animalis* and later to a new species *Bifidobacterium lactis*. The species *B. lactis* was later shown not to fulfill the criteria for a species and was instead included in *Bifidobacterium animalis* as a subspecies. *B. lactis* BB-12® is therefore classified as *Bifidobacterium animalis* ssp. *lactis*. Despite a change in the name over the years, the strain *B. lactis* BB-12® ® has not changed. The strain has been deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) under the accession number DSM 15954.

The colony morphology of *B. lactis* BB-12® is round with smooth edge, curved elevation, and smooth and shiny surface. The appearance on MRS medium after anaerobic incubation for 3 days at 37°C is white, non–transparent. Microscopically, *B. lactis* BB-12® is Gram positive, rod-shaped with single, pairs, or branched rods. The strain is non-motile and does not form endospore.

The taxonomic lineage of *B. lactis* BB-12® is:
Kingdom Bacteria, Phylum Actinobacteria, Class Actinobacteria, Order Bifidobacteriales, Family Bifidobacteriaceae, Genus *Bifidobacterium*, Species *Bifidobacterium animalis*, Subspecies *Bifidobacterium animalis* subsp. *lactis*, Strain *Bifidobacterium animalis* ssp. *lactis*, BB-12®. A subspecies-specific core genome was recently identified by in silico analysis for both B. animalis subspecies, revealing the existence of subspecies-defining genes involved in carbohydrate metabolism (Lugli et al., 2019).

Species Identification

The 16S rDNA sequence of BB-12 was compared to a database of 16S rDNA sequences of type strains. The 16S rDNA sequence of the BB-12® strain is identical to the sequence of the type strain of *Bifidobacterium animalis* subsp. *lactis* (GenBank acc. No. NC012815). Therefore, the BB-12® strain is unambiguously identified as *Bifidobacterium animalis* subsp. *lactis*.

2.1.2 Genome Sequencing and Annotation

The complete 'closed genome' of *B. lactis* BB-12® was sequenced, annotated, and published by Garrigues *et al.* (2010). The genome sequence has been deposited in NCBI reference sequence number NC017214 (GenBank accession number CP001853).
The genome sequence of *B. lactis* BB-12® consists of a single circular chromosome of 1.94 million base pairs (Mbp) with a GC content of 60 % and with no plasmids. In brief, the sequence was obtained by use of shotgun cloning into plasmids and cosmids and sequenced by Sanger methodology. Gaps in the sequence identified after alignment of the contigs were closed by use of PCR and primer walking. (Garrigues et al, 2010).

The strain BB-12® was also sequenced in house at Chr. Hansen A/S by purifying total DNA, Illumina sequencing and assembly by use of published methods (Agersø et. al, 2018). The 'draft Genome' consisted of 30 contigs with a total contig length of 1.92 Mbp and a GC content of 60 %.

For the assessment, the 'closed genome' was used and the results were verified by use of the 'draft genome'. Both genome sequences of *B. lactis* BB-12® were subjected to annotation using published methods. The *B. lactis* BB-12® 'closed genome' contained 1,612 protein encoding genes (PEGs) and 64 RNAs, and the 'draft genome' 1,638 PEGs and 55 RNAs.

Search Against Antibiotic Resistance Gene Databases

To identify genes with high identity to previously published antibiotic resistance genes, the annotated closed genome and the draft genome for *B. lactis* BB-12® was analyzed against a curated database of antibiotic resistance genes. The database focus on acquired antibiotic resistance genes from the scientific literature and covers both Gram-positive and Gram-negative bacteria including pathogenic species. The analysis detected the tetracycline resistance gene *tet*(W). No other antibiotic resistance genes were detected. Moreover, the strain was found to be sensitive to all antibiotics except tetracycline, where a minimal inhibitory concentration one two-fold over the EFSA cutoff value was observed (EFSA Journal, 2018).

It is well known from the scientific literature that many *Bifidobacterium* species and all *B. animalis* subsp. *lactis* show low level resistance to tetracycline (Yazid *et al.* 2000; D'Aimmo *et al.* 2007; Mättö *et al.* 2007). This low-level resistance has been shown to be due to the presence of a *tet*(W) gene, which is present in the chromosome of all *B. animalis* subsp. *lactis* strains described to date (Gueimonde *et al.* 2010; Milani *et al.* 2013). Based on the genetic context in which the *tet*(W) gene is located in the *B. lactis* BB-12® chromosome, a potential transfer of the gene is considered to be unlikely as the region flanking the *tet(*W) is conserved in all *B. animalis* ssp. *lactis* present in the NR NCBI data base (100% coverage and 99-100% identity). Several studies have investigated if the *tet*(W) gene in *B. lactis* in general and specifically in the *B. lactis* BB-12® strain is transmissible and no evidence of transmissibility has been shown for the *tet*(W) gene (Masco *et al.* 2006; Saarela *et al.* 2007; Gueimonde *et al.* 2010; IPLAIC4 in this

reference is the *B. lactis* BB-12® strain Raeisi *et* al. 2018; Polit et. al. 2018). Moreover, since *B. lactis* BB-12® does not contain any plasmids and no bacteriophages are known to infect the species, the risk of transfer of *tet*(W) to other microorganisms is negligible.

Search Against the Virulence Factor Database and Phenotypic Test

The annotated closed genome and the draft genome of *B. lactis* BB-12® was analyzed against a published database containing virulence factors and other genes related to pathogenicity and toxicity from 30 different pathogens including Gram-positive pathogens such as *Enterococcus*, *Staphylococcus*, *Streptococcus* and *Listeria*. All hits except one were associated with stress regulation (Clp), heat shock proteins, biosynthesis, capsule formation or transport systems. No hits were assessed to be virulence factors and all hits could be regarded as niche factors (Hill et al. 2012) or involved in housekeeping within the cell, since these genes are also found in commensal bacteria.

One hit was annotated as 'Response regulators consisting of a CheY-like receiver domain and a winged-helix DNA-binding domain'. The protein encoding gene had closest identity (54.4% amino acid identity over 92.3% coverage, 5.4% gaps and with an E-value of $1.11*10^{-75}$) to PhoP described as a possible two component system response transcriptional positive regulator involved in sensing Mg^{2+} starvation in Mycobacterium tuberculosis and Gram-negative bacteria. (Pérez E et. al, 2001) This may enhance intracellular growth of such bacteria, but the gene is not itself a virulence gene. Moreover, the gene from *B. lactis* BB-12® was also present in all 21 *Bifidobacterium animalis* genomes in the NCBI NR database with 100% coverage and identity (94-100%). Therefore, the gene can be considered a niche factor and is not considered a safety concern.

In addition to the *in-silico* genome screening, phenotypic tests for cytotoxicity and hemolysis were also performed. Results of these phenotypic tests showed that *B. lactis* BB-12® did not cause cytotoxic activity in a Vero cell assay and the strain is non-hemolytic.

In conclusion, the *in-silico* genome screening for potential virulence factors and other genes related to pathogenicity, virulence or toxicity in *B. lactis* BB-12®. did not reveal any virulence or toxicity genes or other genes of safety concern which further indicates the safety of *B. lactis* BB-12®.

2.1.3 Phenotypic Properties and Strain Mechanisms

The phenotypic characteristics and mechanisms of *B. lactis* BB-12® have been established through extensive in vitro testing. The characteristics and science behind this probiotic strain have been reviewed by Jungersen et al. (2014).

Carbohydrate Fermentation Profile and Metabolic Characteristics

The intestinal microbiota plays an important role in metabolic activities such as fermentation of undigested carbohydrates, lipid metabolism and glucose homeostasis.

Bifidobacterium are chemoorganotrophs with a fermentative metabolism. The carbohydrate fermentation profile of *B. lactis* BB-12® using API 50 CHL medium, is shown in Table 1. *B. lactis* BB-12® forms L(+) lactic acid, acetic and succinic acid. This strain shows negative catalase and oxidase reactions. No deleterious metabolic activities caused by *Bifidobacterium* strains have been reported.

Table 1. Carbohydrate Fermentation Profile of *Bifidobacterium animalis* ssp. *lactis* BB-12®

Control	-	Esculine	-
Glycerol	-	Salicine	±
Erythritol	-	Cellobiose	-
D-Arabinose	-	Maltose	+
L-Arabinose	-	Lactose	+
Ribose	+	Melibiose	+
D-Xylose	-	Saccharose	±
L-Xylose	-	Trehalose	-
Adonitol	-	Inuline	-
β-Methyl-xyloside	-	Melezitose	-
Galactose	-	D-Raffinose	±
D-Glucose	+	Amidon	-
D-Fructose	-	Glycogen	-
D-Mannose	-	Xylitol	-
L-Sorbose	-	β-Gentiobiose	±
Rhamnose	-	D-Turanose	-
Dulcitol	-	D-Lyxose	-
Inositol	-	D-Tagatose	-
Mannitol	-	D-Fucose	-
Sorbitol	-	L-Fucose	-
α-Methyl-D-mannoside	-	D-Arabitol	-
α-Methyl-D-glucoside	-	L-Arabitol	-
N-acetyl glucosamine	-	Gluconate	-
Amygdaline	+	2-keto-gluconate	-
Arbutine	-	5-keto-gluconate	-

Antibiotic resistance

Minimum inhibitory concentrations (MICs) of 9 antibiotics were determined for *B. lactis* BB-12® according to the ISO 10932 | IDF 223 international standard (Table 2). These MICs were compared with the cut-off values established for *Bifidobacterium* by the European Food Safety Authority (EFSA Journal 2018).

Table 2: MIC values for *Bifidobacterium animalis* ssp. *lactis* BB-12®

	Antibiotic	**MIC in µg/ml**	**EFSA cut-off values in µg/ml[a]**
Aminoglycoside	Gentamicin	32-64	64
	Kanamycin	256	n.r.
	Streptomycin	64-128	128
Tetracycline	Tetracycline	16	8
Macrolide	Erythromycin	0.25	1
Lincosamide	Clindamycin	0.06	1
Chloramphenicol	Chloramphenicol	2	4
β-lactam	Ampicillin	0.12-0.25	2
Glycopeptide	Vancomycin	0.5	2

n.r.: not required to be tested by EFSA. a: EFSA cut-off values for *Bifidobacterium* group as listed in 'Guidance on microorganisms used as feed additives or as production organisms' (EFSA Journal 2018, 16(3):5206)

The *B. lactis* BB-12® strain is sensitive to most of the antibiotics tested with MIC values that are less than or equal to EFSA 2018 cut-off values for *Bifidobacterium* group. The MIC value for tetracycline is one two-fold dilution above the EFSA cut-off value, although, that is considered acceptable due to the technical variation of the phenotypic method as also recognized by EFSA in several published opinions. The reduced susceptibility is most likely due to the presence of the tetracycline resistance gene *tet*(W) (EFSA Journal 2018).

Production of biogenic amines and L- and D-Lactate

The strain *B. lactis* BB-12® was tested for production of histamine, tyramine, cadaverine and putrescin using an in-house procedure based on published methods and no production of the four biogenic amines were detected. The Bifidobacteria including Bifidobacterium animalis subsp. lactis use a unique pathway of hexose catabolism, which produces primarily acetate and L-lactate (Encyclopedia of Food Sciences and Nutrition 2ed, 2003 p463-47). This fermentation pathway is known as the "Bifidobacterium shunt" or the "fructose-6-phosphate pathway". The strain Bifidobacterium (BB-12®) was tested experimentally in order to confirm this.

B. lactis BB-12® was tested for production of L- and D-lactate. The ratio between L- and D-Lactic acid was found to be over 95% of the lactate produced was the L-enantiomer.

Conclusion of Genomic Sequence Safety Assessment

The genome sequence of the probiotic strain *B. lactis* BB-12® was analyzed for presence of unwanted genes by *in silico* genome assessment. No such genes were found, except for one true antibiotic resistance gene, *tet*(W), coding for low level tetracycline resistance. This gene has for a long time been known to be present often in *Bifidobacterium* strains and has been found in all *B. animalis* subsp. *lactis* strains investigated so far. The possible transmissibility of the gene has been assessed *in silico* and in several studies aimed at facilitating transfer; however, no transfer has been seen and the risk of transfer of *tet*(W) has been judged to be negligible. Moreover, the strain showed only low level of resistance to tetracycline and was sensitive to all other antibiotics tested for, did not show cytotoxic activity and was non-hemolytic. On the basis of the present genome analysis and phenotypic analysis, it is concluded that the probiotic strain *B. lactis* BB-12®, can be considered to be safe.

Acid and Bile Tolerance

Gastric acid and bile play an important role in the body's defense against ingested microorganisms, capable of killing and controlling gastrointestinal exposure to many pathogens. However, this same defense mechanism can also disable potentially beneficial microbes. For probiotic effects that are dependent on viability and physiological activity in the intestine, the survival of the probiotic in the presence of gastric acid and bile of the upper gastrointestinal tract is critical.

B. lactis BB-12® demonstrated high survival rates when exposed to *in vitro* condition at pH 2-5. This characteristic was shown to be due in part, to the low pH induction of H^+-ATPase activity, an enzyme complex involved in maintaining intracellular pH homeostasis in bacteria (Vernazza et al. 2006). In another study, *B. lactis* BB-12® showed high pH tolerance after three hours exposure at pH 3 and pH 2 (Vinderola et al. 2003).

Testing on tolerance to bile salts revealed that the bile resistance of *B. lactis* BB-12® was moderate, showing 24% growth at 1% bile compared to a control (Vinderola et al. 2003). In another study, *B. lactis* BB-12® did not grow well at 1% bile but demonstrated high survival rates (Vernazza et al. 2006). *B. lactis* BB-12® showed both growth and deconjugation of sodium tauro-deoxycholate and sodium glyco-deoxycholate; this strain also grew in the presence of sodium tauro-cholate and sodium glyco-cholate without showing any deconjugation (Vinderola et al. 2003). Using an artificial gut model system (TIM-1) simulating passage through gastric acid and upper intestinal bile, 60%–80% of the *B. lactis* BB-12® in a normal capsular dose remains viable in the tested condition.

The above data suggests that the majority of *B. lactis* BB-12® bacteria may survive gastric acid and bile after consumption by humans. These properties enhance the potential of *B. lactis* BB-12® to provide a health benefit to the host.

Bile Salt Hydrolase

Following the harsh and acidic gastric environment, bile salts of the small intestine present the next challenge for live probiotics. *B. lactis* BB-12® contains the gene coding for bile salt hydrolase, an enzyme that is important for coping with the high bile salt concentrations in the small intestine. This enzyme is present and active in *B. lactis* BB-12® always, which is documented by both microarray analyses and protein studies using 2-D gel electrophoresis (Garrigues et al. 2005). Having such an enzyme provides an advantage for the strain as it allows a quick response to high bile salt concentrations and thus facilitates the viable passage from the small intestine to the large intestine.

Mucus Adhesion

Adhesion of probiotic microorganisms to the intestinal mucosa is considered a prerequisite for colonization, pathogen inhibition, immune interactions, and barrier function enhancement. The adhesion of *B. lactis* BB-12® to intestinal mucosa was determined *in vitro*. The adhesion models used were polycarbonate-well plates, with or without mucin, and different configurations of Caco-2 and/or HT29-MTX cell cultures. Compared to several probiotic strains tested, *B. lactis* BB-12® displayed the highest level of adhesion to both untreated wells, as well as mucin-treated wells. Though at a lower level, *B. lactis* BB-12® also adhered to Caco-2 cultures, HT29-MTX cells, and co-cultures of Caco-2:HT29-MTX (Laparra et al. 2009). A number of studies revealed that *B. lactis* BB-12® has demonstrated high adherence properties in various *in vitro* settings as follows: it adhered well to fecal mucus isolated from several species, ranging from 10% in dog mucus to 30% in human mucus (Rinkinen et al. 2003); *B. lactis* BB-12® had a high level of binding to immobilized human and bovine intestinal mucus glycoproteins (He et al. 2001); among 60 human intestinal bifidobacteria isolates, *B. lactis* BB-

12® demonstrated similar or better adhesion to mucus in a comparative *in vitro* test (internal study); *B. lactis* BB-12® displayed excellent adhesion properties to fecal mucus prepared from infants and children after rotavirus diarrhea or from healthy counterparts (Juntunen et al. 2001).

Pathogen Inhibition

The ability to inhibit pathogens is one of the three main mechanisms of *B. lactis* BB-12® besides barrier function enhancement and immune interactions. Results from several *in vitro* studies show that *B. lactis* BB-12® is capable of inhibiting gastrointestinal pathogens through production of antimicrobial substance as well as through competition for mucosal adhesion.

Production of antimicrobial substances from *B. lactis* BB-12® was tested against *Bacillus cereus*, *Clostridium difficile*, *Clostridium perfringens* Type A, *Escherichia coli* ATCC 4328, *Enterococcus faecalis*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp *enterica* serovar Typhimurium, *S. enterica* subsp. *enterica* serovar Typhi, *Shigella flexneri*, *Shigella sonnei*, and *Candida albicans*. *B. lactis* BB-12® displayed antagonistic activity against eight out of the twelve tested pathogens (Martins et al. 2009). In batch and continuous culture fermentation systems, a combination of *B. lactis* BB-12® and prebiotics was able to inhibit *Campylobacter jejuni* and/or *E. coli*. The results suggested that the acetate and lactate produced by *B. lactis* BB-12® play a role in the antagonistic action, rather than the ability to lower pH. *B. lactis* BB-12® demonstrated good displacement of *C. difficile*, *B. vulgatus*, *E. aerogenes*, *L. monocytogenes* and to a minor degree *C. histolyticum*, *S. enterica* and *S. aureus* (Collado et al. 2007a). *B. lactis* BB-12® alone or in combination with *L. rhamnosus* LGG® significantly reduced the adhesion of pathogenic strains (*S. enterica* serovar Typhimurium, *C. perfringens*, *C. difficile*, and *E. coli* K2) to pig intestinal mucus (Collado et al. 2007b).

Laursen et al. (2017) investigated the effects of a six-month placebo-controlled probiotic intervention with *B. lactis* BB-12® and *Lactobacillus rhamnosus* LGG® on gut microbiota composition and diversity in more than 200 Danish infants and concluded that probiotic administration during early life did not change gut microbiota community structure or diversity, despite active proliferation of the administered probiotic strains. Thus, the inhibitory effect of *B. lactis* BB-12® strain on pathogens is most likely caused by acetic and lactic acid. The production of these inhibitory compounds does not affect the main commensal bacterial groups in the gut.

Barrier Function Enhancement

Barrier function enhancement is one of the central and generally accepted mechanisms of probiotics. Maintenance of an intact and functional mucus layer and epithelial cell lining in

the gastrointestinal tract is critical for health. The effect of *B. lactis* BB-12® fermentation products on tight junction integrity was performed by measuring the transepithelial electric resistance of the Caco-2 cells. A high resistance of the tight junction indicates that the epithelial lining has a good integrity. The fermentation products from *B. lactis* BB-12® increased the tight junction resistance significantly above that of the untreated control, and in all cases, it induced the greatest increase in transepithelial electric resistance compared to other strains tested. These *in vitro* observations indicate that *B. lactis* BB-12® may increase tight junction strength and protect against disruption of the epithelial barrier function (Commane et al. 2005).

Immune Interactions

Immune interaction is increasingly being acknowledged as a substantial probiotic mechanism. Probiotics are capable of communicating with and affecting the immune system through immune cells located in the intestine since seventy to eighty percent of the immune cells are associated with the gut mucosa. Observations from the studies below demonstrated that *B. lactis* BB-12® is able to interact with the immune cells and may have a beneficial impact on the immune function.

The effect of twelve *Bifidobacterium* strains on the maturation process of dendritic cells derived from human monocytes was studied *in vitro*. *B. lactis* BB-12® was able to induce maturation of dendritic cells measured by surface expression markers. Expression of cytokines varied to a great extent depending on the strain, however, *B. lactis* BB-12® demonstrated induction of IL-12 and TNF-α to a high degree and IL-10 to a low degree. In PBMCs, *B. lactis* BB-12® induced high levels of IL-10, IFN-γ and TNF-α (Lopez et al. 2010). In a study to assess the ability of nine different probiotic strains to induce maturation and expression of cytokine/chemokine in human dendritic cells, *B. lactis* BB-12® was able to induce all cytokines tested (IL-1β, IL-6, IL-10, IL-12 and IFN-γ). The response was dose-dependent and increased with higher dose (Latvala et al. 2008). An *in-vitro* study investigated if fecal precipitates obtained during consumption of *B. lactis* BB-12®, induced an anti-inflammatory response in a macrophage-like cell line. It was found that fecal precipitates tended to elicit a higher TNF-α response during the period of *B. lactis* BB-12® consumption, compared to pre- and post-consumption (Matsumoto et al. 2007).

2.2 Method of Manufacture

2.2.1 Production Process

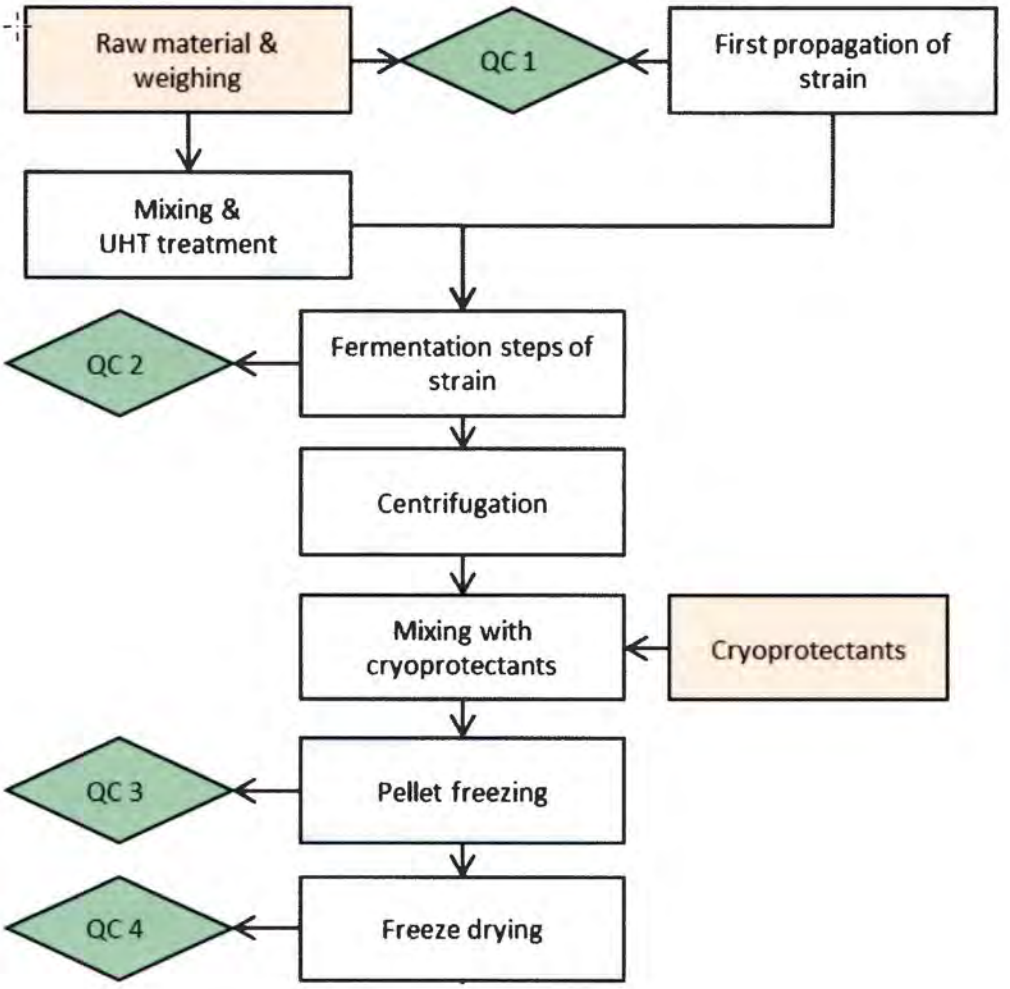
All production steps are performed in accordance with current Good Manufacturing Practices and under an approved HACCP plan. The production process starts with inoculation of the microorganism into the growth substrate (propagation of strain). The media and

cryoprotectants used in the production process are primarily based on carbohydrates, amino acids, vitamins and minerals that are suitable for human consumption with history of safe use.

The fermentation takes place under anaerobic conditions and is controlled by pH and temperature. Fermentation is stopped by cooling when the microbiological growth has ceased. The microorganisms are harvested and concentrated by centrifugation using a separator and thereafter mixed with cryoprotectants and frozen into pellets in liquid nitrogen. At this step, the microorganisms can be frozen as frozen direct vat set. Alternatively, the frozen pellets are lyophilized (freeze drying) into granules and packed as freeze-dried direct vat set. The drying principle is based on sublimation of the liquid from the frozen material. The frozen direct vat set, and freeze-dried direct vat set are used for dairy and other food productions. Figure 1 shows the production process flow.

The freeze-dried granules may be grinded to a powder and blended with excipients to a standardized cell count and sold as an individual product. The powder may also be blended with other strains and excipients before it is filled into the appropriate dosage forms such as capsules, tablets or sticks.

Figure 1. Production Process Flow Chart



The individual productions steps are as follows:

1. *Production of media for fermentation.* The media ingredients used in the manufacturing process are primarily carbohydrates, amino acids, vitamins and minerals that are safe and suitable for human consumption.

2. *Inoculation and fermentation.* From Chr. Hansen's Culture Collection, *B. lactis BB-12®* working cell bank (inoculation culture) is propagated throughout different production steps. This includes the first propagation from a small vial followed by a number of fermentation processes using the above-mentioned media for fermentation. Upon completion of the fermentation processes the bacterial cells are harvested and proceed to the concentration step.

3. *Concentration and mixing with cryoprotectants.* The bacterial cells are harvested and concentrated by centrifugation using a separator. The concentrated bacterial cells are then mixed with cryoprotectants. The cryoprotectants used are mainly carbohydrates and amino acids that are safe and suitable for human consumption.

4. *Freezing into pellets.* The bacterial cell suspension mixture is frozen into pellets.

5. *Freeze-drying.* The frozen pellets are lyophilized resulting in very low water activity and ensuring stability of the culture. The freeze-dried granules may be ground to a powder and blended with excipients to a standardized cell count and sold as an individual product. The powder may also be blended with other strains and excipients before it is filled into the appropriate product forms such as capsules, tablets or sticks.

2.3 Analytical Program and Product Specifications

B. lactis BB-12® Product Information sheet is attached in Appendix 1.

Production batches of *B. lactis* BB-12® are thoroughly tested throughout the production process as described below by identification, viability and Quality Program:

- *Strain characterization.* Strain is characterized by colony and cell morphology. The strains are identified according to the current recognized and accepted taxonomy by appropriate molecular testing techniques. During strain characterization, other valuable characteristics are studied such as temperature tolerance, antibiotic resistance profile, bile sensitivity, immunology and salt tolerance. Genotypically, strain is characterized by DNA fingerprinting and plasmid content.

- *Identification of strain.* An unambiguous identification test is used to confirm the identity of the probiotic strain used by Chr. Hansen before fermentation. The method used is a DNA fingerprinting by pulse-field gel electrophoresis (PFGE).

- *Viability (Total Cell Count (CFU)).* Viability is measured as Colony-forming units per gram (CFU/g) of individual lyophilized bulks, probiotic blends and finished products. Furthermore, a stability program is implemented to document the stability of the final product during shelf life.

- *Microbial purity.* The microbial purity of products is determined in accordance with the product release specification criteria.

- *Quality Program.* Chr. Hansen's extensive Quality Program includes a FSSC 22000 standard and hygienic monitoring program. This program serves to verify the process control of the production facility. It includes testing surfaces of process equipment and air quality to document the cleanliness of production as well as analyzing total aerobic microbial count, and coliform bacteria.

- *Allergen Control.* Chr. Hansen controls 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 GMP and HACCP programs that are FSSC 22000 certified at all of our production sites. 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.

- *Release of product.* Finally, all products are tested and released according to a product release specification to guarantee the identity, total count, and purity of the microorganisms (Table 3). Certificates of Analysis of three non-consecutive batches of *B. lactis* BB-12® are in Appendix 2.

Table 3. Release Specifications for *Bifidobacterium animalis* ssp. *Lactis* BB-12®

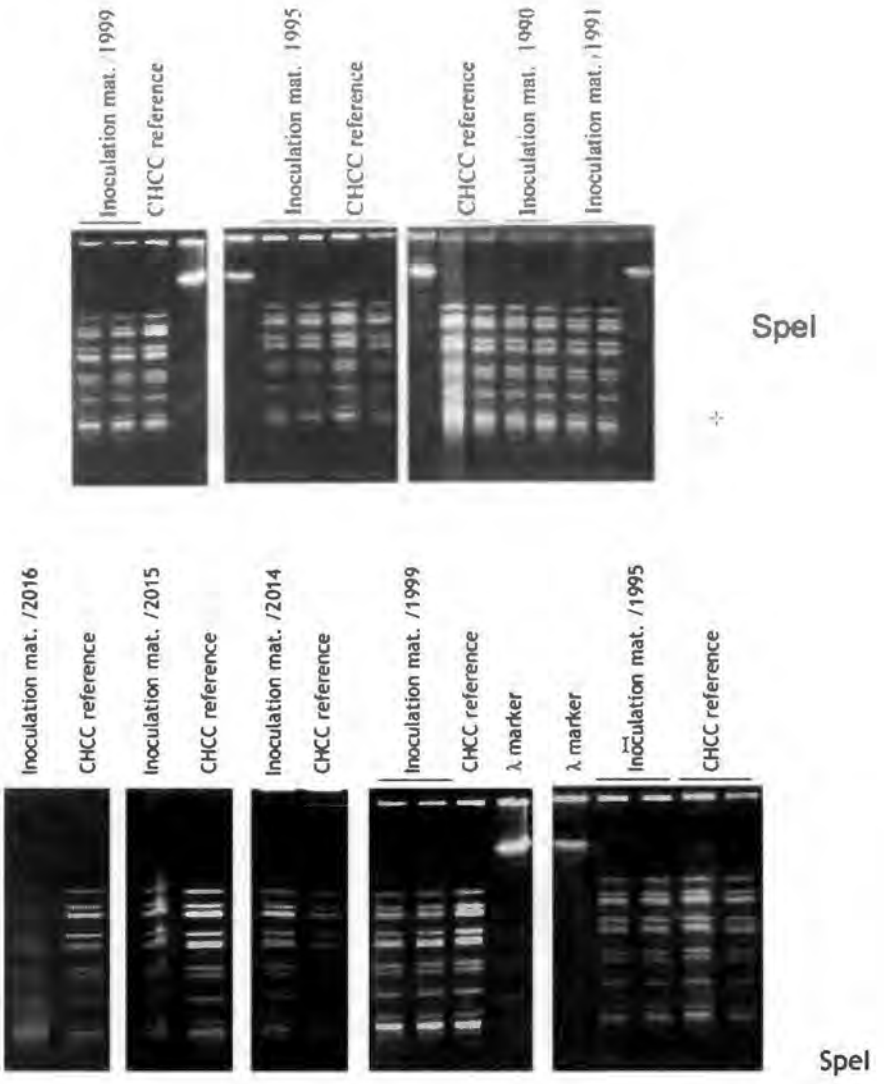
Criterion	Specification	Method
Description	Fine powder	
Color	White to light beige	
Odor	Representative	
Taste	Representative	
Viable Cell Count	≥ $1x10^{11}$ CFU/g	ISO 29981/IDF 220
Microbiological		
Non-Lactic Cell Count	< 500 CFU/g	ISO 13559/IDF 153:2002
Enterococci	<100 CFU/g	NMKL no 68. 2011
Enterobacteriaceae	<10 CFU/g	SOP-03912
Staphylococcus (coagulase +)	<10 CFU/g	SOP-04746
Salmonella	absent	AOAC 2004,03
Listeria	absent	AOAC 2004.06.2008
Molds and Yeast	<10 CFU/g	SOP-02839

2.4 Stability and Viability

2.4.1 Genetic Stability During Storage

Genetic stability of *B. lactis* BB-12® has been demonstrated by DNA fingerprinting comparing the stock culture in the cell bank and various batch of inoculation material produced since 1990 (Figure 2).

Figure 2. Identical DNA Fingerprinting of *Bifidobacterium animalis* ssp *lactis* BB-12® Reference Stock Material (CHCC Reference) and Inoculation Materials Produced Since 1990



2.4.2 Published Studies on *B. lactis* BB-12® Survival and Viability in Various Products

Saarela et al. (2005) investigated the stability of *B. lactis* BB-12® during freeze-drying, storage, and acid and bile exposure. The procedure was performed by using a milk-free culture medium and cryoprotectants (sucrose, betaine, or reconstituted skim milk as control) to produce cells for nonmilk-based applications. For stability studies freeze-dried powders were stored at 37°, 5° and -20°C for 2– 6 months. The sucrose-formulated *B. lactis* BB-12® showed excellent stability during storage at refrigerated (5°C) and frozen temperatures (-20°C) for 5–6 months. During this time the reduction in viability of the cells freeze-dried with sucrose was maximally log 0.4 CFU/g. During an accelerated storage stability test at 37°C, cells freeze-dried with sucrose had good survival (cell numbers within 1.2 log values after 2 months storage). *B. lactis* BB-12® also had a good survival during exposure to pH 3 and 1% bile acids. Betaine proved to be a poor cryoprotectant compared with sucrose.

Simpson et al. (2005) examined the intrinsic tolerance of nine *Bifidobacterium* species to heat and oxygen, and survival following spray drying and during storage. *B. animalis* ssp. *lactis* (including *B. lactis* BB-12®) showed high tolerance to heat (57°C) and moderate tolerance to oxygen. When subjected to spray drying, *B. lactis* BB-12® had survival values ranging from 72% to 79%. In a viability experiment, *B. lactis* BB-12® did not show significant reduction in viability after 30 days of storage at 4°C. At 15°C, *B. lactis* BB-12® had no significant decline after 30 days but a significant decline was recorded by 90 days. At 25°C, the effect was noted after 30 days. The authors concluded that the study identified a group of closely related *Bifidobacterium* species with a distinctive tolerance to heat and oxygen that included the commercial probiotic strain *B. lactis* BB-12®. These species had high initial survival following spray drying and maintained viability during storage at refrigerated temperatures.

The survival of *B. lactis* BB-12® in ice cream was reported by a number of studies. Magarinos et al. (2007) inoculated ice cream with *B. lactis* BB-12® and the product was stored at -25°C for 60 days. *B. lactis* BB-12® showed a logarithmic decrease of 10% at the end of experiment. Akalin et al. (2008) examined the survival of *B. lactis* BB-12® in low-fat ice cream supplemented with oligofructose or inulin, stored at -18°C for 90 days. Freezing process caused a significant decrease in the viability of *B. lactis* BB-12® however, the minimum level of 10^6 CFU/g was maintained for *B. lactis* BB-12® during storage only in ice cream with oligofructose. Homayouni et al. (2008) evaluated the survival of free or encapsulated *B. lactis* BB-12® in synbiotic ice cream containing 1% resistant starch, stored at -20°C for 180 days. The viability of the free state *B. lactis* BB-12® decreased from 4.1×10^9 CFU/ml at day one to 1.1×10^7 CFU/ml after 180 days. Encapsulation of *B. lactis* BB-12® with calcium alginate beads raised the survival rate 30% during the same storage duration and condition.

The stability and survival of *B. lactis* BB-12® in various food products have also been reported. In non-fermented frozen vegetarian (soy) dessert, *B. lactis* BB-12® survived the 6-month storage trial at population of 10^7 CFU/g or greater. The target number of viability in this study after the storage condition was 10^6 CFU/g (Heenan et al. 2004). In milk, *B. lactis* BB-12® produced in different ways (variables being fermentation time, pH during drying, and cryoprotectant) had comparable stability, whereas in juice, sucrose-protected cells survived better than reconstituted skim milk-protected cells. The acid and bile tolerance were better in cells added to milk compared with those in phosphate buffered saline or juice. Despite good culturable stability in milk, the acid and bile tolerance of cells decreased during the storage (Saarela et al. 2006). In cream cheese, the viable count of *B. lactis* BB-12® remained above the desirable 10^6 CFU/g in all the trial during the period evaluated (8°C ± 0.5 for 45 days) (Alves et al. 2013). In a fermented soy product, the effect of inulin and okara on *B. lactis* BB-12® viability was investigated throughout 28 days of storage at 4°C. Population of *B. lactis* BB-12® remained above 8 log CFU/g between the first day and 28th day of storage in different soy products. The addition of inulin and okara flour in fermented soy product did not influence the probiotic viability during the storage period (Bedani et al. 2013).

Part 3

Part 3. Dietary Exposure

B. lactis BB-12® is intended to be added as probiotic microorganism to a variety of conventional foods consistent with current good manufacturing practices (cGMPs). It is intended to be consumed by the general population. Intended applications include but are not limited to the following: milk and dairy products, such as yogurt and other fermented milk products; dairy alternatives (fermented oat milk, fermented soy milk, fermented almond milk, fermented coconut milk); beverages such as juice and protein shakes; shelf-stable products such as bars (granola bars, protein bars, meal replacement bars); confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings); and breakfast cereals (RTE and hot).

To allow for loss of viability over time and to ensure at least $5x10^{10}$ CFU/serving in conventional food products shelf life, the initial addition level of *B. lactis* BB-12® may be as high as $5x10^{11}$ CFU/serving.

The number of *B. lactis* BB-12® in conventional foods and in supplement forms will decline over the shelf-life since it will not proliferate in the products to which it is added. In a number of products, *B. lactis* BB-12® is expected to be present at concentration of 10^{8} to 10^{10} CFU/serving at the time of consumption. The maximum ingestion of *B. lactis* BB-12® through conventional foods is likely to be less than 10^{11} CFU/day based on the assumption that the average consumption of a healthy individual is approximately 20 servings of all combined food per day. Intake of 10^{11} CFU/day would be achieved by those who consume 10 servings of food containing 10^{10} CFU of *B. lactis* BB-12® per day.

Part 4

Part 4. Self-limiting Levels of Use

B. lactis BB-12® does not have any self-limiting intake levels under the conditions of use described in this GRAS notification, other than it is restricted to applications that can sustain living *B. lactis* BB-12® for the intended level throughout the shelf life of the product.

Part 5

Part 5. Experience Based on Common Use in Food Before 1958

The basis for the GRAS conclusion for *B. lactis* BB-12® is based on scientific procedures and not based on common use in food before 1958.

Part 6

Part 6. Narrative

6.1 History of Safe Use and Recognition of Safety by Regulatory Authorities

6.1.1 History of Consumption of *B. animalis* ssp. *lactis* BB-12®

B. lactis BB-12® is a strain that was specially selected by Chr. Hansen for the production of probiotic dairy products. It has been used in infant formula, dietary supplements, and fermented milk products worldwide and is clinically very well documented. This strain is technologically well suited, expressing fermentation activity, high aerotolerance, good stability and a high acid and bile tolerance. Furthermore, *B. lactis* BB-12® does not have adverse effects on taste, appearance or on the mouth feel of the food and is able to survive in the probiotic food until consumption (Jungersen et al. 2014).

Lactic acid bacteria, including bifidobacteria, are consumed in enormous quantities primarily through consumption of fermented foods. It has been reported that *B. animalis* ssp. *lactis* is the most common *Bifidobacterium* utilized as a probiotic in commercial dairy products in North America and Europe (Barrangou et al. 2009; Briczinski et al. 2009). Morgensen et al. (2002) estimated that the average European ingests about $2.2x10^{12}$ lactic acid bacteria/year, which is equivalent to $6x10^{9}$ lactic acid bacteria/day. They also reviewed 54 cases of endocarditis in which lactic acid bacteria were isolated and none of these isolates was a *Bifidobacterium*. Sanders (2006) reported that *Bifidobacterium* species, including *B. animalis* ssp. *lactis*, have a long history of safe use where no cases of clinical infection have been reported. A Medline search of "*Bifidobacterium*" and "sepsis" conducted by Hammerman et al. (2006) that went back 15 years yielded once case of sepsis caused by *Bifidobacterium longum* in a 19 years old man after acupuncture. The subject had not ingested probiotics and completely recovered within 10 days.

No serious adverse events have been reported in vulnerable populations, such as preterm and full-term infants, pregnant and lactating women and hospitalized patients. In these populations, *B. lactis* BB-12® has been consumed in daily dosages ranging from approximately 0.1 to 100 billion colony forming units (cfu). Supplementation periods have ranged from 2 weeks to 12 months. The dosage forms have been milk powder, dairy products or dietary supplements in the form of capsules. There were no adverse events have been reported in healthy populations as well. In this population, *B. lactis* BB-12® has been consumed in daily dosages ranging from 0.1 billion to 50 billion cfu. Supplementation periods have ranged from 1 week to 7 months. The dosage forms have been milk powder, dairy products or dietary supplements in the form of capsules.

6.1.2 Safety Evaluations of *Bifidobacterium animalis* ssp. *lactis* BB-12® by Authoritative Bodies

In Europe, strains belonging to the species *Bifidobacterium animalis* have been granted Qualified Presumption of Safety (QPS) status in 2008 (EFSA, 2010). The QPS concept was developed in 2007 to provide a harmonized generic pre-evaluation to support safety risk assessments of microorganisms intentionally introduced into the food chain. The identity, body of knowledge, safety concerns and antimicrobial resistance of valid taxonomic units were assessed. The QPS status is given if the taxonomic group does not raise safety concerns or, if safety concerns exist, can be defined and excluded. The list of QPS recommended biological agents is updated annually, *Bifidobacterium animalis* has remained valid up to the latest 2018 list (EFSA BIOHAZ Panel, 2017).

In Denmark, *B. lactis* BB-12® has obtained approvals by the Danish Veterinary and Food Administration for use in food products and by the Danish Medicines Agency for use as a natural remedy/ herbal medicinal product. In other countries in Europe, *B. lactis* BB-12® is approved by the Swedish, Polish and Austrian authorities as one of the probiotic bacteria in a medicinal product.

In the USA, FDA stated that the agency had no questions regarding a GRAS notice submitted by Nestle USA for the use of *B. lactis* BB-12® and *Streptococcus thermophilus* strain Th4 as ingredients in milk-based infant formula intended for consumption by infants four months and older at levels not to exceed good manufacturing practice (GRN 49, 2002). At the time of the GRAS notice and the FDA response, neither Nestle nor the agency was aware that strain *B. lactis* BB-12® harbors the *tet(W)* gene encoding for resistance to tetracycline. Nestle convened an expert panel which concluded, based on consideration of the distribution of *tet(W)* in food and microbes, the potential for gene transfer, antibiotic susceptibility, and clinical consequences of exposure to the *tet(W)* gene, that the presence of the gene in *B. lactis* BB-12® has no impact on the safety of the bacterium for its intended use. The agency accepted this determination with no questions (FDA, 2005).

The safety of B. *lactis* BB-12® was further evaluated using the decision tree of Pariza et. al. (2015). Based on the outcome of the decision tree for determining safety of microbial cultures for consumption by human and animals (Appendix 3), including strain characterization, genome sequencing, screening for undesirable attributes and metabolites, and experimental evidence of safety by appropriately designed safety evaluation studies. Chr. Hansen concluded that *B. lactis* BB-12® is non-pathogenic, non-toxigenic and is safe for use as a probiotic microorganism in the foods and beverages listed in this notification.

6.2 Risk Assessment of the Consequences of *tet*(W) gene in *B. animalis* ssp. *lactis* BB-12®

6.2.1 Direct Consequence of *tet*(W) gene

The presence of antibiotic resistance in probiotic bacteria is controversial due to potential direct and indirect consequences to safety. A direct consequence of tetracycline resistance is that this antibiotic cannot be used to cure any infections caused by *B. lactis* BB-12® However, since *B. lactis* BB-12® has a long history of safe use with over 30 years without a single reported case of infection, the risk of acquiring a *B. lactis* BB-12® infection where subsequent treatment failure is caused by the presence of *tet*(W) is considered to be negligible. In the hypothetical event that *B. lactis* BB-12® were to cause an infection, this could be treated with any number of other antibiotics to which *B. lactis* BB-12® is susceptible. Antibiotics which are highly active against *Bifidobacterium* and which are used for treating infections caused by anaerobes include amoxicillin (alone or with clavulanic acid), imipenem, clindamycin, and cefofitin (Moubareck et al. 2005).

6.2.2 Indirect Consequence of *tet*(W) gene

An indirect consequence of the presence of the *tet*(W) gene is that it could be transferred to other microorganisms rendering them resistant to tetracycline. However, due to the structure of the chromosome around *tet*(W) in *B. lactis* BB-12®, which is described below, such transfer is considered to be highly unlikely.

At least 38 different genes conferring resistance to tetracycline identified in Gram-positive and Gram-negative bacterial species, and some of these are widespread in nature (Roberts, 2005). In a study by Villedieu et al. (2003), tetracycline resistant bacteria represented 11% of the total cultivable microflora in saliva and plaque samples from 20 healthy adults. In almost every case, the resistance could be correlated with the presence of an acquired *tet* gene. Thus, acquired tetracycline resistance is extremely common in the human microflora. All *B. animalis* ssp *lactis* strains described to date in the scientific literature are resistant to tetracycline.

There are two major classes of acquired resistance to tetracycline (Roberts, 2005). One type of resistance is caused by a ribosome protection mechanism whereby the ribosome is altered so tetracycline cannot reach its active site. The other type of resistance is caused by an increased efflux of tetracycline from the cell whereby the internal concentration of tetracycline does not reach levels sufficient to inhibit cell growth. Genes encoding ribosome protection include *tet*(M) and *tet*(W) while genes giving increased efflux include *tet*(A) and *tet*(K). Resistance to tigecycline, the critically important antibiotic, is attributed to increased efflux of tetracycline (Alekshun and Levy, 2007, WHO list). Tigecycline is effective against microbes containing ribosome protection proteins such as *tet*(M) (Livermore, 2005) and we have

confirmed in our own laboratories that the *tet*(W) gene in *B. lactis* BB-12® does not confer resistance to tigecycline.

The *tet*(W) gene which encodes a ribosome protection protein was first described in *Butyrivibrio fibrisolvens* (Barbosa *et al.*, 1999) where it resides on a conjugative transposon (Melville *et al.*, 2004). It has subsequently been described in a number of other genera including *Bifidobacterium*. The *tet*(W) gene is quite conserved while the flanking regions are quite different between genera. Transferability is dependent on the structure of the surrounding DNA sequences. Because the genome sequence of *B. lactis* BB-12® has been determined, the exact genetic structure in the vicinity of the *tet*(W) gene is known. There are no plasmids in *B. lactis* BB-12® and *tet*(W) is located on the chromosome. The GC content of the *tet*(W) gene is 53% whereas the overall GC content of the *B. lactis* BB-12® genome is 60%. DNA flanking the *tet*(W) gene has a GC content around 53% indicating that the event that led to the acquisition of *tet*(W) involved additional DNA. Upstream of *tet*(W) is a transposase gene and a short gene of unknown function both with the same GC content as *tet*(W). The gene following the gene of unknown function encodes GMP synthase and has the same high GC content as the rest of the *B. lactis* BB-12® chromosome. The genes downstream of *tet*(W) have GC contents that suggest they are not part of the acquired DNA. Thus, *B. lactis* BB-12® appears to have acquired three genes from an unknown donor strain. No insertion sequences or remnants of insertion sequences are detected downstream of *tet*(W) indicating that *tet*(W) is not part of a transposon. Moreover, these flanking regions were present in all B. animalis subsp. lactis in the NR NCBI database with 100 coverage and 99-100% identity suggestion this area to be conserved in the subspecies.

Transfer of *tet*(W) from one strain to another has been described in several organisms. These include: *Butyrivibrio fibrisolvens* where the gene is part of a conjugative transposon (Melville et al. 2004; 10^{-2} -10^{-5} transconjugants/donor); *A. pyogenes* where it is associated with an origin of transfer and a mobilization system (Billington et al. 2002; 10^{-9} - 10^{-11} transconjugants/donor); and *B. longum* strain F8 where it is located in a structure that resembles a transposon (Kazimierczak et al. 2006, $< 2 \times 10^{-7}$ transconjugants /recipient). The transfer from *B. longum* was to a *Bifidobacterium adolescentis* strain and analysis of the transconjugants showed they had a site-specific integration of *tet*(W) into a region of the *B. adolescentis* chromosome which is identical to the corresponding region of the *B. longum* chromosome. Thus, it is very unlikely that this mechanism of transfer would be able to transfer *tet*(W) to species that were not members of genus *Bifidobacterium*.

No evidence of transferability of the *tet*(W) could be obtained from the following studies: *Rothia* sp. (Villedieu et al. 2007; $< 10^{-9}$ transconjugants/donor); *A. pyogenes* (Billington et al. 2006, $< 5 \times 10^{-10}$ transconjugants /donor); *Bifidobacterium* species not specified (Aires et al. 2007; detection limit not specified); *B. longum* (Florez et al. 2006,

detection limit not specified); *S. ruminantium* (Scott et al. 2000, detection limit not specified); *M. multiacidus* (Scott et al. 2000, detection limit not specified); *Roseburia* sp. (Kazimierczak et al. 2006, detection limit not specified); *Clostridium* sp. (Kazimierczak et al. 2006, detection limit not specified); *B. thermophilum* (Mayrhofer et al. 2007, detection limit not specified); *B. pseudolongum* (Mayrhofer et al. 2007, detection limit not specified) and *B. animalis* ssp *lactis* (Masco et al. 2006, detection limit not specified). Several studies have investigated if the *tet*(W) gene in *B. lactis* in general and specifically in the *B. lactis* BB-12® strain is transmissible and no evidence of transmissibility has been shown for the *tet*(W) gene (Masco *et al.* 2006; Saarela *et al.* 2007; Gueimonde *et al.* 2010; IPLAIC4 in this reference is the *B. lactis* BB-12® strain Raeisi *et* al. 2018; Polit et. al. 2018). Moreover, since *B. lactis* BB-12® does not contain any plasmids and no bacteriophages are known to infect the species, the risk of transfer of *tet*(W) to other microorganisms is negligible.

In addition, unpublished results from Chr. Hansen's research laboratories failed to show any transfer of *tet*(W) from *B. lactis* BB-12® to various recipients (detection limits varied from 10^{-7} to 10^{-9} transconjugants/donor).

Thus, although the *tet*(W) gene is acquired by a number of microorganisms, transfer from these microorganisms to others does not normally occur. In the few cases where transfer has been shown to occur, it was associated with genetic elements which are likely to mediate this transfer. These genetic elements are not present in *B. lactis* BB-12®.

In order for a genetic transfer to occur, direct contact between the donor and recipient is required. The most relevant habitat for *B. lactis* BB-12® is the gastrointestinal tract of humans and other mammals; high levels are also found in some probiotic dairy products. High levels of this strain have not been described in other environments. In a study to investigate the potential role of exposure of *Bifidobacterium* isolates to acid and bile stress on the transfer of a *tet*(W) gene, to *Enterococcus faecalis*, No *E. faecalis* transconjugants were obtained after mating with either stressed or unstressed *Bifidobacterium* (Polit, et. al. 2018). Thus, it is highly unlikely that *B. lactis* BB-12® can transfer *tet*(W) to microorganisms which are not normal inhabitants of the GI tract, normally found in food products, nor because of exposure to gastrointestinal stresses.

An alternative method of gene transfer is transduction which is mediated by bacteriophage. Bacteriophage attacking *B. animalis* subsp *lactis* have not been described. Bacteriophage are normally quite specific in their host ranges so even if the bacteriophage do exist, they are not likely to interact with any organisms outside the species *B. animalis*. Thus, it is considered extremely unlikely that transduction would be a mechanism which would mediate transfer of *tet*(W) to any other species.

6.2.3 Consequences of a Potential Transfer of *tet*(W) gene

Even though it is considered very unlikely that *tet*(W) can transfer from *B. lactis* BB-12® to other microbes, it is important to consider the consequences of such potential transfer. Based on the *in vitro* experiments that have been done and the genetic structure in the vicinity of *tet*(W) in the *B. lactis* BB-12® chromosome, it is estimated that the frequency of transfer will be considerably less than 10^7 transconjugants/donor. *B. lactis* BB-12® does not colonize the GI tract so the number of cells present at any given time depends on the consumption of products containing *B. lactis* BB-12® (Larsen *et al.*, 2006). A normal level of *B. lactis* BB-12® in the GI tract of a person consuming a product containing *B. lactis* BB-12® would be in the range of 10^6 to 10^8 viable cells per gram of feces. Thus, the number of transconjugants which could theoretically arise would be considerably less than 0.1 to 10 per gram of feces. This low number of transconjugants must be compared to the number of tetracycline resistant bacteria that are already present in feces.

In a recent survey of *Escherichia coli* in stool samples from infants, 12% were resistant to tetracycline and levels up to 10^9 tetracycline-resistant *E. coli* per gram of feces were reported (Karami et al. 2006). In a survey done in 1983, 67% of clinical *Bacteroides fragilis* isolates were resistant to tetracycline (Tally et al. 1985). This corresponds to a level of more than 10^9 tetracycline-resistant *Bacteroides fragilis* per gram of feces. Tetracycline resistance is also very common among *Enterococcus* isolated from various sources (Aarestrup et al. 2000; Aarestrup et al. 2002; Marcovei and Zurek, 2006) including humans. Tetracycline resistance levels greater than 60% were found which corresponds to up to 10^8 tetracycline-resistant *Enterococcus* per gram of feces.

The oral cavity contains a large number of bacteria and it is estimated that we swallow close to 10^{11} bacteria every day (Wilson, 2005). Among the approximately 50% which can be cultivated, 11% are resistant to tetracycline (Villedieu et al. 2003) corresponding to approximately 10^{10} tetracycline resistant bacteria being swallowed every day. During the consumption of foods containing *B. lactis* BB-12® there is a transient exposure to material containing up to 10^9 *B. lactis* BB-12® per gram in the oral cavity. Even if BB-12® were present for sufficient time for a conjugation event to occur, the resulting tetracycline resistant bacteria would represent an exceedingly small proportion of the tetracycline resistant bacteria already present in the oral cavity.

This survey of the frequency of tetracycline resistance among various bacterial species is far from exhaustive but illustrates that there is a very high level of tetracycline resistance among the bacteria composing the normal human microflora. The contribution of a hypothetical transfer of *tet*(W) from *B. lactis* BB-12® is vanishingly small in comparison. Even if the

assumed transfer level were several orders of magnitude higher, the contribution would still be extremely small compared to what is already present.

6.2.4 Conclusion of Risk Assessment of *tet*(W) gene

In conclusion, *B. lactis* BB-12® has been used as a food supplement and as part of probiotic food products for over 30 years without a single reported case of infection. Since *B. lactis* BB-12® is sensitive to a number of antibiotics normally used to treat infections, the direct risk posed by the presence of *tet*(W) in *B. lactis* BB-12® is considered to be negligible. Indirect effects can occur if the *tet*(W) gene is transferred to another microorganism and that microorganism causes a health effect which cannot be cured by the use of tetracycline. Based on the genetic structure in the vicinity of the *tet*(W) gene in the *B. lactis* BB-12® chromosome, such transfer is considered to be extremely unlikely. The consequences of a potential transfer to organisms which *B. lactis* BB-12® is likely to encounter are assessed and found to be negligible in comparison to the high level of bacteria already present in the human body which are tetracycline resistant. Since the likelihood of an adverse event caused by the presence of *tet*(W) in *B. lactis* BB-12® is assessed to be extremely low and the consequences of such adverse events assessed to be negligible; it is concluded that *B. lactis* BB-12® containing the *tet*(W) gene does not pose any significant health risk and is safe under the intended conditions of use proposed in this GRAS notification.

6.3 Clinical Studies Evaluating Safety of *Bifidobacterium animalis* ssp. *lactis* BB-12®

6.3.1 Studies of *Bifidobacterium animalis* ssp. *lactis* BB-12® in Infants

Isolauri et al. (2000) investigated the potential of probiotic to control allergic inflammation at an early age in a randomized, double blind, placebo-controlled trial. The probiotics used were *B. lactis* BB-12® ($1x10^9$ CFU/g formula powder) or *L. rhamnosus*, LGG® ($3x10^8$ CFU/g). A total of 27 infants (mean age 4.6 months) who manifested atopic eczema were randomized to receive *B. lactis* BB-12® (n=9), LGG® (n=9), or placebo (n=9) in extensible hydrolyzed formula; the intervention was conducted for 2 months. The severity of atopic eczema, growth, concentration of circulating cytokines and urinary concentration of methyl-histamine and eosinophilic protein X were determined. Infants received probiotic formula showed significant improvement in skin condition, in parallel with a reduction in the concentration of soluble CD4 in serum and eosinophilic protein X in urine. No adverse events were reported in the study. The authors concluded that specific strain of probiotic may counteract inflammatory responses beyond the intestinal milieu and probiotic approach may offer a new direction in the treatment and prevention of allergy.

Kankaanpää et al. (2002) investigated whether the positive outcome of probiotic in alleviating allergic symptoms would be associated with the differential absorption and

utilization of dietary polyunsaturated fatty acid (PUFA). The study involved 15 infants (4-8 months old) referred to a pediatric clinic on the basis of atopic eczema. Through a randomized double-blind study design, the infants were divided into 3 groups of feeding: extensively hydrolyzed formula supplemented with *B. lactis* BB-12® ($1x10^9$ CFU/g formula powder; n=5); the same formula supplemented with *L. rhamnosus*, LGG® ($3x10^8$ CFU/g; n=5); or non-supplemented control (n=5). Blood samples were collected at the first clinical examination before the start of the study and at subsequent clinical examinations. In plasma neutral lipids, α-linolenic acid proportions were reduced by the probiotic supplementation. In phospholipids, LGG® formula did not influence α-linolenic acid proportions, while *B. lactis* BB-12® formula increased the proportion of α-linolenic acid. The authors concluded that some physiological effects of probiotics may be associated with physiological interactions between probiotics and dietary PUFA. No adverse events were reported in the study.

Along the line with a relationship between probiotic and allergic inflammation, Kirjavainen et al. (2002) conducted a randomized, placebo-controlled trial to assess whether the efficacy of bifidobaterial supplementation in the treatment of allergy relate to modulation of intestinal microbiota. The study included 21 infants with early onset atopic eczema of whom 8 were intolerant to extensively hydrolysed whey formula (EHF, referred to as the highly sensitized group), and 13 were tolerant (sensitized group). In the sensitized group, 7 were weaned to EHF with *B. lactis* BB-12® supplementation ($1x10^9$ cfu *B. lactis* BB-12®/ g; the mean daily intake of *B. lactis* BB-12® was $8x10^{10}$ CFU/kg body weight) and 6 to EHF without probiotic. At the end of intervention, infants in the highly sensitized group had greater numbers of lactobacilli/enterococci than those in the sensitized group. Serum total IgE concentration correlated directly with *E. coli* counts in all infants and with bacteroides counts in the highly sensitized group. Probiotic supplementation decreased the numbers of *E. coli* and protect against an increase in bacteroides number. The authors concluded that bifidobacterial supplementation appears to modify the gut microbiota in a manner that may alleviate allergic inflammation. No adverse events were reported in the study.

In a randomized, double-blind, placebo-controlled study to evaluate the efficacy of *B. lactis* BB-12® in preventing acute diarrhea in infants (Chouraqui et al. 2004), 90 healthy infants (sex ratio 1:1) age <8 months received either a biologically acidified infant formula supplemented with viable *B. lactis* BB-12® (10^6 CFU/g powder, equivalent to $1.5x10^8$ CFU/L; n=46) or a commercial, non-acidified formula (n=44; control). The infants received at least 10^8 cfu of bifidobacteria per day, depending on the volume ingested for the duration of their stay in the residential childcare centers (at least 4 months). Fewer infants receiving *B. lactis* BB-12® experienced acute diarrheal disease during the study compared to control, but the difference was not statistically significant. There was a statistically insignificant trend for shorter episodes of diarrhea in the *B. lactis* BB-12® group. Feeding infants with *B. lactis* BB-12® reduced their risk of getting diarrhea by a factor of 1.9. Analysis of the cumulative incidence of diarrheal

episodes showed a trend that the first onset of diarrhea occurred later in the *B. lactis* BB-12® group. All infants tolerate the formulas well. Adequate growth was recorded in all infants, with no difference between groups. There were no serious adverse effects associated with either formula. The only clinical problems noted were spitting and regurgitation, which occurred in 11% of the infants receiving *B. lactis* BB-12® and 13% of those receiving control formula.

The tolerance and safety of long-term consumption of probiotics was evaluated by Saavedra et al. (2004) in a prospective, double-blind, randomized, placebo-controlled study of healthy infants aged 3-24 months. Two levels of *B. lactis* BB-12® and *Streptococcus thermophilus* were tested: the high supplement contained $1x10^7$ cfu of each *B. lactis* BB-12® and *S. thermophiles*/ g formula powder; and the low-supplement contained $1x10^6$ cfu of both probiotics/ g of formula powder. One hundred eighteen infants (58 male, 60 female) were randomized to receive non-supplemented formula (control, n=40), standard milk-based formula containing high supplement (n=39), or low supplement (n=39) for the duration of 18 months to identify any adverse effects, and to examine the effects on growth, general clinical status, and intestinal health. The supplemented formulas were well accepted and were associated with a lower frequency of reported colic or irritability ($p < 0.001$) and a lower frequency of antibiotic use ($p < 0.001$) compared to the non-supplemented formula. There was no significant difference in the frequency of reporting of loose stools, fever and vomiting associated with loose or watery stools, or discomfort passing bowel movements. The frequency of reporting of colic or irritability was significantly lower in both supplemented groups than in the placebo group. There were no significant differences between groups in growth, health care attention seeking, daycare absenteeism, or other health variables. The authors concluded that long-term consumption of formulas supplemented with *B. lactis* BB-12® and *S. thermophilus* was well tolerated and safe.

Weizman et al. (2005) investigated the effect of *B. lactis* BB-12® and *Lactobacillus reuteri* in preventing infections in healthy infants attending child care centers. The participants (n=201), age 4-10 months, were similar regarding gestational age, birth weight, gender, and previous breastfeeding. The infants were assigned randomly to formula supplemented with *B. lactis* BB-12® (n=73), *L. reuteri* (n=68), or non-supplemented control (n=60) for the duration of 12 weeks. Compared to probiotic group, the control group had significantly more febrile and diarrhea episodes, and longer duration of diarrhea. The *L. reuteri* group, compared with *B. lactis* BB-12® or controls, had a significant decrease of number of days with fever, clinic visits, child care absences, and antibiotic prescriptions. Rate and duration of respiratory illnesses did not differ significantly between groups. Adverse effects were not noticed in any of the participants. Throughout the study, growth parameters (weight, length, and head circumference) were satisfactory, with no significant differences between groups. All the other secondary outcome measures pertaining to behavior and stooling parameters did not reveal any significant differences between groups. There were no cases of bloody stools. The authors

concluded that infants fed a formula supplemented with *B. lactis* BB-12® or *L. reuteri* had fewer and shorter episodes of diarrhea, with no effect on respiratory illnesses.

The effect of *B. lactis* BB-12® on intestinal microbiota of preterm infants were examined by Mohan et al. (2006) in a randomized, double blind, placebo-controlled trial involving 69 preterm infants born with a gestational age of <37 week. The infants were randomized into the placebo (n=32) or the *B. lactis* BB-12® (n=37) group. The probiotic group received $1.6x10^9$ cfu of *B. lactis* BB-12® on day 1 to 3 and $4.8x10^9$ cfu from day 4 onward. The administration of the study preparation started on the first day after birth and continued for 21 days. The study ended at the 35th day after birth or when the infant was discharged from the hospital, if earlier. Fecal samples were collected as fresh as possible during the study period and both culture-dependent and culture-independent methods were used to study the gut microbiota. Bifidobacterial numbers were significantly higher in the probiotic than in the placebo group. The infants supplemented with *B. lactis* BB-12® also had lower viable counts of *Enterobacteriaceae* and *Clostridium* spp. than the infants in the placebo group. *B. lactis* BB-12® did not reduce the colonization by antibiotic-resistant organisms in the study population. No adverse effect was observed in any of the infants supplemented with *B. lactis* BB-12®.

The results of the above study on body weight, fecal pH, acetate, lactate, calprotectin, and IgA in the same population of preterm infants were reported in 2008 (Mohan et al. 2008). In antibiotic-treated infants, probiotic supplementation resulted in a higher body weight and the fecal pH was significantly lower compared with placebo. The fecal concentrations of acetate and lactate were 42% and 38% higher, respectively, in the probiotic group than in the placebo group. Fecal calprotectin was lower in the probiotic group, while fecal IgA was higher in this group compared with the placebo group. The authors concluded that dietary supplementation of preterm infants with *B. lactis* BB-12® starting early after birth led to an increase in fecal acetate, lactate, and total fecal IgA and to a decrease in fecal calprotectin. A significantly higher body weight in response to probiotic supplementation was only observed in infants treated with antibiotics.

Rautava et al. (2006) evaluated whether probiotics might promote mucosal immunologic maturation in formula-fed infants. Eighty-one healthy term infants who needed artificial feeding before the age of 2 were randomized to receive infant formula supplemented with either $1x10^{10}$ cfu of each *B. lactis* BB-12® and *L. rhamnosus* LGG®/ g formula powder (n=32) or placebo (n=40) daily until the age of 12 months. The follow-up was completed by 72 of 81 infants enrolled. Blood samples were obtained at the ages of 3, 7, and 12 months. At 12 month of age, the serum concentrations of sCD14 were higher in infants receiving probiotics compared to infants receiving placebo. Administration of the probiotics *B. lactis* BB-12® and LGG® at the time of introduction of cow's milk in the infant's diet resulted in cow's milk–specific IgA antibody responsiveness that may be the result of increased production of sCD14. The authors

concluded that results of the study provide an insight to the mechanisms through which probiotics may promote immunologic maturation in infancy. There were no adverse events reported in the study.

The tolerance and safety of *B. lactis* BB-12® in combination with *Lactobacillus paracasei* ssp. *paracasei* CRL-431 in a prebiotic-containing infant formula was assessed in a randomized controlled trial by Vlieger et al. (2009). A group of 126 healthy term infants (age < 7 days) were randomized to receive a prebiotic (galactooligossacharides)-containing starter formula supplemented with *B. lactis* BB-12® and *L. paracasei* CRL-431 (n=67) or the same formula without probiotics (n=59) for the first 3 months of life. Parents of the infants who completed the first part of the study were asked to continue the use of the study formula for another 3 months. Normal growth occurred in all infants; there were no statistically significant differences for weight gain, length and head circumference. 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, antibiotic use and visits to the general practitioner. No serious adverse events were reported that could be related to the study formula. Adverse events as reported by the parents were vomiting, diarrhea, constipation, colic, and rash. Fewer infants in the probiotics group had developed a rash in the first 3 months (5 vs. 12 in control group; $p<0{\cdot}05$). No differences were seen in other adverse effects between the two groups in both the first and second trimester. The authors concluded that the use of a prebiotic-containing starter formula supplemented with *B. lactis* BB-12® and *L. casei* CRL-431 in early infancy is safe, well tolerated and has no adverse effects on growth and infant behavior.

Taipale et al. (2011) conducted a randomized, double-blind, placebo-controlled study to evaluate the impact of *B. lactis* BB-12® on the risk of acute infectious diseases in healthy newborn infants. One hundred six 1-month-old infants were randomly assigned to receive *B. lactis* BB-12® -containing tablet (n=55) or a control tablet (n=54). The tablets were administered to the infants twice a day (daily dose of *B. lactis* BB-12® was $10x10^9$ cfu) with a novel slow-release pacifier or a spoon from the age of 1–2 months to 8 months. Fecal samples were collected at the age of 8 months. Breastfeeding habits, pacifier use, dietary habits, medications and all signs and symptoms of acute infections were registered, and adverse effects were recorded in detail. No significant differences between *B. lactis* BB-12® and control groups were observed in reported gastrointestinal symptoms, otitis media or use of antibiotics. However, the infants receiving *B. lactis* BB-12® were reported to have experienced fewer respiratory infections than the control infants. No serious adverse effects were detected during the administration period. Two infants receiving *B. lactis* BB-12® withdrew from the study as a result of GI complaints. One infant in the control group was diagnosed with atopic eczema, and his physician recommended the family to discontinue from the intervention. The authors

concluded that controlled administration of *B. lactis* BB-12® in early childhood may reduce respiratory infections.

The effect of *B. lactis* BB-12® on intestinal immunity and inflammation in full term healthy infants was studied by Holscher et al. (2012). A total of 145 six- week-old healthy, full-term infants (2-6 weeks old) were enrolled in a prospective, randomized, double-blind, controlled clinical trial with 2 groups studied in parallel to a breastfed comparison group (n=52). The formula-fed infants were randomized to partially hydrolyzed whey formula (n=43) or the same formula containing 10^6 cfu of *B. lactis* BB-12® per gram of formula powder (n=50) for 6 weeks. Randomization of formula-fed infants was stratified by vaginal vs Cesarean section. The breastfed reference group was exclusively breastfed and followed for 6 weeks starting at 6 weeks of age. Stool samples were collected in each study visit at 2 and 6 weeks; secretory and specific IgA (prior to and following immunization), fecal pH, calprotectin, and lactate were analyzed from stool samples. All infants consuming probiotic increased anti-poliovirus-specific IgA. Among vaginally delivered formula fed infants, probiotic consumption increased fecal IgA. In cesarean-delivered infants probiotic consumption tended to increase anti-rotavirus-specific IgA. One serious event unrelated to study formula occurred (hospitalization for respiratory syncytial virus). The authors concluded that infants consuming formula with *B. lactis* BB-12® produced feces with detectable presence of *B. lactis* BB-12® and augmented sIgA concentration. Cesarean-delivered infants consuming *B. lactis* BB-12® had heightened immune response, as evidenced by increased anti-rotavirus- and anti-poliovirus-specific IgA following immunization.

The effect of *B. lactis* BB-12® on oral colonization of mutans streptococci was evaluated by Taipale et al. (2012) involving 106 infants age 1-2 months old. In this study, tablets containing *B. lactis* BB-12® ($1x10^{10}$ CFU/tablet, n=38), xylitol (n=37), or sorbitol (n=31) were randomly administered to 106 participants using a novel slow-release pacifier or a spoon. The pacifier contained a pouch into which the tablet was inserted. The families were informed that they could receive tablets and pacifiers until the child was 24 months old. The infants visited the health care centers at 1, 8, and 24 months of age; where in the last 2 visits a clinical oral examination was performed and oral microbial samples were collected. The levels of lactobacilli and yeast did not differ between the groups. *B. lactis* BB-12® cell counts barely exceeding the detection limit were found in three of the oral samples of the 8-month-old children; the 2-year samples did not contain *B. lactis* BB-12®. The early administration *B. lactis* BB-12® did not result in permanent oral colonization of this probiotic or significantly affect mutant streptococci colonization in the children. No serious adverse effects were detected during the administration period. Two infants receiving *B. lactis* BB-12® and 1 infant receiving sorbitol withdrew from the study as a result of gastrointestinal complaints. One infant in the xylitol group was diagnosed with atopic eczema, and his physician recommended that the family discontinue the intervention.

A post-trial analysis on *B. lactis* BB-12® on occurrence of dental carries, from the same study population was conducted using data collected on clinical examination and questionnaires at the age of 4 years (Taipale et al. 2013). Of the 106 randomized infants who fulfilled all inclusion criteria and started intervention, 32 of 38 in the *B. lactis* BB-12® group, 33 of 37 in the xylitol group, and 29 of 31 in the sorbitol group completed the 4-year follow up. Mean duration of tablet delivery was 14.9±6.7 months, and this did not differ between the groups. No differences were detected between the study groups in the occurrence of enamel caries or obvious dentinal caries. Administration of *B. lactis* BB-12® in infancy does not seem to increase or decrease the occurrence of caries by 4 years of age in a low-caries population. The authors concluded that, "The result thus suggests that the early administration of BB-12® should be safe with regard to the future dental health of the child.... the early colonization of mutans streptococci and visible plaque in a young child's dentition are strongly associated with the occurrence of caries."

TABLE 4: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN INFANTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Isolauri (2000)	Randomized, double-blind, placebo-controlled trial to evaluate the potential of probiotic to control allergy inflammation at early age.	27 infants (mean age 4.6 months), manifested atopic eczema during breast feeding. BB-12®: 9	$1x10^9$ cfu BB-12®/g; $3x10^8$ cfu LGG®/g formula powder	2 months	Patients given probiotic formula showed significant improvement in skin condition, in parallel with a reduction in the concentration of soluble CD4 in serum and eosinophilic protein X in urine. No adverse events were reported in the study.
Kankaanpaa (2002)	Randomized, double-blind, placebo-controlled trial to investigate if probiotic efficacy in alleviating allergic symptoms is associated with utilization of dietary PUFA.	15 infants (4 – 8 months) with atopic eczema. BB-12®: 5 LGG®: 5	$1x10^9$ cfu BB-12®/g; $3x10^8$ cfu LGG®/g formula powder	2 months	In plasma neutral lipids, α-linolenic acid proportions were reduced by the probiotic supplementation. In phospholipids, LGG® formula did not influence α-linolenic acid proportions, while *B. lactis* BB-12® formula increased the proportion of α-linolenic acid. Some physiological effects of probiotics may be associated with physiological interactions between probiotics and dietary PUFA (polyunsaturated fatty acids). No adverse events were reported in the study.
Kirjavainen (2002)	Randomized, placebo-controlled trial to assess whether the efficacy of bifidobaterial supplementation in the treatment of allergy relate to modulation of intestinal microbiota.	21 infants with early onset atopic eczema. BB-12®: 7	$1x10^9$ cfu BB-12®/g of extensively hydrolyzed whey formula. Mean daily intake: $8x10^{10}$ CFU/kg body weight.	Not clear	Infants in the highly sensitized group (HSG) had greater numbers of lactobacilli/enterococci than those in the sensitized group. Sehrum total IgE concentration correlated directly with *E. coli* counts in all infants and with bacteroides counts in the HSG. Probiotic supplementation decreased the numbers of *E. coli* and protected against an increase in bacteroides number. No adverse events were reported in the study.
Chouraqui (2004)	Randomized, double-blind, placebo-controlled study to evaluate the efficacy of *B. lactis* BB-12® in preventing acute diarrhea in infants.	90 healthy infants (sex ratio 1:1) age <8 months. BB-12®: 46	10^6 cfu BB-12®/g powder, equivalent to $1.5x10^8$ CFU/L, in formula (infants received at least 10^8 CFU BB-12®/day)	At least 4 months (infants received formula for the duration of their stay in the center).	Compared to control group, infants fed BB-12® had less diarrhea and trended towards shorter episodes of diarrhea. No serious adverse events associated with either formula; adequate growth was recorded in all infants. The authors concluded that *B. lactis* BB-12® has some protective effects against acute diarrhea in healthy children.

TABLE 4: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN INFANTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Saavedra (2004)	Prospective, double-blind, randomized, placebo- controlled study to evaluate tolerance to formulas containing 2 levels of probiotics on growth, general clinical status, and intestinal health in healthy infants, and to identify any adverse effects.	118 infants (3 – 24 months). BB-12®: n=39 (high-supplement); n=39 (low-supplement)	High supplement: $1x10^7$ cfu of each BB-12® and *S. thermophiles*/g formula powder; Low-supplement: $1x10^6$ cfu of both probiotics/g of formula powder.	18 months	The supplemented formulas were well accepted and were associated with a lower frequency of reported colic or irritability and a lower frequency of antibiotic use than the non-supplemented formula. There were no significant differences between groups in growth, health care attention seeking, daycare absenteeism, or other health variables
Weizman (2005)	Randomized, double-blind, placebo-controlled trial to investigate the effect of *B. lactis* BB-12® and *Lactobacillus reuteri* in preventing infections in infants attending child care centers.	201 healthy term infants age 4-10-month-old. BB-12®: 73	$1x10^7$ cfu of BB-12®/g of formula powder.	12 weeks	Compared to probiotic group, the control group had significantly more febrile and diarrhea episodes, and longer duration of diarrhea. The *L reuteri* group, compared with *B. lactis* BB-12® or controls, had a significant decrease of number of days with fever, clinic visits, child care absences, and antibiotic prescriptions. Rate and duration of respiratory illnesses did not differ significantly between groups. Adverse effects were not noticed in any of the participants.
Mohan (2006)	Randomized, double blind, placebo-controlled trial to investigate the role of *B. lactis* BB-12® in modifying gut bacteria.	69 preterm infants (<37 gestation week). BB-12®: 37	$1.6x10^9$ cfu of BB-12® on d 1–3 after births and $4.8x10^9$ cfu from day 4 onwards daily in 1 ml solution of water.	21 days	Bifidobacterial numbers were significantly higher in the probiotic than in the placebo group. The infants supplemented with *B. lactis* BB-12® also had lower viable counts of *Enterobacteriaceae* and *Clostridium* spp. than the infants in the placebo group. *B. lactis* BB-12® did not reduce the colonization by antibiotic-resistant organisms in the study population. No adverse effect was observed in any of the infants supplemented with *B. lactis* BB-12®

TABLE 4: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN INFANTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Mohan (2008)	Randomized, double blind, placebo-controlled trial to examine whether the oral application of *B. lactis* BB-12® may improve selected indicators of health status in pre- term infants.	69 preterm infants (<37 gestation week). BB-12®: 37	$1.6x10^9$ cfu of BB-12® on d 1–3 after births and $4.8x10^9$ cfu from day 4 onwards daily in 1 ml solution of water.	21 days	In antibiotic-treated infants, probiotic supplementation resulted in a higher body weight and the fecal pH was significantly lower compared with placebo. The fecal concentrations of acetate and lactate were 42 and 38% higher, respectively, in the probiotic group than in the placebo group. Fecal calprotectin was lower in the probiotic group, while fecal IgA was higher in this group compared with the placebo group. No adverse events were reported in the study.
Rautava (2006)	Double-blind, placebo-controlled trial to evaluate if probiotics might promote mucosal immunologic maturation in formula-fed infants.	81 infants who needs artificial feeding before the age of 2 month. BB-12® and LGG®: 32	$1x10^{10}$ cfu of both BB-12® and LGG® in formula	From study enrollment until the age of 12 month	At 12 months of age, the serum concentrations of sCD14 were higher in infants receiving probiotics compared to infants receiving placebo. Administration of the probiotics LGG® and *B. lactis* BB-12® at the time of introduction of cow's milk in the infant's diet resulted in cow's milk–specific IgA antibody responsiveness that may have been the result of increased production of sCD14. No adverse events were reported in the study.
Vlieger (2009)	Randomized, controlled trial to assess the safety and tolerance of probiotic *B. lactis* BB-12® and *Lactobacillus paracasei* ssp. *paracasei* CRL-431 to a prebiotic-containing infant formula in healthy, term infants.	126 healthy newborn infants (age < 7 days) BB-12® + *L. paracasei* CRL-431 = 67	$1x10^7$ cfu of each BB-12® and *L. paracasei* CRL-431/g of powder formula. Prebiotic: galacto-oligosaccharides, 24 g/100 ml.	6 months	Normal growth occurred in all infants; no statistically significant differences for weight gain, length and head circumference were reported. 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, antibiotic use, visits to the general practitioner and number of adverse events. The use of a prebiotic-containing starter formula supplemented with *L. casei* CRL-431 and *B. lactis* BB-12® in early infancy is safe, well tolerated and has no adverse effects on growth and infant behavior.

TABLE 4: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN INFANTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Taipale (2011)	Randomized, double-blind, placebo-controlled study to evaluate the impact of *B. lactis* BB-12® on the risk of acute infectious diseases.	109 newborn 1-month-old infants. BB-12®: 55	$5x10^9$ cfu of BB-12® in a tablet; 2 tablets were given per day via a slow-release pacifier.	8 months	No significant differences between *B. lactis* BB-12® and control groups were observed in reported gastrointestinal symptoms, otitis media or use of antibiotics. However, the infants receiving *B. lactis* BB-12® were reported to have experienced fewer respiratory infections than the control group infants. No serious adverse effects were detected during the administration period. Two infants receiving *B. lactis* BB-12® withdrew from the study as a result of GI complaints. One infant in the control group was diagnosed with atopic eczema, and his physician recommended the family to discontinue from the intervention.
Holscher (2012)	Randomized, double-blind, placebo-controlled trial to investigate the effect of *B. lactis* BB-12® on intestinal immunity and inflammation.	145, six-week-old, full-term, healthy infants. BB-12®: 50	10^6 cfu BB-12®/g	6 weeks	All infants consuming probiotic increased anti-poliovirus-specific IgA. Among vaginally delivered formula fed infants, probiotic consumption increased fecal IgA. In cesarean-delivered infants probiotic consumption tend to increase anti-rotavirus-specific IgA. One study formula–unrelated serious adverse event occurred (hospitalization for respiratory syncytial virus).
Taipale (2012)	Randomized, double-blind, placebo-controlled trial to study the effect of *B. lactis* BB-12® on oral colonization of mutant streptococci and *B. lactis* BB-12®.	106 infants (age 1-2 months); oral sample collection at age 8 months and 2 years. BB-12®: 38	$1x10^{10}$ cfu of BB-12®/day in tablets, administered in a slow-release pacifier or a spoon.	Between 8 -24 months	The levels of lactobacilli and yeast did not differ between the groups. *B. lactis* BB-12® cell counts barely exceeding the detection limit were found in three of the oral samples of the 8-month-old children; the 2-year samples did not contain *B. lactis* BB-12®. The early administration of *B. lactis* BB-12® did not result in permanent oral colonization of this probiotic or significantly affect mutant streptococci colonization in the children. No serious adverse effects were detected during the administration period.

TABLE 4: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN INFANTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Taipale (2013)	Post-trial analysis on administration of *B. lactis* BB-12® on carries occurrence at four years of age.	106 infants (age 1-2 months); data collected at age 4 years. BB-12®:32	$1x10^{10}$ cfu of BB-12®/day in tablets, administered in a slow-release pacifier or a spoon.	Between 8 -24 months	No differences were detected between the study groups in the occurrence of enamel caries or obvious dentinal caries. Administration of *B. lactis* BB-12® in infancy does not seem to increase or decrease the occurrence of caries by 4 years of age in a low-caries population. No adverse events were reported in the study.

6.3.2 Studies of *B. animalis* ssp. *lactis* BB-12® in Children

Merenstein et al. (2010), in a randomized, double blind, placebo-controlled trial, administered *B. lactis* BB-12® in yogurt-based drinks to 87 healthy children between the age of 1 and 3 years for duration of 90 days. The number of children randomized in the study was 197 of whom 95 of them were allocated as control group. The primary objective was to determine if consumption of a probiotic in yogurt drink decreases absences from daycare due to illnesses. The secondary objective was to determine if probiotic-containing yogurt drink improves overall parental satisfaction due to decreased absences from work and an overall healthier child. The yogurt drink contained a combination of active cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The active drink was supplemented with $1x10^{10}$ cfu of *B. lactis* BB-12®. There were no significant differences in the days of missed school per group throughout the study. Additionally, there were no differences in any secondary outcomes among the groups. Six total adverse events were reported for the study, three in each group, involving 5 study subjects. One subject had both diarrhea and dermatitis at the same time. This participant was in the active group. There were no serious adverse events in either group reported throughout the entire study.

Merenstein et al. (2011), in another randomized, double blind, placebo-controlled trial in a population of children aged 2-4 years investigated the potential of *B. lactis* BB-12® in decreasing absences of children from attending daycare/ school centers. A total of 172 healthy children were randomized, 91 children were allocated to consume a yogurt drink containing $1x10^{10}$ cfu of *B. lactis* BB-12®, and 81 children were allocated to a control group (the same yogurt drink without *B. lactis* BB-12®). The primary outcome, missed days of school because of illness per 100 days, was similar in both the active and control groups. Eleven total adverse events were reported for the study and there were no statistically significant differences between the groups, with 8 in the active group and 3 in control group. Five subjects had diarrhea or loose stools in the active group compared with zero in the control group. There were no serious adverse events in either group reported throughout the entire study. The authors concluded that the probiotic-containing yogurt-based beverage studied did not decrease absences because of illnesses in daycare/school for healthy children ages 2–4 years.

Hojsak et al. (2015) investigated the role of *B. lactis* BB-12® in preventing nosocomial infections in the acute hospital settings. A total of 727 hospitalized children age 1-18 years were randomly allocated to receive placebo (n=365) or *B. lactis* BB-12® (n=362) at a dose of $1x10^{9}$ cfu daily, mixed with water and consumed under pediatric supervision for the duration of hospital stay. Nosocomial infections were defined as infections that occurred more than 48 hours after hospital admission and that were not present at the time of admission. There were no differences in incidence or duration of common nosocomial infections between groups, incidence, duration, and severity of gastrointestinal and respiratory tract infections, duration of hospitalization, and the use of antibiotics. There were no adverse events occurred throughout

the study period. The authors concluded that the use of *B. lactis* BB-12® failed to prevent nosocomial infections in an acute-setting pediatric hospital in children who were more than 1 year of age.

Hojsak et al. (2016) also conducted a randomized, double-blind, placebo-controlled trial to investigate the role of *B. lactis* BB-12® in the prevention of common infections in 201 healthy children who attend day care centers. During the 3-month intervention, the children (median age 4.6 years) were randomly allocated to receive placebo (n=106) or *B. lactis* BB-12® (n=104) in a sachet. The daily dose of *B. lactis* BB-12® was $1x10^9$ cfu, mixed with meal (such as milk, water, yogurt) or spread on a spoon of yogurt and immediately consumed. Parents were asked to fill a diary on a daily basis and were contacted by physicians every 7-10 days. There was no difference observed between probiotic and placebo groups on common infections, duration of symptoms, number of children with gastrointestinal and respiratory tract infections, absence from day care center due to infections, and use of antibiotics. There were no adverse events recorded during the study period. The authors concluded that in the performed study, *B. lactis* BB-12® has no effect on the prevention of gastro intestinal and respiratory tract infections in healthy children who attend day care centers.

In a randomized, double-blind, placebo-controlled trial, Taipale et al. (2016) studied the impact of *B. lactis* BB-12® administration on the risk of acute infectious diseases in healthy children. One hundred and nine 1-month-old infants were assigned randomly to a probiotic group receiving *B. lactis* BB-12® containing tablet ($5x10^9$ CFU/tablet; n=55) or a placebo (n=54). The test tablets were administered to the infants twice daily until the age of 2 years with a novel slow-release pacifier or a spoon. Breastfeeding habits, pacifier use, dietary habits, medication, and all signs and symptoms of acute infections were registered in dairies by parents and in questionnaires by trained professionals. No serious adverse effects were detected during administration period. The infants receiving *B. lactis* BB-12® were reported to have experienced fewer respiratory tract infections than the controls. No significant differences between the groups were observed in reported gastrointestinal symptoms, otitis media, or fever. The authors concluded that administration of *B. lactis* BB-12® in early childhood may reduce respiratory tract infections.

Tan et al. (2017) determined the safety of *B. lactis* BB-12® supplemented yogurt in healthy children through a phase I, double-blinded, randomized controlled study involving 60 children aged 1-5 years. Participants were randomly assigned to consume *B. lactis* BB-12® - supplemented yogurt ($1x10^{10}$ cfu; n=29) or non-supplemented control yogurt (n=31) daily for 10 days. The primary outcome was to assess safety and tolerability, as determined by the number of reported adverse events. A total of 186 non-serious adverse events were reported, with no significant differences between the control and *B. lactis BB-12®* groups. No significant changes due to probiotic treatment were observed in the gut microbiota of the study cohort. The

authors concluded that *B. lactis* BB-12® -supplemented yogurt is safe and well-tolerated when consumed by healthy children.

TABLE 5: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN CHILDREN					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Merenstein (2010)	Cluster-randomized, double blind, placebo-controlled trial to determine if consumption of yogurt containing *B. lactis* BB-12® improves health in children ages 1–3 years attending daycare/ school centers.	183 healthy children (age 1-3 years) attending daycare/ school at least 3 days a week. BB-12®: 87	$1x10^{10}$ cfu of BB-12® / day in strawberry flavored dairy drink.	90 days	There were no significant differences in the days of missed school per group throughout the study. Additionally, there were no differences in any secondary outcomes among the groups (if probiotic-containing yogurt- based drink improves overall parental satisfaction due to decreased absences from work and an overall healthier child). Six total adverse events were reported for the study, three in each group, involving 5 study subjects. One subject had both diarrhea and dermatitis at the same time. This participant was in the active group. There were no serious adverse events in either group reported throughout the entire study.
Merenstein (2011)	Randomized, double blind, placebo-controlled trial to determine if consumption of yogurt containing *B. lactis* BB-12® decreases absences in children 2-4 years attending daycare/ school centers.	172 healthy children (age 2-4 years). BB-12®: 91	$1x10^{10}$ cfu of BB-12® day in strawberry yogurt-based drink.	100 days	The primary outcome, missed days of school because of illness per 100 days, was similar in both the active and control groups. Eleven total adverse events were reported for the study and there were no statistically significant differences between the groups, with 8 in the active group and 3 in control group. Five subjects had diarrhea or loose stools in the active group compared with zero in the control group. There were no serious adverse events in either group reported throughout the entire study.
Hojsak (2015)	Randomized, double-blind, placebo-controlled trial to investigate the role of *B. lactis* BB-12® in preventing nosocomial infections in the acute hospital setting.	727 hospitalized children (aged 1-18 years). BB-12®: 362	$1x10^{9}$ cfu of BB-12® day (mixed with 20 ml water, consumed under pediatric supervision).	For the entire duration of hospital stay	There were no differences in incidence or duration of common nosocomial infections between groups. There was also no difference between intervention and placebo groups on incidence, duration, and severity of gastrointestinal and respiratory tract infections, duration of hospitalization, and the use of antibiotics. There were no adverse events recorded in the patients.

TABLE 5: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN CHILDREN					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Hojsak (2016)	Randomized, double-blind, placebo-controlled trial to investigate the role of *B. lactis* BB-12® in the prevention of common infections in healthy children who attend day care centers.	210 healthy children (median age 4.6 years) who attend day care centers. BB-12®: 104	$1x10^9$ cfu of BB-12®/ day, mixed with meal (milk, water, yogurt, or spread on a spoon of yogurt and immediately consumed).	3 months	No difference was observed between probiotic and placebo groups on common infections, duration of symptoms, number of children with gastrointestinal and respiratory tract infections, absence from day care center due to infections, and use of antibiotics. There were no adverse events recorded during the study period .
Taipale (2016)	Randomized, double-blind, placebo-controlled trial to study the impact of administration of *B. lactis* BB-12® on the risk of acute infectious disease in healthy children.	109 infants (1-month-old) BB-12®: 55	$1x10^{10}$ cfu of BB-12®/ day in tablets, administered in a slow-release pacifier or a spoon.	2 years	The infants receiving *B. lactis* BB-12® were reported to have experienced fewer respiratory tract infections than the controls. No significant differences between the groups were observed in reported gastrointestinal symptoms, otitis media, or fever. No serious adverse effects were detected during the administration period. The authors concluded that administration of *B. lactis* BB-12® in early childhood may reduce respiratory tract infections.
Tan (2017)	Phase I, double-blinded, randomized controlled study to determine safety of *B. lactis* BB-12® supplemented yogurt in healthy children	60 healthy children (1-5 years) BB-12®: 30	$1x10^{10}$ cfu of BB-12®/ day in yogurt drink	10 days	A total of 186 non-serious adverse events were reported, with no significant differences between the control and *B. lactis* BB-12® groups. No significant changes due to probiotic treatment were observed in the gut microbiota of the study cohort. The authors concluded that *B. lactis* BB-12®-supplemented yogurt is safe and well-tolerated when consumed by healthy children.

6.3.3 Studies of *B. animalis* ssp. *lactis* BB-12® in Adults

Sullivan et al. (2003) compared the effect of clindamycin on the intestinal flora in subjects ingesting yogurt with added probiotics in a randomized, double-blind, placebo-controlled trial. A total of 24 healthy subjects (age 21-48 years) received clindamycin daily for 7 days and yogurt containing $1x10^8$ cfu of each *L. acidophilus* NCFB 1748, *B. lactis* BB-12® and *L. paracasei* F19 for 14 days; fecal samples were collected before and after administration of clindamycin. At the end of intervention, the probiotic microorganisms evaluated in this study prevented ecological disturbances in the numbers of intestinal *Bacteroides fragilis* group species during clindamycin administration. One subject developed diarrhea in connection with the study and one subject reported looser stool. Both subjects belonged to the active group and new stool samples were tested for *C. difficile*. No growth of *C. difficile* could be confirmed in the sample from the first subject but the sample was cytotoxin positive. The individual was later treated with metronidazole to be relived from the symptoms. In the second case, neither growth of *C. difficile* nor production of cytotoxin could be verified and the symptoms disappeared spontaneously.

The effects of multispecies probiotics and prebiotic oligofructose (synbotic) on bacterial translocation, gastric colonization, systemic inflammation, and septic morbidity in elective surgical patients were assessed by Anderson et al. (2004). Patients (age 47-80 years, n=137) were enrolled two weeks prior to elective abdominal surgery and randomized to receive the synbiotic (n=72) or placebo (n=65). The synbiotic group received probiotics in a dose of one capsule three times a day (a capsule contains $4x10^9$ cfu of *B. lactis* BB-12®, *L. acidophilus* LA-5, L. *bulgaricus*, and *S. thermophilus*) and a prebiotic (16 g oligofructose powder dissolved in a cupful of water) twice daily. The treatment continued until patients were discharge from hospital. There were no significant differences between the synbiotic and control groups in bacterial translocation, gastric colonization, systemic inflammation, or septic complications. There was no difference in the incidence of septic morbidity between the placebo and synbiotic groups. The most common sites of infection were the urinary tract (32%), respiratory tract (24%), and surgical wound (22%). Fourteen patients (10%) died within 30 days of surgery, of which five were in the placebo group and nine in the synbiotic group (p=0.354). The authors concluded that, "In this study, synbiotics had no measurable effect on gut barrier function in elective surgical patients. Further studies investigating the place of pre- and probiotics in clinical practice are required."

The same synbiotic preparation as the above study was evaluated for its influence on gut barrier function and sepsis in critically ill patients through a randomized controlled trial (Jain et al. 2004). A total of 90 patients admitted to the intensive care unit (ICU) were randomized to receive the synbiotic preparation or placebo (n=45 into each group).

After 1 week of therapy, patients in the synbiotic group had a significantly lower incidence of potentially pathogenic bacteria and multiple organisms in their nasogastric aspirates compared to control. There were no significant differences between the groups in terms of intestinal permeability, septic complications or mortality. The authors concluded that the administration of synbiotic in critically ill patients favorably altered the microbial composition of the upper gastrointestinal tract but had no effect on intestinal permeability and was not associated with measurable clinical benefit.

In a human trial combined with *in vitro* test, Wang et al. (2004) examined the potency of yogurt containing *B. lactis* BB-12® and *L. acidophilus* LA-5 in inhibiting growth of *H. pylori.* As many as 70 adults infected with *H. pylori* were enrolled in the study; 59 received the yogurt with probiotics (mean age 39±10 years; 22 male) and 11 received placebo (mean age 33±9 years; 5 male). The dose of probiotics was $1x10^7$ cfu of each *L. acidophilus* LA-5 and *B. lactis* BB-12®, twice daily in yogurt. The *in vitro* test showed that *B. lactis* BB-12® exerted an inhibitory effect against H. pylori; whereas LA-5 did not show any effect. Administration of yogurt supplemented with probiotics decreased the urease activity of *H. pylori* after 6 weeks of therapy. The authors concluded that regular intake of yogurt containing *B. lactis* BB-12® and *L. acidophilus* LA-5 effectively suppressed *H. pylori* infection in humans. No adverse events were described in the study.

Laake et al. (2005) performed a randomized, double blind, placebo-controlled study to confirm the effect of probiotic on symptoms and endoscopic appearance in ulcerative colitis (UC) patients. Fermented milk containing $1x10^8$ cfu of each *B. lactis* BB-12® and *L. acidophilus* LA-5 was given daily for 4 weeks to 51 UC patients. Stool samples were collected for examination of lactobacilli, bifidobacteria, fungi, and pH, during and after intervention. Endoscopic evaluation was performed before, during, and after intervention. The number of lactobacilli and bifidobacteria increased significantly during intervention; involuntary defecation, and abdominal cramps were significantly decreased in the UC/ileal-pouch-anal-anastomosis (IPAA) group. Blood test, fecal fungi, and fecal pH did not change significantly during intervention. No adverse events were reported in the study. The authors concluded that the effect of probiotics in this study confirmed their previously reported effect of probiotics on clinical symptoms and endoscopic score in a smaller double blind, randomized controlled study.

A dose response study to investigate the effects of *B. lactis* BB-12® and *Lactobacillus paracasei* ssp. *paracasei* (CRL-431) on adult blood lipids and bowel habits was performed by Larsen et al. (2006). In a randomized, placebo-controlled, double-blinded design, 75 healthy adults age 18-40 years were divided into 5 groups and received either placebo or probiotic doses of 10^8, 10^9, 10^{10} or 10^{11} CFU/day. *B. lactis*

BB-12® and CRL-431 were mixed in equal amounts in the desired dose. The study lasted for 7 weeks, with a 2 weeks run-in period, followed by 3 weeks of intervention and 2 weeks wash-out. Blood and fecal samples were collected before the run in, prior and after intervention, and at the end of the wash-out period. Participants recorded their bowel habits daily in a diary throughout the 7 weeks intervention and reported if they experienced adverse events. The fecal recovery of *B. lactis* BB-12® increased significantly with increasing dose, while CRL-431 was not recovered in any of the fecal samples. There was no significant changes in the fecal microbiota composition between the probiotic placebo groups. A significant linear increase in fecal consistency (looser stool) with increasing probiotic dose was observed. No overall dose–response effect was found on the blood lipids. The side effects reported were flatulence (68%), abdominal bloating (37%) and headache (22%; in the run-in period without changes during intervention or wash out). No participants reported adverse side effects during the intervention. The authors concluded that "the increasing dose of probiotics was well tolerated and did not seem to cause any adverse side effects. Increasing dose of probiotics showed an effect on bowel habits, with the observed changes before and after the intervention within a normal range."

In a randomized, placebo-controlled trial, Sheu et al. (2006) tested whether prior treatment with probiotics improved the efficacy of quadruple therapy in eradicating residual *H. pylori* after failed triple therapy. One hundred thirty-eight patients whom have failed in triple therapy were assigned to either a probiotic plus quadruple therapy group (n=69; mean age 46.4; female 31) or a quadruple therapy only group (n=69; mean age 48.9; female 30). The patients received 1 week of quadruple therapy with or without a 4-week pretreatment with yogurt containing probiotics ($1x10^9$ cfu of each *B. lactis* BB-12® *L. acidophilus* LA-5, *L. bulgaricus*, and *S. thermophilus*). The probiotic plus quadruple therapy group had a higher *H. pylori* eradication rate than did the quadruple therapy only group. Probiotics decreases *H. pylori* loads despite antimicrobial resistance, thus improving the efficacy of quadruple therapy in eradicating residual *H. pylori*. The adverse events (nausea or vomiting, constipation, diarrhea, and metallic taste) were lower in the probiotic plus quadruple therapy group than in the quadruple therapy-only group ($p<0.05$). The authors noted that data from this study provided support that pre-treatment with probiotics (AB-yogurt) may diminish the side effects of quadruple therapy while serving as a rescue regimen for failed triple therapy.

In a randomized, double-blind, placebo-controlled study with *L. acidophilus* LA-5 and *B. lactis* BB-12®, Wildt et al. (2006), investigated the effect of this probiotic combination on patients with collagenous colitis (CC). Twenty-nine subjects were randomized, 21 to the probiotic group and 8 to the placebo group for 12 weeks. The primary end point was a reduction in bowel frequency of $\geq$ 50% per week. The

secondary end points were change in bowel frequencies, stool consistency, stool weight, histopathology, and abdominal bloating and pain. The study showed a reduction in bowel frequency per week in six of the 21 subjects in the probiotic group, and one out of the eight in the placebo group. There were no differences seen between the two groups for the secondary end points. However, post hoc analysis showed a median reduction in bowel frequency per week from 32 to 23, a reduction in number of days with liquid stools per week from six days to one day, and an increase in the number of days with solid stools per week in the probiotic group. Thus, the authors concluded that although the probiotic had no significant effect on the chosen end points, due to the post hoc analysis demonstrating amelioration of the symptoms, this probiotic treatment may potentially influence the disease course of CC.

The effect of *B. lactis* BB-12® in a synbiotic mixture to modulate gut microbiota and preserve intestinal barrier function in patients undergoing colectomy was investigated by Reddy et al. (2007). Eighty-eight patients were randomly assigned to the following treatments: group 1 had mechanical bowel preparation (MBP) only; group 2 had neomycin +MBP; group 3 had synbiotics + neomycin + MBP; and group 4 had synbiotics + neomycin but no MBP. The prebiotic used was 15 g oligofructose powder twice daily and the probiotic preparation was 4×10^9 cfu of *Lactobacillus acidophilus* LA-5, *Lactobacillus bulgaricus, B. lactis* BB-12® and *Streptococcus thermophilus* in a capsule three times daily. Fecal samples were obtained from within the lumen of the resected colectomy specimen. The combination of MBP, neomycin and synbiotics reduces the prevalence of faecal *Enterobacteriaceae* and bacterial translocation. There was no significant difference between the groups in intestinal permeability, inflammatory response or septic morbidity. Postoperative septic morbidity was noted in 15 of 88 patients, 11 patients had wound infections, 7 patients had lower respiratory tract infections, and 3 patients had an intra-abdominal collection. There were no in-hospital deaths recorded.

Saarela et al. (2007) conducted a trial involving 20 healthy adults to investigate the effects of tetracycline group antibiotic on GI survival and tetracycline susceptibility of *B. lactis* BB-12® and *L. acidophilus* LA-5. The antibiotic group, consisting of 10 patients (mean age 42 years) suffering from respiratory tract infections, consumed doxycycline and probiotic capsule (total number of *L. acidophilus* LA-5 log 9.6 and *B. lactis* BB-12® log 9.0 in a daily dose) for 2 weeks. The control group (n=10, mean age 41 years) consumed probiotic capsules only for the same duration. Fecal samples were collected at three sampling points: Day 0 for controls (before starting the probiotic intervention) and Days 0–2 (most often Day 1) for subjects on antibiotic therapy (the first fecal sample after consultation with general practitioner); 1 week and 2-week samples (for both antibiotic and control groups), respectively. An additional sample (1 month

after discontinuation of therapy) was obtained from four individuals in the antibiotic group. *L. acidophilus* and B. *animalis subsp. lactis* were isolated from fecal sample and tested for susceptibility to antibiotics. Doxycycline consumption did not have a large impact on the survival of *B. lactis* BB-12® and LA-5, but it showed detrimental effect on the bifidobacterial population in the gut. Concomitant ingestion of probiotic bacteria LA-5 and *B. lactis* BB-12® with tetracycline antibiotic did not generate a safety risk regarding the possible transfer of tetracycline resistance genes to the ingested strains over the time period of this study. No adverse events were described in the study.

The possibility of *B. lactis* BB-12® consumed with ice-cream to affect the salivary levels of mutans streptococci and lactobacilli in 24 healthy adults (mean age 20 years) was examined in a randomized, double-blind, crossover study by Çaglar et al. (2008). The experimental period comprised four consecutive times: Period 1, run in (1 week); Period 2, intervention (10 days); Periods 3, washout (2 weeks); Period 4, intervention (10 days). During the intervention periods, subjects were instructed to eat a cup of ice-cream per day containing either probiotic ($1x10^7$cfu of *B. lactis* BB-12®) or a control ice- cream without viable bacteria. No tooth brushing was allowed for 1 h after intake. Samplings of whole saliva were taken before and after period 2 and 4. A statistically significant reduction of salivary mutans streptococci was recorded after consumption of the *B. lactis* BB-12® ice-cream. A decline of high mutans streptococci counts was also seen after intake of the control ice-cream, but the difference compared to baseline was not statistically significant. The salivary lactobacilli levels were unaltered after both regimes. No side or adverse effects were reported during the course of the study.

Kajander et al. (2008) investigated the effects of multispecies probiotic supplementation (*Lactobacillus rhamnosus,* LGG®, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS and *B. lactis* BB-12®) on abdominal symptoms, quality of life, intestinal microbiota and inflammatory markers in irritable bowel syndrome (IBS) in a randomized, placebo-controlled 5-month intervention. A total of 86 IBS patients were randomized to receive either multispecies probiotic supplementation ($1x10^7$ cfu of each strain daily in milk-based drink; n=43; mean age 50; 41 female, 2 male) or placebo (the same milk-based drink without probiotics; n=43; mean age 46; 39 female, 4 male). The IBS symptoms and bowel habits were recorded in a diary. Patients with ongoing IBS medication were allowed to continue the medication and adverse events were recorded. Fecal and blood samples were collected at three time points: at baseline, halfway through the study, and at the end of the study.

At the end of intervention, the composite IBS score decreased 14 points from baseline with the probiotic group vs. 3 points with placebo ($p=0.0083$). The microbiota similarity index increased with the probiotic supplementation, while it decreased with

placebo. No differences were seen in C-reactive protein. Most adverse events in both groups were symptoms of the gastrointestinal or respiratory tract (probiotic: 62%; placebo: 65%). Other events reported in the probiotic group were an eye operation, an atherosclerotic finding in the carotid artery, an inflamed mole, cystitis and tenosynovitis. Reported events in the placebo group were oral herpes, breathing difficulties, hyperthyroidism, backache, a foot operation, an inflamed operation wound, vaginitis and a prophylactic treatment against intestinal worms. Four of the adverse gastrointestinal events (all in the placebo group) were considered to have a possible connection with the study, whereas the rest of the events were evaluated as having no connection with the test drink. The authors concluded that," This multispecies probiotic seems to be an effective and safe option to alleviate symptoms of irritable bowel syndrome, and to stabilize the intestinal microbiota."

The influence of *B. lactis* BB-12® on endogenous *Bifidobacterium* and their association with parameters of immune function in elderly subjects was characterized by Ouwehand et al. (2008) in a randomized, double-blind, placebo-controlled trial involving 55 participants living in nursing homes. This study was conducted within the framework of a randomized control trial of 209 elderly by Pitkala et al. (2007). The average age of the study population was 84.3±0.98 years, of which 81.5% were female. Fecal samples were taken at the start of the study, at 3 and 6 months. The participants received three treatments: oat-based drink supplemented with 10^9 CFU/day *B. longum* 2C and 46 (intervention group); the drink without supplementation (placebo group); and the drink with 10^9 CFU/day *B. lactis* BB-12® (control group). A subset of samples from 55 elderly was selected based on availability of a complete or an almost complete set of fecal and serum samples (intervention, n=19; control n=18; placebo n=18) for bifidobacteria composition and serum cytokines analysis. Negative correlations were observed between the levels of *Bifidobacterium* species and the pro-inflammatory cytokine TNF-α and the regulatory cytokine IL-10. The anti-inflammatory TGF-β1 levels were increased over time in all three groups, and the presence of *B. breve* correlated with higher serum TGF-β1 levels. The authors concluded that modulation of the fecal *Bifidobacterium* may provide a means of influencing inflammatory responses. No adverse events were described in the study.

De Vrese et al. (2011) reported the effect of fermented milk product containing lactobacilli and bifidobacteria in reducing antibiotic-associated diarrhea and *H. pylori* activity. Eighty-eight *H. pylori*-infected but otherwise healthy adults age 18-65 years (43 male; 45 female) participated in the randomized, double-blind study were given the following products: 1) yogurt containing *B. lactis* BB-12®, *Lactobacillus acidophilus* LA-5 and *S. thermophilus* ($1x10^6$ cfu of each strain; n=30); 2) the same product but pasteurized after fermentation as control (n=29); or 3) chemically acidified and curded

milk (n=29) in order to exclude any effect from living or dead probiotic or yogurt bacteria. The subjects consumed 2x125g/ day of the respective experimental milk product for 5 weeks. At the end of study period, all milk products decreased *Helicobacter* activity by 18 to 45% without significant differences between groups. The observed decrease in *H. pylori* activity seems to be not or not only due to probiotic bacteria but (rather) to components of acidified milk (most probably lactic acid). Fruit-yogurt-like fermented milk products with living probiotic bacteria significantly shorten the duration of antibiotics-associated diarrhea and improve gastrointestinal complaints. No adverse events were reported in the study.

In a randomized, placebo-controlled, double blind, parallel dose–response study, Savard et al. (2011) investigated the impact of two levels of *B. lactis* BB-12® and *L. acidophilus* LA-5 consumption on fecal bacterial counts in healthy adults. Fifty-eight volunteers age 18-55 years were randomly assigned to receive 10^9 or 10^{10} cfu of *B. lactis* BB-12® + LA-5 + 40 mg of green tea extract/ day in yogurt (Yoptimal or Yoptimal-10, respectively), or placebo (no probiotic, no starter and no green tea extract). During the supplementation period of 4 weeks, subjects had to consume 100 g of yoghurt (Yoptimal, n=20; Yoptimal-10, n=18; or placebo, n=20) once a day. Fecal samples were collected from each volunteer at week 0 and 4. Results of quantitative PCR showed significant increases in bifidobacteria, and lactobacilli counts with the Yoptimal groups compared to placebo. The dose of 10^9 cfu of *B. lactis* BB-12®, *B. lactis* BB-12® and LA-5 in Yoptimal can survive in the intestinal tract as evidenced by their presence in feces. No significant difference was observed between treatments in volunteers' weight, waist girth, blood pressure, fasting plasma triglyceride and HDL-C concentrations, as well as cholesterol/HDL- cholesterol ratio. A significant increase in plasma cholesterol levels was observed in the placebo group but the levels remained stable in the two probiotic yoghurt groups. The frequency of all adverse events was the same between the three treatments. Flatulence at week 4 was reported to occur more frequently than at baseline for all three treatments.

Wildt et al. (2011) investigated the clinical effect of *B. lactis* BB-12® and *Lactobacillus acidophilus* LA-5 to maintain remission in 32 ulcerative colitis patients. In the randomized, double-blind, placebo-controlled study, 20 patients received the probiotic mixture ($1.5x10^{11}$ cfu of *B. lactis* BB-12® and LA-5 daily in capsules) and 12 patient received placebo for 52 weeks. Patients were evaluated clinically at weeks 0, 4, 16, 28, 40, 52; blood and fecal samples were collected in some of the visits. All patients kept a standardized diary throughout the study period. After one year of treatment, five patients (25%) in the probiotic group and one patient (8%) in the placebo group maintained remission. The median time to relapse was 125.5 days (range 11–391 days) in the probiotic group and 104 days (range 28–369 days) in the placebo group

respectively. The overall safety and tolerance of the probiotic mixture and placebo was good with no serious adverse events. The reported adverse events included flatulence, abdominal bloating and pain, changes in fecal consistency, musculoskeletal, tiredness, incontinence, stress, oral blisters, eye redness, headache, dizziness, influenza, gastroenteritis, cystitis, and pneumonia. Gastrointestinal symptoms were reported equally in both groups and a relation between probiotics and gastrointestinal side effects could not be established.

Palaria et al. (2012) examined the effect of *B. lactis* BB-12® (in combination with prebiotic oligofructose) on fecal bacterial counts of healthy adults, focusing on bifidobacteria, clostridia, and enterobacteria. Fifty-two subjects (average age 31 years) participated in the randomized, crossover, placebo-controlled trial which was divided into 5 periods: pre-feeding (1 week), feeding (3 weeks), washout (4 weeks), second feeding (3 weeks), and final washout period (4 weeks). The pre-feeding period was a control period, during which the subjects were not given any yogurt drink. During the second feeding period, there was a crossover of the feeding design. Subjects were randomly assigned to two groups, A (probiotic, n=26) and B (placebo, n=26), but only 46 subjects completed the study (group A finished with 24 subjects and group B with 22 subjects). Subjects consumed yogurt containing $10^9 - 10^{10}$ cfu of *B. lactis* BB-12® and 1 g inulin or placebo during feeding period and fecal samples were collected at 14-time points from 46 subjects who completed the study. No significant differences in numbers of bifidobacteria, clostridia, or enterobacteria were observed between the probiotic and placebo groups during any of the feeding periods. Subgrouping subjects based on lower initial bifidobacterial numbers or higher initial clostridial numbers showed corresponding significant differences between the synbiotic yogurt and placebo groups. The authors concluded that the synbiotic yogurt can increase bifidobacterial numbers and decrease clostridial numbers (but not enterobacterial numbers) in some individuals. There was no adverse events described in the study.

The ability of *B. lactis* BB-12® or *L. casei* 431 to modulate the immune system was evaluated by Rizzardini et al. (2012) using a vaccination model in healthy subjects. A randomized, double-blind, placebo-controlled, parallel-group study was conducted in 211 subjects (56% females, mean age 33·2 years) who consumed $1x10^9$ cfu of *B. lactis* BB-12® in capsule (n=54) or *L. casei* 431 in dairy drink (n=59) or placebo (both capsule, n= 52 or drink forms, n=56) for 6 weeks. A seasonal influenza vaccination was given after 2 weeks of feeding period; plasma and saliva samples were collected at baseline and after 6 weeks. The subjects were contacted by phone 10 weeks after the end of supplementation for safety assessment. At the end of intervention, changes from baseline in vaccine-specific plasma IgG, IgG1 and IgG3, and increases for vaccine-specific secretory IgA in saliva and total antibody concentration were significantly greater in both

probiotic groups vs. the placebo group. No differences were found for plasma cytokines or innate immune parameters. In 49 subjects, 98 adverse events were assessed as related to the study products; the pattern and incidence of adverse events were similar between the groups. The most prevalent of adverse events were high fever (26% of events), rhinitis (13% of events) and severe malaise (12% of events). No adverse events led to discontinuation and no serious adverse events occurred during the study. The authors concluded that supplementation with *B. lactis* BB-12® or *L. casei* 431 may be an effective means to improve immune function by augmenting systemic and mucosal immune responses to challenge.

The effect of *B. lactis* BB-12® in combination with *Lactobacillus rhamnosus* GG on health-related quality of life in healthy adults affected by upper respiratory infections (URI) was assessed by Smith et al. (2013). A total of 231 apparently healthy college students (age 18-25 years) experiencing URI were randomized to receive placebo (n=117) or probiotic-containing powder (daily dose of $1x10^9$ cfu of each LGG® and *B. lactis* BB-12®, n=114) for 12 weeks. Subjects were asked to complete a survey to assess health related quality of life (HRQL) during URI, and self-report if they missed any school or work as a consequence of a URI. The duration of URI was 33% longer in the placebo group compared to the probiotics group (P=0·001) and severity scores were 34% (30 points) higher for the placebo group compared to the probiotics group (P=0·0003). Significantly fewer days of illness and significantly lower severity scores indicate a higher HRQL in the probiotics group compared to the placebo group. The number of missed school days was significantly higher for the placebo group compared to the probiotics group; while no difference was observed in the number of missed work days. A total of forty-three adverse events were reported during the study period: the most common were diarrhea or vomiting (45% in placebo and 55% in probiotic), followed by flatulence and bloating (57% placebo and 43% probiotic). There were no significant differences between groups for adverse events, and no serious adverse events were reported. The authors concluded that combination of LGG® and *B. lactis* BB-12® may be beneficial for mitigating decrements in HRQL during URI in college students living on campus in residence halls.

In a randomized, placebo-controlled trial, Dickerson et al. (2014) examine whether combination of *B. lactis* BB-12® and LGG® supplementation can reduce symptom severity in patients with schizophrenia receiving antipsychotic treatment. Sixty-five outpatients with moderately severe schizophrenia (age 18-65 years) were randomized to probiotic ($1x10^9$ cfu of each LGG® and *B. lactis* BB-12® daily in tablet, n=33) or placebo (n=32). A total of 58 participants completed the trial which lasted for 14 weeks. There were no significant differences in the syndrome scale between the probiotic and placebo groups. Patients in probiotic group were less likely to develop

severe bowel difficulty over the course of the trial. There were a total of 4 serious adverse events reported: 3 psychiatric hospitalizations for an increase in psychotic symptoms, 1 medical hospitalization for dehydration. These serious adverse events involving 2 participants in probiotic group and 1 in placebo group. The probiotic supplementation was well-tolerated with no difference between groups in the number of persons with adverse events: upper respiratory illness (placebo 10, probiotic 12), gastrointestinal symptoms such as constipation, heartburn, nausea, stomach cramps, and/ or flatulence (12 in each groups), diarrhea (placebo 6, probiotic 9). No participant in the probiotic group discontinued the study because of a serious or non-serious adverse event. Measures of analysis of variance showed no significant differences in the psychiatric symptoms between the probiotic and placebo groups, however, patients in probiotic group were less likely to develop severe bowel difficulty over the course of the trial. The authors concluded that probiotic supplementation may help prevent a common somatic symptom associated with schizophrenia.

Eskesen et al. (2015) reported the effect of *B. lactis* BB-12® on defecation frequency and gastrointestinal wellbeing in healthy adults. A total of 1248 subjects with low defecation frequency and complaints of general abdominal discomfort were included in the randomized, double-blind, placebo-controlled trial. Subjects were randomized to $1x10^9$ cfu (n=343) or $1x10^{10}$ cfu of *B. lactis* BB-12® (n=452) or a placebo capsule (n=453) once daily for 4 weeks and completed a diary on bowel habits, relief of abdominal discomfort and symptoms. All adverse events, defined as any medical occurrence, were recorded. Significantly higher defecation frequency was observed in the *B. lactis* BB-12® group compared with placebo. Effects on defecation frequency were similar for the two doses tested, suggesting that a ceiling effect was reached with the one billion dose. Gastrointestinal well-being, defined as relief of abdominal discomfort, did not show significant differences. There were 337 adverse events recorded during the study in 337 (18.7%) of subjects. Of these, 17 events were assessed as related to the study treatment. The majority of related events were gastrointestinal disorders, which were expected as one important inclusion criteria in participants was abdominal discomfort. There were no obvious differences between the treatment groups in the number of adverse events or the number of subjects with events. Three adverse events were defined as serious, which were not related to the study treatment. The authors concluded that *B. lactis* BB-12® is considered safe and that consumption of *B. lactis* BB-12® improves the gastrointestinal health of individuals whose symptoms are not sufficiently severe to consult a doctor.

Consumption of *B. lactis* BB-12® in yogurt was reported to reduce expression of TLR-2 on peripheral blood-derived monocytes and pro-inflammatory cytokines in adults by Meng et al. (2015). The randomized, partially blinded, 4-period crossover study

evaluated *B. lactis* BB-12® at 7 doses of log 10 ±0.5 cfu per day in 30 healthy adults aged 18-40 years for 4 weeks. The subjects received four treatments in random order: yogurt smoothie alone, smoothie with added *B. lactis* BB-12® before or after fermentation, or *B. lactis* BB-12® in capsule form. At baseline and after each 4-week treatment, peripheral blood mononuclear cells were isolated. No adverse effects were reported on any of the treatments, and compliance was 99.2 %. Participants who consumed the yogurt smoothie with *B. lactis* BB-12® added post-fermentation had significantly lower expression of TLR-2 and reduction in TNF-α secretion. The authors concluded that the study findings not only demonstrate a potential anti-inflammatory effect of *B. lactis* BB-12® in healthy adults, but also indicate that the delivery matrix influences the immunomodula-tory properties of *B. lactis* BB-12®.

In a phase I, randomized, double-blinded, controlled study, Merenstein et al. (2015) determined the safety of *B. lactis* BB-12® in healthy adults who were prescribed antibiotics for respiratory infections. Forty participants (age 18-65 years, concurrently taking penicillin-class antibiotic regimen for a respiratory infection, whom general health was ensured by trained practitioners) were randomly assigned to consume 4 ounces of either *B. lactis* BB-12® -supplemented yogurt ($1x10^{10}$ cfu, n=19) or non-supplemented control yogurt (n=21) daily for 10 days. To assess the safety of the interventions, follow-up interviews were conducted at days 6, 11, 15, and 180, and a second physical examination was performed on day 14. A total of 165 adverse events were reported in this study. There were 98 adverse events reported in the control group and 67 adverse events reported in the *B. lactis* BB-12® group. There were also no reported allergic reactions or hypersensitivity to the yogurts. No serious adverse events were reported and no participant deaths occurred. There were no participant withdrawals from the study due to adverse events. In a small subset of patients, changes in whole blood expression of genes associated with regulation and activation of immune cells were detected in the *B. lactis* BB-12® - supplemented group. The authors concluded that *B. lactis* BB-12® - supplemented yogurt is safe and well tolerated when consumed by healthy adults concurrently taking antibiotics.

Toiviainen et al. (2015) evaluated the impact of orally administered lozenges with *B. lactis* BB-12® and *Lactobacillus rhamnosus* GG on plaque accumulation, gingival health and the oral microbiota in healthy subjects. The study was a randomized, controlled, double-blind trial. Sixty-two healthy university students with salivary mutans streptococci counts $\geq 10^3$ CFU/ml consumed lozenges containing a combination of LGG® and *B. lactis* BB-12® (test group, n= 29) or lozenges without added probiotics (control group, n= 31) for 4 weeks. At baseline and at the end of the test period, the plaque index and gingival index were determined. The probiotic lozenge decreased both plaque index and gingival index while no changes were observed in the control group. No probiotic-

induced changes were found in the microbial compositions of saliva in either group. Gastrointestinal problems were reported by two subjects which did not appear to be connected with the consumption of the lozenge. The authors concluded that the probiotic lozenge improved the periodontal status without affecting the oral microbiota.

A study by Linn et al. (2018) investigated the effect of *L. acidophilus* LA-5 and *B. lactis* BB-12® on radiation-induced diarrhea (RID), an acute side effect of radiotherapy in the treatment of cervical cancer. In this study, 54 patients were randomized into probiotic or placebo groups and were double-blinded. The probiotic group were instructed to take one capsule containing 1.75 billion live bacteria three times a day, beginning from the first day until the end of radiotherapy. The placebo group received identically appearing capsules containing starch and the same schedule. The patients were assessed daily during radiotherapy and follow-up weekly for three weeks after radiotherapy. The incidence of diarrhea was reduced in the probiotic group (53.8%) compared to the placebo group (82.1%) and the mild-to-moderate and severe diarrhea were significantly reduced in the probiotic group. The use of an anti-diarrhoeal medication was significantly reduced in the probiotic group as well and the difference in grade 2 abdominal pain, episodes of abdominal pain in days were significantly reduced. The authors concluded that supplementation of probiotic is an easy and effective way to reduce the incidence of severity of RID in cervical cancer patients.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Sullivan (2003)	Randomized, double-blind, placebo-controlled trial to compare the effect of clindamycin on the intestinal flora in subjects ingesting yogurt with added probiotics.	24 healthy subjects (age 21-48 years). Probiotic group (with BB-12®): 12	$1x10^8$ cfu of each *L. acidophilus* NCFB 1748, BB-12® and *L. paracasei* F19	14 days	The probiotic microorganisms evaluated in this study prevented ecological disturbances in the numbers of intestinal *Bacteroides fragilis* group species during clindamycin administration. No adverse events were described in the study.
Anderson (2004)	Prospective, randomized, controlled trial to assess the effects of synbotic on bacterial translocation, gastric colonization, systemic inflammation, and septic morbidity in elective surgical patients.	137 patients undergoing abdominal surgery. Synbiotic (including BB-12®): 72	$4x10^9$ cfu of each BB-12®, *L. acidophilus* LA-5, *L. bulgaricus*, and *S. thermosphilus* 3 times a day in capsules. Prebiotic: 16 g oligofructose, twice daily.	Until patients were discharged from hospital	There were no significant differences between the synbiotic and control groups in bacterial translocation, gastric colonization, systemic inflammation, or septic complications. There was no difference in the incidence of septic morbidity between the placebo and synbiotic groups. The commonest sites of infection were the urinary tract, respiratory tract, and surgical wound. Fourteen patients died within 30 days of surgery; five were in the placebo group and nine in the synbiotic group. No difference between synbiotic and control groups (p=0.354).
Jain (2004)	Randomized, controlled trial to determine whether administration of symbiotic could alter gut barrier function in critically ill patients and thus reduce sepsis.	90 patients admitted to intensive care units (ICU). Synbiotic (including BB-12®): 45	$4x10^9$ cfu of each BB-12®, *L. acidophilus* LA-5, *L. bulgaricus*, and *S. thermosphilus* 3 times a day in capsules. Prebiotic: 16 g oligofructose, twice daily.	Until patients were discharged from hospital.	Patients in the synbiotic group had a significantly lower incidence of potentially pathogenic bacteria and multiple organisms in their nasogastric aspirates compared to control. There were no significant differences between the groups in terms of intestinal permeability, septic complications or mortality.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS

Reference	Study Design & Objective	Description of Subjects	Daily Dose and Delivery of Probiotics	Duration	Results
Wang (2004)	Human trial and in vitro test to examine whether yogurt supplemented with *B. lactis* BB-12® and *L. acidophilus* LA-5 could inhibit *H. pylori* growth.	70 adults infected with *H. pylori*. BB-12® + LA-5: 59	$1x10^7$ cfu of each L. acidophilus LA-5 and BB-12®, twice daily in yogurt.	6 weeks	*B. lactis* BB-12® exerted an in vitro inhibitory effect against *H. pylori*, whereas LA-5 did not show an effect. Administration of yogurt supplemented with probiotics decreased the urease activity of *H. pylori* after 6weeks of therapy. No adverse events were described in the study.
Laake (2005)	Randomized, double blind, placebo-controlled study to confirm the effect of probiotic on symptoms and endoscopic appearance in ulcerative colitis (UC) patients.	51 UC patient (10 w/ FAP, operated on with IPAA); 6 UC patients operated on for IRA.	$1x10^8$ cfu of each BB-12® and LA-5 daily	4 weeks	The number of lactobacilli and bifidobacteria increased significantly during intervention; involuntary defecation abdominal cramps were significantly decreased in the UC/IPAA group. Blood test, fecal fungi, and fecal pH did not change significantly during intervention. No adverse events were reported in the study.
Larsen (2006)	Randomized, placebo-controlled, double-blinded, parallel dose–response study to investigate the dose–response effects of probiotic supplementation on blood lipids, recovery from feces and bowel habits.	75 healthy young adults (age 18-40 years). BB-12® + *L. paracasei* CRL-431: 15 per dosing group	10^8,10^9,10^{10} or 10^{11} cfu of each BB-12® and CRL-431/day in capsules.	3 weeks	The fecal recovery of *B. lactis* BB-12® increased significantly with increasing dose. CRL-431 was not recovered in any of the fecal samples. Supplementation with probiotics did not change the fecal bacterial composition. A significant linear increase in fecal consistency (looser stool) with increasing probiotic dose was observed. No overall dose–response effect was found on the blood lipids. High doses of probiotics were well tolerated.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Sheu (2006)	Randomized, placebo-controlled trial to test whether prior treatment with yogurt supplemented with probiotics improved the efficacy of quadruple therapy in eradicating residual H. pylori after failed triple therapy.	138 *H. pylori* infected patients. Probiotic group (with BB-12®): 69	$1x10^9$ cfu of each BB-12®, *L. acidophilus* LA-5, *L. bulgaricus*, and *S. thermophilus*/ day in yogurt	4 weeks	The yogurt-plus-quadruple therapy group had a higher *H. pylori* eradication rate than did the quadruple therapy-only group. Probiotics decreases *H. pylori* loads despite antimicrobial resistance, thus improving the efficacy of quadruple therapy in eradicating residual *H. pylori*. The adverse effects of quadruple therapy were lower in the yogurt-plus-quadruple therapy group than in the quadruple therapy-only group.
Wildt (2006)	Randomized, double-blind, placebo-controlled trial on the effects of *L. acidophilus* LA-5 and *B. lactis* BB-12® on collagenous colitis	29 patients with CC. Probiotic group (with BB-12®): 21		12 weeks	The study showed a reduction in bowel frequency per week, but no differences were seen between the two groups for the secondary end points. Post hoc analysis did show a median reduction in bowel frequency per week, a reduction in number of days with liquid stools per week, and an increase in the number of days with solid stools per week in the probiotic group.
Reddy (2007)	Randomized clinical trial to investigate the combined effect of synbiotic, neomycin, and mechanical bowel preparation (MBP) on intestinal barrier function in patients undergoing colectomy.	88 patients undergoing colectomy. Synbiotic (including BB-12®): 20 + 22	$4x10^9$ cfu of *L.acidophilus* LA-5, *L. bulgaricus*, *B. lactis* BB-12®, *S. thermophilus* 3 x daily in capsules. Prebiotic: 15 g oligofructose twice daily in capsules.	Not stated	The combination of MBP, neomycin and synbiotics reduces the prevalence of faecal Enterobacteriaceae and bacterial translocation; however, this was not associated with a reduction in inflammatory response or septic morbidity in this study. Fifteen postoperative septic morbidity, 11 wound infections, 3 intra-abdominal collection were noted; all events were not different in all groups. There were no in-hospital deaths.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Saarela (2007)	Human trial to investigate the effects of tetracycline group antibiotic on GI survival and tetracycline susceptibility of *B. lactis* BB-12® and *L. acidophilus* LA-5.	20 healthy adults (mean age 41.5 years) suffering from respiratory tract infections. BB-12® + *L. acidophilus* LA-5: 10	$10^9 - 10^{10}$ cfu of BB-12® and *L. acidophilus* LA-5, 3 times daily in capsules	2 weeks	Concomitant ingestion of probiotic bacteria *L. acidophilus* LA-5 and *B. lactis* BB-12® with tetracycline antibiotic did not generate a safety risk regarding the possible transfer of tetracycline resistance genes to the ingested strains over the time period of this study. No adverse events were described in the study.
Çaglar (2008)	Randomized, double-blind, crossover study to examine whether short-term consump-tion of ice-cream containing bifido-bacteria can affect the salivary levels of mutans streptococci and lactobacilli in young adults.	24 healthy adults (mean age 20 years). BB-12®: 23	$1x10^7$cfu of BB-12®/day ice cream.	0 days	A statistically significant reduction of salivary mutans streptococci was recorded after consumption of the probiotic ice-cream. A decline of high mutans streptococci counts was also seen after intake of the control ice-cream, but the difference compared to baseline was not statistically significant. The salivary lactobacilli levels were unaltered after both regimes. No side or adverse effects were reported during the course of the study.
Kajander (2008)	Randomized, placebo-controlled study to investigate multi species probiotics effect on irritable bowel syndrome (IBS).	86 IBS patients. BB-12®+other strains: 43	$1x10^7$ cfu of each BB-12®, LGG®, *L. rhamno-sus* Lc705, *P. freudenreichii* ssp. *shermanii* JS daily in milk-based drink.	5 months	The composite IBS score decreased 14 points from baseline with the probiotic group vs. 3 points with placebo (p=0.0083). The microbiota similarity index increased with the probiotic supplementation, while it decreased with placebo. No differences were seen in C-reactive protein. No significant adverse events were recorded.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Ouwehand (2008)	Randomized, double-blind placebo- controlled study to characterize the influence of *B. lactis* BB-12® on endogenous *Bifidobacterium* and their association with parameters of immune function in elderly subjects.	55 elderly adults in nursing homes (average age: 84.3±0.98 years). BB-12®: 18	$1x10^9$ cfu of BB-12®/day in oat-based drink.	6 months	Negative correlations were observed between the levels of *Bifidobacterium* species and the pro-inflammatory cytokine TNF-α and the regulatory cytokine IL-10. The anti-inflammatory TGF-b1 levels were increased over time in all three groups, and the presence of *B. breve* correlated with higher serum TGF-b1 levels. Modulation of the faecal *Bifidobacterium* may provide a means of influencing inflammatory responses. No adverse events were described in the study.
De Vrese (2011)	Randomized, double-blind, controlled trial to investigate probiotic effects and reduction of the antibiotic-associated diarrhea in *H. pylori* infected but otherwise healthy subjects.	88 *Helicobacter pylori*-infected but otherwise healthy adults (age 18-65 years). BB-12®: 30	$1x10^6$ cfu of each BB-12®, *L. acidophilus* LA-5, and *S. thermophiles*/g of yoghurt-like low fat milk. Consumption: 2x125 g/ day	5 weeks	All milk products decreased *Helicobacter* activity by 18 to 45% without significant differences between groups. The observed decrease in *H. pylori* activity seems to be not or not only due to probiotic bacteria but (rather) to components of acidified milk (most probably lactic acid). Fruit-yogurt-like fermented milk products with living probiotic bacteria significantly shorten the duration of antibiotics-associated diarrhea and improve gastrointestinal complaints. No adverse events were reported in the study.
Savard (2011)	Randomized, placebo-controlled, double blind, parallel dose–response study to investigate the impact of *B. lactis* BB-12® and *L. acidophilus* LA-5 consumption on fecal bacterial counts in healthy adults.	58 healthy adults (age 18-55 years). BB-12® +LA-5: 38	10^9 or 10^{10} cfu of BB-12® and LA-5 and 40 mg of green tea extract/ day in yogurt (Yoptimal)	4 weeks	There were significant increases in bifidobacteria counts with the optimal treatment as compared to baseline. A significant increase in plasma cholesterol levels was observed in the placebo group but the levels remained stable in the two probiotic yoghurt groups. The frequency of all adverse events was the same between treatments. Flatulence at week 4 was reported to occur more frequently than at baseline for all treatments.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Wildt (2011)	Randomized double-blind placebo-controlled trial to investigate the clinical effect of treatment with L. acidophilus LA-5 and *B. lactis* BB-12® to maintain remission in patients with ulcerative colitis.	32 patients with ulcerative colitis. Probiotic (with BB-12®): 20	$1.5x10^{11}$ cfu of BB-12® and *L. acidophilus*, LA-5/ day in capsules	52 weeks	Five patients (25%) in the probiotic group and one patient (8%) in the placebo group maintained remission after 1 year of treatment. The median time to relapse was 125.5 days (range 11–391 days) in the probiotic group and 104 days (range 28–369 days) in the placebo group respectively. The probiotic combination was overall well tolerated.
Palaria (2012)	Randomized, double-blind, crossover, placebo- controlled study to investigate the effect of symbiotic yogurt (*B. lactis* BB-12® + inulin) on levels of fecal Bifidobacteria, Clostridia, and Enterobacteria.	46 healthy adults (average age 31 years). BB-12®: 22	$10^9 - 10^{10}$ cfu of BB-12® and 1 g inulin/day in yogurt	2 times 3 weeks of feeding period.	Synbiotic yogurt can increase bifidobacterial numbers and decrease clostridial numbers (but not enterobacterial numbers) in some individuals, but it cannot modulate these microbial groups in the majority of individuals in the study. No adverse events were described in the study.
Rizzardini (2012)	Randomized, double-blind placebo- controlled study to investigate the ability of *B. lactis* BB-12® and *L. casei* 431 to modulate the immune system using a vaccination model in healthy subjects.	211 healthy subjects (mean age 32.2 years). BB-12®: 35	$1x10^9$ cfu of BB-12® in capsule or *L. casei* 431 in drink/day	6 weeks	Changes from baseline in vaccine-specific plasma IgG, IgG1 and IgG3, and increases for vaccine-specific secretory IgA in saliva and total antibody concentration were significantly greater in both probiotic groups vs. the placebo group. In 49 subjects, 98 adverse events (AE) were assessed as related to the study products; the pattern and incidence of AE were similar between the groups. The most prevalent of AE were high fever (26% of events), rhinitis (13% of events) and severe malaise (12% of events). No AE led to discontinuation and no serious AE occurred during the study.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Smith (2013)	Prospective, randomized, double-blind, placebo-controlled trial to assess the effect of LGG® and *B. lactis* BB-12® on health-related quality of life (HRQL) in college students affected by upper respiratory infections (URI).	231 apparently healthy students (age 18-25) BB-12®+LGG®: 101	$1x10^9$ cfu of each LGG® and BB-12® per day in a small foil stick.	12 weeks	Probiotic group showed significantly shorter duration of URI indicating higher HRQL during infection and missed significantly fewer school days compared to placebo group. Number of missed work days was not different between groups. There were no significant differences between groups for adverse events, and no serious adverse events were reported.
Dickerson (2014)	Randomized, placebo-controlled trial to examine whether probiotic supplementation can reduce symptom severity in patients with schizophrenia receiving antipsychotic treatment.	65 outpatients with moderately severe schizophrenia (age 18-65 years). BB-12®+LGG®: 33	$1x10^9$ cfu of each LGG® and BB-12®/ day in tablet.	14 weeks	No significant differences in the syndrome scale between the probiotic and placebo groups. Patients in probiotic group were less likely to develop severe bowel difficulty over the course of the trial. There was a total of 4 serious events; the probiotic was well tolerated with no difference between groups in the number of persons with adverse event. No participant in the probiotic group discontinued the study because of a serious or non-serious adverse event.
Eskesen (2015)	Randomized, double-blind, placebo-controlled trial to evaluate the effect of *B. lactis* BB-12®, on defecation frequency and gastrointestinal well-being in healthy adults.	1248 healthy adults (18-70 years) with a low defecation frequency and complaints of general abdominal discomfort. BB-12®: 795	$1x10^9$ or $1x10^{10}$ cfu of BB-12® capsule daily with breakfast	4 weeks	Significantly higher defecation frequency was observed in the *B. lactis* BB-12® group compared with placebo. Effects on defecation frequency were similar for the two doses tested, suggesting that a ceiling effect was reached with the one billion dosage. In total, 18·7% subjects experienced non-serious adverse events (AE) during the study. The majority of the related events were GI disorders. There were no differences between the treatment groups in the number of AE or the number of subjects with events. Based on these data, the *B. lactis* BB-12® probiotic strain is considered safe.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Meng (2015)	Randomized, partially blinded, crossover study to evaluate the effects of *B. lactis* BB-12® on immune responses delivered via different matrices (yogurt vs. capsule from).	30 healthy adults (age 18-40 years). BB-12®: 30	Log 10 ±0.5 cfu of BB-12®/day in yogurt smoothies or capsule.	4 weeks	*B. lactis* BB-12® interacted with peripheral myeloid cells via Toll-like receptor 2 (TLR-2). Delivery matrix influences the immunomodulatory properties of *B. lactis* BB-12®. No adverse effects were reported on any of the treatments, and compliance was 99.2 %.
Merenstein (2015)	Phase I, randomized, double-blinded, controlled study to determine the safety of *B. lactis* BB-12® in healthy adults who were prescribed antibiotics for respiratory infections.	40 generally healthy adults (age 18 – 65 years) concurrently taking antibiotics. BB-12®: 19	$1x10^{10}$ cfu of BB-12®/ day in yogurt drink	0 days	A total of 165 non-serious adverse events were reported, with no differences between the control and *B. lactis* BB-12® groups. In a small subset of patients, changes in whole blood expression of genes associated with regulation and activation of immune cells were detected in the *B. lactis* BB-12® - supplemented group. *B. lactis* BB-12® - supplemented yogurt is safe and well tolerated when consumed by healthy adults concurrently taking antibiotics.
Toiviainen (2015)	Randomized, double-blind, controlled trial to evaluate the effects of orally administered LGG® and *B. lactis* BB-12® on plaque accumulation, gingival health and the oral microbiota in healthy subjects.	62 healthy university students with salivary mutans streptococci counts $\geq 10^3$ CFU/ml. LGG® + BB-12®: 29	$2x10^9$ cfu of each LGG® and BB-12®	4 weeks	The probiotic lozenge decreased both plaque index and gingival index while no changes were observed in the control group. No probiotic-induced changes were found in the microbial compositions of saliva in either group. Gastrointestinal problems were reported by two subjects which did not appear to be connected with the consumption of the lozenge.

TABLE 6: *Bifidobacterium animalis* ssp. *lactis* BB-12® CLINICAL STUDIES IN ADULTS					
Reference	**Study Design & Objective**	**Description of Subjects**	**Daily Dose and Delivery of Probiotics**	**Duration**	**Results**
Linn (2018)	Randomized, double-blind, placebo-controlled study on the effects of probiotics for the prevention of acute radiation-induced diarrhea among cervical patients.	54 patients undergoing radiotherapy for cervical cancer. BB-12®: 27	1.75 billion bacteria three times daily in capsule form	Duration of radiotherapy treatment plus three weeks follow up.	The incidence of diarrhea, mild-to-moderate and severediarrhea was reduced in the probiotic group. The use of an anti-diarrhoeal medication was significantly reduced in the probiotic group as well and the difference in grade 2 abdominal pain, episodes of abdominal pain in days were significantly reduced. The authors concluded that supplementation of probiotic is an easy and effective way to reduce the incidence of severity of RID in cervical cancer patients.

6.3.4 Review Articles Regarding the Safety of *B. animalis* ssp. *lactis* BB-12®

A systematic review of randomized controlled trials by Szajewska et al. (2013) aimed to determine the effects infant formula supplemented with *B. lactis* BB-12® and *Lactobacillus rhamnosus* GG compared with non-supplemented formula administered in early infancy (<4 months of age) on the growth of healthy infants. Seven out of nine studies that met the inclusion criteria assessed the effects on growth of infant formula supplemented with *B. lactis* BB-12®. Compared with non-supplemented controls, supplementation of infant formula with *B. lactis* BB-12® had no effect on weight gain, length gain, or head circumference gain. The authors concluded that, "The effect on growth is an important part of the safety evaluation of any product used in infants. Supplementation of infant formulae with *B. lactis* results in growth similar to what is found in infants fed non-supplemented formulae."

Van den Nieuwboer et al. (2014) systematically evaluated safety aspects of probiotic and synbiotic administration in young infants (0-24 months of age). Of 139 studies identified, only 65 (57 original studies, 8 follow-up studies) of them met the inclusion criteria and thus were included in the safety analysis. Most studies were published between 2008 and 2012. The safety profile of the administered probiotics and synbiotics were assessed by means of the reported adverse events and analyzed according to their nature and quantity. Adverse events are defined as the occurrence of complications or illnesses or worsening of the condition throughout the study. Adverse Events were categorized according to the Common Terminology Criteria for Adverse Events (CTCAE version 4.0, NIH, 2009) classification system. A total of 10,056 infants, between 0 and 24 months of age, were enrolled in the 57 eligible clinical intervention studies. As many as 5,643 infants were assigned to the treatment arm and 4,413 infants to the placebo arm, with a drop-out rate of 10.3 and 10.6% respectively. The strain *B. lactis* BB-12® was administered to 309 participants. On average, the infants received a total of 2.79×10^{10} cfu of probiotic(s)/day ranging from a maximal dosage of 2×10^{12} cfu with *L. rhamnosus* GG to the lowest dosage of 7×10^{6} cfu with *S. thermophilus* (unspecified). All formula-fed infants received a total of 1×10^{10} cfu per day.

The most common reported adverse events were 'diarrhoea', 'respiratory infections', 'gastrointestinal infections', 'sepsis' and 'fever', which were also the main clinical outcomes to be influenced by probiotics. No study reported a bacteremia or fungemia associated with the ingested probiotics. The authors concluded that, "...probiotic administration to infants between 0 and 24 months is safe with regard to the evaluated strains in infants with a particular health status or susceptibility. Most adverse events and serious adverse events were considered unrelated to the study product, and there were no major safety concerns. Almost all studies concluded that none of the

adverse effects were related to the study product; the study products are generally well tolerated."

Bifidobacteria colonize the orogastrointestinal tract and rarely cause invasive human infections. However, an increasing number of bifidobacterial blood culture isolates has lately been observed in Norway. To investigate the pathogenicity of the Bifidobacterium species responsible for bacteremia, a study (Esaiassen et al. 2017) was done on Bifidobacterium isolates from 15 patients for whom cultures of blood obtained from 2013 to 2015 were positive. Clinical data was collected and analyzed for phenotypic and genotypic antibiotic susceptibility. All isolates were subjected to whole-genome sequencing. The patients were predominantly in the extreme lower of upper age spectrum and many were severely immunocompromised and a majority of them had gastrointestinal tract-related conditions. This is most likely the largest case series of patients with Bifidobacterium bacteremia for which clinical, microbiological, and genome sequencing data have been described. There were three main clinical characteristics among patients with bacteremia. First, patients were predominantly in the extreme lower or upper age spectrum. Second, the majority of patients had some degree of immune impairment. Third, most (11/15) patients had gastrointestinal tract-related conditions or symptoms. The clinical findings are in line with previous reports on patients with invasive Bifidobacterium infections indicating that they seem to be opportunistic infections in immunocompromised patients, probably secondary to bacterial translocation from the gut.

And from 2002 to 2011, Cohen et al. 2016, evaluated bloodstream infections caused by common probiotic organisms in hematopoietic cell transplant recipients at the Fred Hutchinson Cancer Research Center in Seattle, Washington. Patients with at least one positive blood culture for common probiotic organisms (*Lactobacillus* species, *Bifidobacterium* species, Streptococcus *thermophilus,* and *Saccharomyces* species) within one year post hematopoietic cell transplantation were considered cases. A total of 0.5% of patients developed a blood stream infection from one of these organisms within a year post-hematopoietic cell transplant. However, no *Bifidobacterium* species or *S. thermophilus* were identified and the authors concluded that organisms that are frequently incorporated into over-the-counter probiotics are infrequent causes of bacteremia after HTC.

6.4 Specific Safety Considerations

We are not aware of any safety concern related to *Bifidobacterium animalis* subsp. *lactis*. Moreover, many species in the genus Bifidobacterium including *Bifidobacterium animalis* are on EFSAs list of species with a general presumption of safety (last update June 2018). The following Bifidobacterium species: *B. adolescentis*, *B. animalis; B. longum, B. breve* and *B. bifidum* have, due to the long history of safe use been on the EFSA QPS list since 2007 (EFSA journal, 2018).

6.5 Inconsistent Information

Chr. Hansen Panel have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

6.6 Recognition of Safety by an Authoritative Group of Qualified Experts

The intended use of *B. lactis* BB-12® 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, and concluding that the expected exposure to *B. lactis* BB-12® 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.

6.7 Common Knowledge Elements of GRAS Conclusion

All studies used to establish this GRAS status conclusion have been published in the scientific literature, thus generally available.

6.8 Conclusion

The history of safe use of *B. lactis* BB-12® is strongly supported by a large body of published research. This probiotic strain has been incorporated in a variety of conventional food products and has been consumed as a dietary supplement in the United States and internationally by general population. All the available evidence demonstrates that there is no reason to suspect harm to healthy individuals consuming conventional foods containing *B. lactis* BB-12®. We concluded that the intended use of *B. lactis* BB-12® to be added as an ingredient to a variety of conventional foods consistent with current good manufacturing practice, can be considered GRAS. The basis of this conclusion are scientific procedures set forth under the U.S. Food & Drug Administration Final Rule, 81 FR 54959 and the data and information presented in this notice.

Part 7

Part 7. List of Supporting Data and Information

All data and information used in this GRAS notification to establish safety are generally available.

Aarestrup, F M, H Hasman, L B Jensen, M Moreno, I A Herrero, L Dominguez, M Finn, and A Franklin. 2002. "Antimicrobial Resistance among Enterococci from Pigs in Three European Countries." *Applied and Environmental Microbiology* 68 (8):4127–29. https://doi.org/doi 10.1128/Aem.68.8.4127-4129.2002.

Aarestrup, F. M., Y. Agerso, P. Gerner-Smidt, M. Madsen, and L. B. Jensen. 2000. "Comparison of Antimicrobial Resistance Phenotypes and Resistance Genes in Enterococcus Faecalis and Enterococcus Faecium from Humans in the Community, Broilers, and Pigs in Denmark." *Diagnostic Microbiology and Infectious Disease*. https://doi.org/10.1016/S0732-8893(00)00130-9.

Agersø, Y., Stuer-Lauridsen, B., Bjerre, K., Jensen, M.G., Johansen, E., Bennedsen, M., Brockmann, E., and Nielsen, B. 2018. "Antimicrobial Susceptibility Testing and Tentative Epidemiological Cutoff Values for Five Bacillus Species Relevant for Use as Animal Feed Additives or for Plant Protection". *Appl Environ Microbiol* Sep 17;84(19). pii: e01108-18. doi: 10.1128/AEM.01108-18.

Aires, J., F. Doucet-Populaire, and M. J. Butel. 2007. "Tetracycline Resistance Mediated by tet(W), tet(M), and tet(O) Genes of Bifidobacterium Isolates from Humans." *Applied and Environmental Microbiology* 73 (8):2751–54. https://doi.org/10.1128/AEM.02459-06.

Akalin, A. S., and D. Erişir. 2008. "Effects of Inulin and Oligofructose on the Rheological Characteristics and Probiotic Culture Survival in Low-Fat Probiotic Ice Cream." *Journal of Food Science* 73 (4). https://doi.org/10.1111/j.1750-3841.2008.00728.x.

Alekshun, Michael N., and Stuart B. Levy. 2007. "Molecular Mechanisms of Antibacterial Multidrug Resistance." *Cell*. https://doi.org/10.1016/j.cell.2007.03.004.

Alves, Larissa L., Neila S.P.S. Richards, Paula Mattanna, Diego F. Andrade, Adriano P. S Rezer, Liana I.G. Milani, Adriano G. Cruz, and José A.F. Faria. 2013. "Cream Cheese as a Symbiotic Food Carrier Using Bifidobacterium Animalis Bb-12 and Lactobacillus Acidophilus La-5 and Inulin." *International Journal of Dairy Technology* 66 (1):63–69. https://doi.org/10.1111/j.1471-0307.2012.00880.x.

Anderson, A D G, C E McNaught, P K Jain, and J MacFie. 2004. "Randomised Clinical Trial of Synbiotic Therapy in Elective Surgical Patients." *Gut* 53 (2):241–45. https://doi.org/10.1136/gut.2003.024620.

Barbosa, Teresa M, Karen P Scott, and Harry J Flint. 1999. "Evidence for Recent Intergeneric Transfer of a New Tetracycline Resistance Gene, tet(W), Isolated from Butyrivibrio Fibrisolvens, and the Occurrence of tet(O) in Ruminal Bacteria." *Environmental Microbiology* 1 (1):53–64. https://doi.org/10.1046/j.1462-2920.1999.00004.x.

Barrangou, Rodolphe, Elizabeth P. Briczinski, Lindsay L. Traeger, Joseph R. Loquasto, Melissa Richards, Philippe Horvath, Anne Claire Coûté-Monvoisin, et al. 2009. "Comparison of the Complete Genome Sequences of Bifidobacterium Animalis Subsp. Lactis DSM 10140 and Bl-04." *Journal of Bacteriology* 191 (13):4144–51. https://doi.org/10.1128/JB.00155-09.

Bedani, Raquel, Elizeu Antonio Rossi, and Susana Marta Isay Saad. 2013. "Impact of Inulin and Okara on Lactobacillus Acidophilus La-5 and Bifidobacterium Animalis Bb-12 Viability in a Fermented Soy Product

and Probiotic Survival under in Vitro Simulated Gastrointestinal Conditions." *Food Microbiology* 34 (2). Elsevier Ltd:382–89. https://doi.org/10.1016/j.fm.2013.01.012.

Bennedsen, Mads, Birgitte Stuer-Lauridsen, Morten Danielsen, and Eric Johansen. 2011. "Screening for Antimicrobial Resistance Genes and Virulence Factors via Genome Sequencing." *Applied and Environmental Microbiology* 77 (8):2785–87. https://doi.org/10.1128/AEM.02493-10.

Billington, Stephen J., and B. Helen Jost. 2006. "Multiple Genetic Elements Carry the Tetracycline Resistance Gene tet(W) in the Animal Pathogen Arcanobacterium Pyogenes." *Antimicrobial Agents and Chemotherapy* 50 (11):3580–87. https://doi.org/10.1128/AAC.00562-06.

Billington, Stephen J., J. Glenn Songer, and B. Helen Jost. 2002. "Widespread Distribution of a Tet W Determinant among Tetracycline-Resistant Isolates of the Animal Pathogen Arcanobacterium Pyogenes." *Antimicrobial Agents and Chemotherapy* 46 (5):1281–87. https://doi.org/10.1128/AAC.46.5.1281-1287.2002.

Briczinski, Elizabeth P., Joseph R. Loquasto, Rodolphe Barrangou, Edward G. Dudley, Anastasia M. Roberts, and Robert F. Roberts. 2009. "Strain-Specific Genotyping of Bifidobacterium Animalis Subsp. Lactis by Using Single-Nucleotide Polymorphisms, Insertions, and Deletions." *Applied and Environmental Microbiology* 75 (23):7501–8. https://doi.org/10.1128/AEM.01430-09.

Çaglar, Esber, Ozgur Onder Kuscu, Senem Selvi Kuvvetli, Sule Kavaloglu Cildir, Nuket Sandalli, and Svante Twetman. 2008. "Short-Term Effect of Ice-Cream Containing Bifidobacterium Lactis Bb-12 on the Number of Salivary Mutans Streptococci and Lactobacilli." *Acta Odontologica Scandinavica* 66 (3):154–58. https://doi.org/10.1080/00016350802089467.

Chouraqui, Jean Pierre, Louis Dominique Van Egroo, and Marie Claire Fichot. 2004. "Acidified Milk Formula Supplemented with Bifidobacterium Lactis: Impact on Infant Diarrhea in Residential Care Settings." *Journal of Pediatric Gastroenterology and Nutrition* 38 (3):288–92. https://doi.org/10.1097/00005176-200403000-00011.

Cohen, S.A., Woodfield, M.C., Boyle, N., Stednick, Z., Boeckh, M., and Pergam, S.A., 2016. "Incidenc and outcomes of bloodstream infections among hematopoietic cell transplant recipeints from species commonly reported to be in over-the-counter probiotic formulations." *Transplant Infectious Disease* Vol. 18, Issue 5, pp.. 699-705. https://doi.org/10.1111/tid.12587.

Collado, M. C., J. Meriluoto, and S. Salminen. 2007a. "Role of Commercial Probiotic Strains against Human Pathogen Adhesion to Intestinal Mucus." *Letters in Applied Microbiology* 45 (4):454–60. https://doi.org/10.1111/j.1472-765X.2007.02212.x.

Collado, M. C., Łukasz Grześkowiak, and Seppo Salminen. 2007b. "Probiotic Strains and Their Combination Inhibit in Vitro Adhesion of Pathogens to Pig Intestinal Mucosa." *Current Microbiology* 55 (3):260–65. https://doi.org/10.1007/s00284-007-0144-8.

Commane, Daniel M., Colette T. Shortt, Stefania Silvi, Albert Cresci, Roisin M. Hughes, and Ian R. Rowland. 2005. "Effects of Fermentation Products of pro- and Prebiotics on Trans-Epithelial Electrical Resistance in an in Vitro Model of the Colon." *Nutrition and Cancer* 51 (1):102–9. https://doi.org/10.1207/s15327914nc5101_14.

Dickerson, Faith B., Cassie Stallings, Andrea Origoni, Emily Katsafanas, Christina L. G. Savage, Lucy A. B. Schweinfurth, Joshana Goga, Sunil Khushalani, and Robert H. Yolken. 2014. "Effect of Probiotic Supplementation on Schizophrenia Symptoms and Association With Gastrointestinal Functioning." *The Primary Care Companion For CNS Disorders*. https://doi.org/10.4088/PCC.13m01579.

EFSA (European Food Safety Authority). 2010. Scientific Opinion on the Maintenance of the List of QPS Biological Agents Intentionally Added to Food and Feed (2010 update). *The EFSA Journal*; 8:1-56.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). 2017. Scientific Opinion on the Update of the List of QPS -Recommended Biological Agents Intentionally Added to Food or Feed as Notified to EFSA. *The EFSA Journal*;15(3):4664, 177 pp. doi:10.2903/j.efsa.2017.4664

EFSA Journal, 2018. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 8: suitability of taxonomic units notified to EFSA until March 2018. EFSA journal 2018:16(7):5315. doi: 10.2903/j.efsa.2018.5315

Esaiassen, E., Hjerde, E., Cavanagh, J.P., Simonsen, G.S., and Klingenberg, C. 2017. "Bifidobacterium Bacteremia: Clinical Characteristics and a Genomic Approach to Assess Pathogenicity". *J Clin Microbiol* Jul; 55(7):2234-2248. Doi: 10.1128/JCM.00150-17

Eskesen, Dorte, Lillian Jespersen, Birgit Michelsen, Peter J. Whorwell, Stefan Müller-Lissner, and Cathrine M. Morberg. 2015. "Effect of the Probiotic Strain Bifidobacterium Animalis Subsp. Lactis BB-12®, on Defecation Frequency in Healthy Subjects with Low Defecation Frequency and Abdominal Discomfort: A Randomised, Double-Blind, Placebo-Controlled, Parallel-Group Trial." *British Journal of Nutrition* 114 (10):1638–46. https://doi.org/10.1017/S0007114515003347.

Flórez, Ana Belén, Mohammed Salim Ammor, Pablo Álvarez-Martín, Abelardo Margolles, and Baltasar Mayo. 2006. "Molecular Analysis of tet(W) Gene-Mediated Tetracycline Resistance in Dominant Intestinal Bifidobacterium Species from Healthy Humans." *Applied and Environmental Microbiology* 72 (11):7377–79. https://doi.org/10.1128/AEM.00486-06.

Garrigues, C, B Stuer-Lauridsen, and E Johansen. 2005. "Characterisation of Bifidobacterium Animalis Subsp. Lactis BB-12 and Other Probiotic Bacteria Using Genomics, Transcriptomics and Proteomics." *Australian Journal of Dairy Technology* 60:84–92.

Garrigues, Christel, Eric Johansen, and Martin B. Pedersen. 2010. "Complete Genome Sequence of Bifidobacterium Animalis Subsp. Lactis BB-12, a Widely Consumed Probiotic Strain." *Journal of Bacteriology* 192 (9):2467–68. https://doi.org/10.1128/JB.00109-10.

Garrigues, Christel, Eric Johansen, and Martin B. Pedersen. 2010. "Complete Genome Sequence of Bifidobacterium Animalis Subsp. Lactis BB-12, a Widely Consumed Probiotic Strain." *Journal of Bacteriology* 192 (9):2467–68. https://doi.org/10.1128/JB.00109-10.

Gueimonde, Miguel, Ana Belén Flórez, Angela H A M Van Hoek, Birgitte Stuer-Lauridsen, Per Strøman, Clara G. De Los Reyes-Gavilán, and Abelardo Margolles. 2010. "Genetic Basis of Tetracycline Resistance in Bifidobacterium Animalis Subsp. Lactis." *Applied and Environmental Microbiology* 76 (10):3364–69. https://doi.org/10.1128/AEM.03096-09.

He, F, A C Ouwehand, H Hashimoto, E Isolauri, Y Benno, and S Salminen. 2001. "Adhesion of Bifidobacterium ssp. to Human Intestinal Mucus." *Microbiology and Immunology* 45 (3):259–62. https://doi.org/10.1111/j.1348-0421.2001.tb02615.x.

Heenan, C. N., M. C. Adams, R. W. Hosken, and G. H. Fleet. 2004. "Survival and Sensory Acceptability of Probiotic Microorganisms in a Nonfermented Frozen Vegetarian Dessert." *LWT - Food Science and Technology* 37 (4):461–66. https://doi.org/10.1016/j.lwt.2003.11.001.

Hojsak, Iva, Ana Mo, Agneza Marija Pasini, and Sanja Kola. 2015. "Bifidobacterium Animalis Subsp . Lactis Fails to Prevent Common Infections in Hospitalized Children : A Randomized , Double-Blind ," no. February:680–84. https://doi.org/10.3945/ajcn.114.102004.The.

Hojsak, Iva, Ana Močić Pavić, Tea Kos, Jelena Dumančić, and Sanja Kolaček. 2016. "Bifidobacterium Animalis Subsp. Lactis in Prevention of Common Infections in Healthy Children Attending Day Care Centers -

Randomized, Double Blind, Placebo-Controlled Study." *Clinical Nutrition* 35 (3):587–91. https://doi.org/10.1016/j.clnu.2015.05.004.

Holscher, Hannah D., Laura A. Czerkies, Pamela Cekola, Richard Litov, Marshall Benbow, Sheryl Santema, Dominik D. Alexander, et al. 2012. "Bifidobacterium Lactis Bb12 Enhances Intestinal Antibody Response in Formula-Fed Infants: A Randomized, Double-Blind, Controlled Trial." *Journal of Parenteral and Enteral Nutrition* 36 (1 SUPPL.). https://doi.org/10.1177/0148607111430817.

Homayouni, A., A. Azizi, M. R. Ehsani, M. S. Yarmand, and S. H. Razavi. 2008. "Effect of Microencapsulation and Resistant Starch on the Probiotic Survival and Sensory Properties of Synbiotic Ice Cream." *Food Chemistry* 111 (1):50–55. https://doi.org/10.1016/j.foodchem.2008.03.036.

Inoue, Azusa, Yoshimitsu Murata, Hiroshi Takahashi, Naoko Tsuji, Shingo Fujisaki, and Jun Ichi Kato. 2008. "Involvement of an Essential Gene, mviN, in Murein Synthesis in Escherichia Coli." *Journal of Bacteriology* 190 (21):7298–7301. https://doi.org/10.1128/JB.00551-08.

Isolauri, E, T Arvola, Y Sütas, E Moilanen, and S Salminen. 2000. "Probiotics in the Management of Atopic Eczema." *Clinical and Experimental Allergy : Journal* 30 (11):1604–10. https://doi.org/cea943 [pii].

Jain, Prashant K., Clare E. McNaught, Alexander D.G. Anderson, John MacFie, and Charles J. Mitchell. 2004. "Influence of Synbiotic Containing Lactobacillus Acidophilus La5, Bifidobacterium Lactis Bb 12, Streptococcus Thermophilus, Lactobacillus Bulgaricus and Oligofructose on Gut Barrier Function and Sepsis in Critically Ill Patients: A Randomised Controlled Trial." *Clinical Nutrition* 23 (4):467–75. https://doi.org/10.1016/j.clnu.2003.12.002.

Jungersen, Mikkel, Anette Wind, Eric Johansen, Jeffrey Christensen, Birgitte Stuer-Lauridsen, and Dorte Eskesen. 2014. "The Science behind the Probiotic Strain Bifidobacterium Animalis Subsp. Lactis BB-12®." *Microorganisms* 2 (2):92–110. https://doi.org/10.3390/microorganisms2020092.

Juntunen, M, P V Kirjavainen, A C Ouwehand, S J Salminen, and E Isolauri. 2001. "Adherence of Probiotic Bacteria to Human Intestinal Mucus in Healthy Infants and during Rotavirus Infection." *Clinical and Diagnostic Laboratory Immunology* 8 (2):293–96. https://doi.org/10.1128/CDLI.8.2.293-296.2001.

Kajander, K., E. Myllyluoma, M. Rajilić-Stojanović, S. Kyrönpalo, M. Rasmussen, S. Järvenpää, E. G. Zoetendal, W. M. De Vos, H. Vapaatalo, and R. Korpela. 2008. "Clinical Trial: Multispecies Probiotic Supplementation Alleviates the Symptoms of Irritable Bowel Syndrome and Stabilizes Intestinal Microbiota." *Alimentary Pharmacology and Therapeutics* 27 (1):48–57. https://doi.org/10.1111/j.1365-2036.2007.03542.x.

Kankaanpää, Pasi E., Baoru Yang, Heikki P. Kallio, Erika Isolauri, and Seppo J. Salminen. 2002. "Influence of Probiotic Supplemented Infant Formula on Composition of Plasma Lipids in Atopic Infants." *Journal of Nutritional Biochemistry* 13 (6):364–69. https://doi.org/10.1016/S0955-2863(02)00185-7.

Karami, N, F Nowrouzian, I Adlerberth, and A E Wold. 2006. "Tetracycline Resistance in Escherichia Coli and Persistence in the Infantile Colonic Microbiota." *Antimicrob Agents Chemother* 50 (1):156–61. https://doi.org/50/1/156 [pii]\r10.1128/AAC.50.1.156-161.2006.

Kazimierczak, Katarzyna A., Harry J. Flint, and Karen P. Scott. 2006. "Comparative Analysis of Sequences Flanking tet(W) Resistance Genes in Multiple Species of Gut Bacteria." *Antimicrobial Agents and Chemotherapy* 50 (8):2632–39. https://doi.org/10.1128/AAC.01587-05.

Kirjavainen, Pirkka V., T. Arvola, S. J. Salminen, and E. Isolauri. 2002. "Aberrant Composition of Gut Microbiota of Allergic Infants: A Target of Bifidobacterial Therapy at Weaning?" *Gut* 51 (1):51–55. https://doi.org/10.1136/gut.51.1.51.

Laake, Knut Ole, A. Bjørneklett, G. Aamodt, L. Aabakken, M. Jacobsen, A. Bakka, and M. H. Vatn. 2005. "Outcome of Four Weeks' Intervention with Probiotics on Symptoms and Endoscopic Appearance after Surgical Reconstruction with a J-Configurated Ileal-Pouch-Anal-Anastomosis in Ulcerative Colitis." *Scandinavian Journal of Gastroenterology* 40 (1):43–51. https://doi.org/10.1080/00365520410009339.

Laparra, J. M., and Y. Sanz. 2009. "Comparison of in Vitro Models to Study Bacterial Adhesion to the Intestinal Epithelium." *Letters in Applied Microbiology* 49 (6):695–701. https://doi.org/10.1111/j.1472-765X.2009.02729.x.

Larsen, C. N., S. Nielsen, P. Kæstel, E. Brockmann, M. Bennedsen, H. R. Christensen, D. C. Eskesen, B. L. Jacobsen, and K. F. Michaelsen. 2006. "Dose-Response Study of Probiotic Bacteria Bifidobacterium Animalis Subsp Lactis BB-12 and Lactobacillus Paracasei Subsp Paracasei CRL-341 in Healthy Young Adults." *European Journal of Clinical Nutrition* 60 (11):1284–93. https://doi.org/10.1038/sj.ejcn.1602450.

Laursen, M.F., Laursen, R. P., Larnkjær, A., Michaelsen, K.F., Babl, M.I., and Licht, T. R., 2017. "Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation." *BMC Microbiol* 17;17(1):175. doi: 10.1186/s12866-017-1090-7.

Latvala, Sinikka, Taija E. Pietilä, Ville Veckman, Riina A. Kekkonen, Soile Tynkkynen, Riitta Korpela, and Ilkka Julkunen. 2008. "Potentially Probiotic Bacteria Induce Efficient Maturation but Differential Cytokine Production in Human Monocyte-Derived Dendritic Cells." *World Journal of Gastroenterology* 14 (36):5570–83. https://doi.org/10.3748/wjg.14.5570.

Linn, Y.H., Thu, K.K., and Win, N.H.H. 2018. "Effect of Probiotics for the Prevention of Acute Radiation-Induced Diarrhoea Among Cervical Cancer Patients: a Randomized Double-Blind Placebo-Controlled Study". *Probiotics Antimicrob Proteins*, May 17, pp 1-10. Doi: 10.1007/s12602-018-9408-9.

Livermore, David M. 2005. "Tigecycline: What Is It, and Where Should It Be Used?" *Journal of Antimicrobial Chemotherapy* 56 (4):611–14. https://doi.org/10.1093/jac/dki291.

López, Patricia, Miguel Gueimonde, Abelardo Margolles, and Ana Suárez. 2010. "Distinct Bifidobacterium Strains Drive Different Immune Responses in Vitro." *International Journal of Food Microbiology* 138 (1–2):157–65. https://doi.org/10.1016/j.ijfoodmicro.2009.12.023.

Lugli, G.A., Mancino, W., Milani, C., Duranti, S., Mancabelli, L., Napoli, S., Mangifesta, M., Viappiani, A., Anzalone, R., Longhi, G., van Sinderen, D., Ventura, M., and Turroni, F. 2019. Dissecting the evolutionary development of the species Bifidobacterium animalis through comparative genomics analysis. Appl Environ Microbiol 85: e02806-18. https://doi.org/10.1128/AEM.02906-18.

Macovei, Lilia, and Ludek Zurek. 2006. "Ecology of Antibiotic Resistance Genes: Characterization of Enterococci from Houseflies Collected in Food Settings." *Applied and Environmental Microbiology* 72 (6):4028–35. https://doi.org/10.1128/AEM.00034-06.

Magariños, Haroldo, Sade Selaive, Marcia Costa, Mirtza Flores, and Olivia Pizarro. 2007. "Viability of Probiotic Micro-Organisms (Lactobacillus Acidophilus La-5 and Bifidobacterium Animalis Subsp. Lactis Bb-12) in Ice Cream." *International Journal of Dairy Technology* 60 (2):128–34. https://doi.org/10.1111/j.1471-0307.2007.00307.x.

Masco, Liesbeth, K. Van Hoorde, E. De Brandt, J. Swings, and G. Huys. 2006. "Antimicrobial Susceptibility of Bifidobacterium Strains from Humans, Animals and Probiotic Products." *Journal of Antimicrobial Chemotherapy* 58 (1):85–94. https://doi.org/10.1093/jac/dkl197.

Matsumoto, M., A. Aranami, A. Ishige, K. Watanabe, and Y. Benno. 2007. "LKM512 Yogurt Consumption Improves the Intestinal Environment and Induces the T-Helper Type 1 Cytokine in Adult Patients with

Intractable Atopic Dermatitis." *Clinical and Experimental Allergy* 37 (3):358–70. https://doi.org/10.1111/j.1365-2222.2007.02642.x.

Mättö, Jaana, Angela H.A.M. van Hoek, Konrad J. Domig, Maria Saarela, Ana Belén Floréz, Elke Brockmann, Ernst Amtmann, Baltasar Mayo, Henk J.M. Aarts, and Morten Danielsen. 2007. "Susceptibility of Human and Probiotic Bifidobacterium Spp. to Selected Antibiotics as Determined by the Etest Method." *International Dairy Journal* 17 (9):1123–31. https://doi.org/10.1016/j.idairyj.2007.01.008.

Mayrhofer, Sigrid, Konrad J Domig, Ernst Amtmann, Angela H a M Van Hoek, Agnes Petersson, Christiane Mair, Helmut K Mayer, and Wolfgang Kneifel. 2007. "Antibiotic Susceptibility of Bifidobacterium Thermophilum and Bifidobacterium Pseudolongum Isolates from Animal Sources." *Journal of Food Protection* 70 (1):119–24. https://doi.org/10.4315/0362-028X-70.1.119.

Melville, Claire M., Romain Brunel, Harry J. Flint, and Karen P. Scott. 2004. "The Butyrivibrio Fibrisolvens tet(W) Gene Is Carried on the Novel Conjugative Transposon TnB1230, Which Contains Duplicated Nitroreductase Coding Sequences." *Journal of Bacteriology* 186 (11):3656–59. https://doi.org/10.1128/JB.186.11.3656-3659.2004.

Meng, Huicui, Zhaoyong Ba, Yujin Lee, Jiayu Peng, Junli Lin, Jennifer A. Fleming, Emily J. Furumoto, Robert F. Roberts, Penny M. Kris-Etherton, and Connie J. Rogers. 2017. "Consumption of Bifidobacterium Animalis Subsp. Lactis BB-12 in Yogurt Reduced Expression of TLR-2 on Peripheral Blood-Derived Monocytes and pro-Inflammatory Cytokine Secretion in Young Adults." *European Journal of Nutrition* 56 (2). Springer Berlin Heidelberg:649–61. https://doi.org/10.1007/s00394-015-1109-5.

Merenstein D.J., Tan, Tina P. , Molokin, Aleksey , Smith, Keisha Herbin , Roberts, Robert F. , Shara, Nawar M. , Mete, Mihriye , Sanders, Mary Ellen, Solano-Aguilar, Gloria, Daniel J. 2015. "Safety of Bifidobacterium Animalis Subsp. Lactis (B. Lactis) Strain BB-12 -Supplemented Yogurt in Healthy Adults on Antibiotics: A Phase I Safety Study." *Gut Microbes* 6 (1):66–77. https://doi.org/10.1080/19490976.2015.1005484.

Merenstein, D. J., K. H. Smith, M. Scriven, R. F. Roberts, M. E. Sanders, and S. Petterson. 2010. "The Study to Investigate the Potential Benefits of Probiotics in Yogurt, a Patient-Oriented, Double-Blind, Cluster-Randomised, Placebo-Controlled, Clinical Trial." *European Journal of Clinical Nutrition* 64 (7). Nature Publishing Group:685–91. https://doi.org/10.1038/ejcn.2010.30.

Merenstein, D., J. Gonzalez, A. G. Young, R. F. Roberts, M. E. Sanders, and S. Petterson. 2011. "Study to Investigate the Potential of Probiotics in Children Attending School." *European Journal of Clinical Nutrition* 65 (4). Nature Publishing Group:447–53. https://doi.org/10.1038/ejcn.2010.290.

Milani, Christian, Sabrina Duranti, Gabriele Andrea Lugli, Francesca Bottacini, Francesco Strati, Stefania Arioli, Elena Foroni, Francesca Turroni, Douwe van Sinderen, and Marco Ventura. 2013. "Comparative Genomics of Bifidobacterium Animalis Subsp. Lactis Reveals a Strict Monophyletic Bifidobacterial Taxon." *Applied and Environmental Microbiology* 79 (14):4304–15. https://doi.org/10.1128/AEM.00984-13.

Mogensen G, S Salminen, J O'Brien, A Ouwehand, W Holzapfel, C Shortt, R Fonden, GD Miller, D Donohue, M Playne, R Crittenden, BB Salvadori, R Zink. 2002. "Food Microorganisms with Health Benefits, Safety Evaluation and Strains with Documented History of Use in Foods." *Bull IDF* 377: 10-19

Mohan, Ruchika, Corinna Koebnick, Janko Schildt, Manfred Mueller, Michael Radke, and Michael Blaut. 2008. "Effects of Bifidobacterium Lactis Bb12 Supplementation on Body Weight, Fecal pH, Acetate, Lactate, Calprotectin, and IgA in Preterm Infants." *Pediatric Research* 64 (4):418–22. https://doi.org/10.1203/PDR.0b013e318181b7fa.

Mohan, Ruchika, Corinna Koebnick, Janko Schildt, Sabine Schmidt, Manfred Mueller, Mike Possner, Michael Radke, and Michael Blaut. 2006. "Effects of Bifidobacterium Lactis Bb12 Supplementation on Intestinal

Microbiota of Preterm Infants: A Double-Blind, Placebo-Controlled, Randomized Study." *Journal of Clinical Microbiology* 44 (11):4025–31. https://doi.org/10.1128/JCM.00767-06.

Moubareck, C., F. Gavini, L. Vaugien, M. J. Butel, and F. Doucet-Populaire. 2005. "Erratum: Antimicrobial Susceptibility of Bifidobacteria (Journal of Antimicrobial Chemotherapy (2005) Vol. 55 (38-44))." *Journal of Antimicrobial Chemotherapy*, https://doi.org/10.1093/jac/dki162.

Nieuwboer, M. van den, E. Claassen, L. Morelli, F. Guarner, and R. J. Brummer. 2014. "Probiotic and Synbiotic Safety in Infants under Two Years of Age." *Beneficial Microbes* 5 (1):45–60. https://doi.org/10.3920/BM2013.0046.

Ouwehand, Arthur C., Nynke Bergsma, Riikka Parhiala, Sampo Lahtinen, Miguel Gueimonde, Harriet Finne-Soveri, Timo Strandberg, Kaisu Pitkälä, and Seppo Salminen. 2008. "Bifidobacterium Microbiota and Parameters of Immune Function in Elderly Subjects." *FEMS Immunology and Medical Microbiology* 53 (1):18–25. https://doi.org/10.1111/j.1574-695X.2008.00392.x.

Palaria, Amrita, Ivy Johnson-Kanda, and Daniel J. O'Sullivan. 2012. "Effect of a Synbiotic Yogurt on Levels of Fecal Bifidobacteria, Clostridia, and Enterobacteria." *Applied and Environmental Microbiology* 78 (4):933–40. https://doi.org/10.1128/AEM.05848-11.

Pariza, M.W., K. Gillies, S.F. Kraak-Ripple, G. Leyer, and A.B. Smith. 2015. "Determining the safety of microbial cultures for consumption by humans and animals". *Reg Tox & Pharm* Vol 73 Issue 1, p. 164-171.

Pérez E, Samper S, Bordas Y, Guilhot C, Gicquel B, Martín C. 2001. An essential role for phoP in Mycobacterium tuberculosis virulence. Mol Microbiol 41:179–87.

Pitkala, K H, T E Strandberg, U H Finne Soveri, a C Ouwehand, T Poussa, and S Salminen. 2007. "Fermented Cereal with Specific Bifidobacteria Normalizes Bowel Movements in Elderly Nursing Home Residents. A Randomized, Controlled Trial." *The Journal of Nutrition, Health & Aging* 11 (4):305–11.

Polit, A., Yang, H., and Amund, D. 2018. Investigating the transmissibility of tet(W) in bifidobacteria exposed to acid and bile stress. Biosci Microbiota Food Health. 2018;37 (2): 39-43.

Raeisi, S.N., Ghoddusi, H.B., Boll, E.J., Farahmand, N., Stuer-Lauriden, Johansen, E., Sutherland, J., and Ouoba, L. I.I., 2018. *Food control* 94:205-211. doi: https://doi.org/10.1016/j.foodcont.2018.07.016.

Rautava, Samuli, Heikki Arvilommi, and Erika Isolauri. 2006. "Specific Probiotics in Enhancing Maturation of IgA Responses in Formula-Fed Infants." *Pediatric Research* 60 (2):221–24. https://doi.org/10.1203/01.pdr.0000228317.72933.db.

Reddy, B. S., J. MacFie, M. Gatt, C. N. Larsen, S. S. Jensen, and T. D. Leser. 2007. "Randomized Clinical Trial of Effect of Synbiotics, Neomycin and Mechanical Bowel Preparation on Intestinal Barrier Function in Patients Undergoing Colectomy." *British Journal of Surgery* 94 (5):546–54. https://doi.org/10.1002/bjs.5705.

Rinkinen, Minna, Elias Westermarck, Seppo Salminen, and Arthur C. Ouwehand. 2003. "Absence of Host Specificity for in Vitro Adhesion of Probiotic Lactic Acid Bacteria to Intestinal Mucus." *Veterinary Microbiology* 97 (1–2):55–61. https://doi.org/10.1016/S0378-1135(03)00183-4.

Rizzardini, Giuliano, Dorte Eskesen, Philip C. Calder, Amedeo Capetti, Lillian Jespersen, and Mario Clerici. 2012. "Evaluation of the Immune Benefits of Two Probiotic Strains Bifidobacterium Animalis Ssp. Lactis BB-12® And Lactobacillus Paracasei Ssp. Paracasei, L. Casei 431 ?? In an Influenza Vaccination Model: A Randomised, Double-Blind, Placebo-Controlled Study." *British Journal of Nutrition* 107 (6):876–84. https://doi.org/10.1017/S000711451100420X.

Roberts, Marilyn C. 2005. "Update on Acquired Tetracycline Resistance Genes." *FEMS Microbiology Letters*. https://doi.org/10.1016/j.femsle.2005.02.034.

Saarela, Maria, I. Virkajärvi, H. L. Alakomi, T. Mattila-Sandholm, A. Vaari, T. Suomalainen, and J. Mättö. 2005. "Influence of Fermentation Time, Cryoprotectant and Neutralization of Cell Concentrate on Freeze-Drying Survival, Storage Stability, and Acid and Bile Exposure of Bifidobacterium Animalis Ssp. Lactis Cells Produced without Milk-Based Ingredients." *Journal of Applied Microbiology* 99 (6):1330–39. https://doi.org/10.1111/j.1365-2672.2005.02742.x.

Saarela, Maria, Ilkka Virkajärvi, Hanna Leena Alakomi, Pia Sigvart-Mattila, and Jaana Mättö. 2006. "Stability and Functionality of Freeze-Dried Probiotic Bifidobacterium Cells during Storage in Juice and Milk." *International Dairy Journal* 16 (12):1477–82. https://doi.org/10.1016/j.idairyj.2005.12.007.

Saarela, Maria, Johanna Maukonen, Atte von Wright, Terttu Vilpponen-Salmela, Andrea J. Patterson, Karen P. Scott, Heikki Hämynen, and Jaana Mättö. 2007. "Tetracycline Susceptibility of the Ingested Lactobacillus Acidophilus LaCH-5 and Bifidobacterium Animalis Subsp. Lactis Bb-12 Strains during Antibiotic/probiotic Intervention." *International Journal of Antimicrobial Agents* 29 (3):271–80. https://doi.org/10.1016/j.ijantimicag.2006.09.020.

Saavedra, Jose M., Adel Abi-Hanna, Nancy Moore, and Robert H. Yolken. 2004. "Long-Term Consumption of Infant Formulas Containing Live Probiotic Bacteria: Tolerance and Safety." *American Journal of Clinical Nutrition* 79 (2):261–67. https://doi.org/00029165.

Sanders, Mary Ellen. 2006. "Summary of Probiotic Activities of Bifidobacterium Lactis HN019." *Journal of Clinical Gastroenterology*. https://doi.org/10.1097/01.mcg.0000225576.73385.f0.

Savard, Patricia, Benoît Lamarche, Marie Eve Paradis, Hélène Thiboutot, Émilie Laurin, and Denis Roy. 2011. "Impact of Bifidobacterium Animalis Subsp. Lactis BB-12 And, Lactobacillus Acidophilus LA-5-Containing Yoghurt, on Fecal Bacterial Counts of Healthy Adults." *International Journal of Food Microbiology* 149 (1). Elsevier B.V.:50–57. https://doi.org/10.1016/j.ijfoodmicro.2010.12.026.

Scott, K P, C M Melville, T M Barbosa, and H J Flint. 2000. "Occurrence of the New Tetracycline Resistance Gene tet(W) in Bacteria from the Human Gut." *Antimicrobial Agents and Chemotherapy* 44 (3):775–77. https://doi.org/10.1128/AAC.44.3.775-777.2000.

Sheu, Bor Shyang, Hsiu Chi Cheng, Ai Wen Kao, Shan Tair Wang, Yao Jong Yang, Hsiao Bai Yang, and Jiunn Jong Wu. 2006. "Pretreatment with Lactobacillus- and Bifidobacterium-Containing Yogurt Can Improve the Efficacy of Quadruple Therapy in Eradicating Residual Helicobacter Pylori Infection after Failed Triple Therapy." *American Journal of Clinical Nutrition* 83 (4):864–69.

Simpson, P. J., C. Stanton, G. F. Fitzgerald, and R. P. Ross. 2005. "Intrinsic Tolerance of Bifidobacterium Species to Heat and Oxygen and Survival Following Spray Drying and Storage." *Journal of Applied Microbiology* 99 (3):493–501. https://doi.org/10.1111/j.1365-2672.2005.02648.x.

Smith, Tracey J., Diane Rigassio-Radler, Robert Denmark, Timothy Haley, and Riva Touger-Decker. 2013. "Effect of Lactobacillus Rhamnosus LGG and Bifidobacterium Animalis Ssp. Lactis BB-12 On Health-Related Quality of Life in College Students Affected by Upper Respiratory Infections." *British Journal of Nutrition* 109 (11):1999–2007. https://doi.org/10.1017/S0007114512004138.

Sullivan, Åsa, Lisbeth Barkholt, and Carl Erik Nord. 2003. "Lactobacillus Acidophilus, Bifidobacterium Lactis and Lactobacillus F19 Prevent Antibiotic-Associated Ecological Disturbances of Bacteroides Fragilis in the Intestine." *Journal of Antimicrobial Chemotherapy* 52 (2):308–11. https://doi.org/10.1093/jac/dkg346.

Szajewska, H, and A Chmielewska. 2013. "Growth of Infants Fed Formula Supplemented with Bifidobacterium Lactis Bb12 or Lactobacillus GG: A Systematic Review of Randomized Controlled Trials." *BMC Pediatrics* 13 (1). BMC Pediatrics. https://doi.org/10.1186/1471-2431-13-185.

Taipale T, K Pienihakkinen, E Isolauri, C Larsen, E Brockmann, P Alanen, J Jokela, and E Soderling. 2011. "Bifidobacterium animalis subsp. lactis BB-12 in reducing the risk of infections in infancy." *British Journal of Nutrition* 105: 409–416. doi:10.1017/S0007114510003685

Taipale, T., K. Pienihäkkinen, P. Alanen, J. Jokela, and E. Söderling. 2013. "Administration of Bifidobacterium animalis subsp. Lactis Bb-12 in Early Childhood: A Post-Trial Effect on Caries Occurrence at Four Years of Age." *Caries Research* 47 (5):364–72. https://doi.org/10.1159/000348424.

Taipale, T., K. Pienihäkkinen, S. Salminen, J. Jokela, and E. Söderling. 2012. "Bifidobacterium Animalis Subsp. Lactis BB-12 Administration in Early Childhood: A Randomized Clinical Trial of Effects on Oral Colonization by Mutans Streptococci and the Probiotic." *Caries Research* 46 (1):69–77. https://doi.org/10.1159/000335567.

Taipale, Teemu J., Kaisu Pienihäkkinen, Erika Isolauri, Jorma T. Jokela, and Eva M. Söderling. 2016. "Bifidobacterium Animalis subsp. Lactis BB-12 in Reducing the Risk of Infections in Early Childhood." *Pediatric Research* 79 (1):65–69. https://doi.org/10.1038/pr.2015.174.

Tally, F. P., G. J. Cuchural, N. V. Jacobus, S. L. Gorbach, K. Aldridge, T. Cleary, S. M. Finegold, G. Hill, P. Iannini, and J. P. O'Keefe. 1985. "Nationwide Study of the Susceptibility of the Bacteroides Fragilis Group in the United States." *Antimicrobial Agents and Chemotherapy* 28 (5):675–77. https://doi.org/10.1128/AAC.28.5.675.

Toiviainen, Aino, Heli Jalasvuori, Emilia Lahti, Ulvi Gursoy, Seppo Salminen, Margherita Fontana, Susan Flannagan, et al. 2015. "Impact of Orally Administered Lozenges with Lactobacillus Rhamnosus GG and Bifidobacterium Animalis subsp. Lactis BB-12 on the Number of Salivary Mutans Streptococci, Amount of Plaque, Gingival Inflammation and the Oral Microbiome in Healthy Adults." *Clinical Oral Investigations* 19 (1):77–83. https://doi.org/10.1007/s00784-014-1221-6.

Vernazza, Claire L., Glenn R. Gibson, and Robert A. Rastall. 2006. "Carbohydrate Preference, Acid Tolerance and Bile Tolerance in Five Strains of Bifidobacterium." *Journal of Applied Microbiology* 100 (4):846–53. https://doi.org/10.1111/j.1365-2672.2006.02832.x.

Villedieu, A, A P Roberts, E Allan, H Hussain, R Mcnab, D A Spratt, M Wilson, and P Mullany. 2007. "Determination of the Genetic Support for tet{W} in Oral Bacteria." *Antimicrobial Agents and Chemotherapy* 51 (6):2195–97.

Villedieu, A., M. L. Diaz-Torres, N. Hunt, R. McNab, D. A. Spratt, M. Wilson, and P. Mullany. 2003. "Prevalence of Tetracycline Resistance Genes in Oral Bacteria." *Antimicrobial Agents and Chemotherapy* 47 (3):878–82. https://doi.org/10.1128/AAC.47.3.878-882.2003.

Vinderola, C. 2003. "Lactic Acid Starter and Probiotic Bacteria: A Comparative "*In Vitro*" Study of Probiotic Characteristics and Biological Barrier Resistance." *Food Res. Int.*, 36:895–904.

Vlieger, Arine M., Afke Robroch, Stef Van Buuren, Jeroen Kiers, Ger Rijkers, Marc A. Benninga, and Rob Te Biesebeke. 2009. "Tolerance and Safety of Lactobacillus Paracasei Ssp. Paracasei in Combination with Bifidobacterium Animalis ssp. Lactis in a Prebiotic-Containing Infant Formula: A Randomised Controlled Trial." *British Journal of Nutrition* 102 (6):869–75. https://doi.org/10.1017/S0007114509289069.

Vrese, Michael De, Holger Kristen, Peter Rautenberg, Christiane Laue, and Jürgen Schrezenmeir. 2011. "Probiotic Lactobacilli and Bifidobacteria in a Fermented Milk Product with Added Fruit Preparation Reduce Antibiotic

Associated Diarrhea and Helicobacter Pylori Activity." *Journal of Dairy Research* 78 (4):396–403. https://doi.org/10.1017/S002202991100063X.

Wang, Kuan Yuan, Shui Nin Li, Chiang Shin Liu, Daw Shyong Perng, Yu Chung Su, Deng Chyang Wu, Chang Ming Jan, Chun Huang Lai, Tsu Nai Wang, and Wen Ming Wang. 2004. "Effects of Ingesting Lactobacillus- and Bifidobacterium-Containing Yogurt in Subjects with Colonized Helicobacter Pylori." *American Journal of Clinical Nutrition* 80 (3):737–41. https://doi.org/80/3/737 [pii].

Weizman, Zvi, Ghaleb Asli, and Ahmed Alsheikh. 2005. "Effect of a Probiotic Infant Formula on Infections in Child Care Centers: Comparison of Two Probiotic Agents." *Pediatrics* 115 (1):5–9. https://doi.org/10.1542/peds.2004-1815.

WHO List. https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia/en/

Wildt, S., Munck, L.K., Vinter-Jensen, L., Hanse, B.F., Nordgaard-Lassen, I., Christensen, S., and Avnstroem, S. 2006. "Probiotic treatement of collagenous colitis: A randomized, double-blind, placebo-controlled trial with *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis*". *Inflamatory Bowel Diseases.* Vol 12, Issue 5, 1 May 2016, p 395-401. https:// doi.org/10.1097/01.MIB.0000218763.99223.49.

Wildt, Signe, Inge Nordgaard, Ulla Hansen, Elke Brockmann, and Jüri J. Rumessen. 2011. "A Randomised Double-Blind Placebo-Controlled Trial with Lactobacillus Acidophilus La-5 and Bifidobacterium Animalis Subsp. Lactis BB-12 for Maintenance of Remission in Ulcerative Colitis." *Journal of Crohn's and Colitis* 5 (2). European Crohn's and Colitis Organisation:115–21. https://doi.org/10.1016/j.crohns.2010.11.004.

Wilson, M. 2005. *Microbial inhabitants of humans: their ecology and role in health and disease.* Cambridge University Press, Cambridge, U.K.

Yazid AM, Ali AM, Shuhaimi M, Kalaivaani V, Rokiah MY, Reezal A. 2000. "Antimicrobial Susceptibility of Bifidobacteria." *Lett. Appl. Microbiol.* 31:57-62.

Appendix 1

CHR. HANSEN

Improving food & health

Probio-Tec® BB-12® Blend-100 V2

Product Information
Version: 1 PI EU EN 05-12-2015

Material no.	713484
Description	Probio-Tec® BB-12® Blend-100 V2 is a standardized white to light beige fine powder consisting of a freeze-dried culture.
Taxonomy	Bifidobacterium BB-12®
Ingredients	Culture and maltodextrin.
Technical Data	**Cell Count** This product has a minimum potency of 100 billion (1.0E+11) CFU (Colony Forming Units) per gram of powder by end of bulk shelf life, provided the product is stored according to "Storage and handling".
Application	This is a semi-finished product for production of dietary supplements or pharma products. A food safety risk assessment has been carried out based on consumption by healthy consumers above 1 year of age. However, the risk assessment of the final product remains the marketer's responsibility. Please observe that the final product might be regulated by food or medicinal product legislation, nationally. If you wish to discuss the legality of use, please contact your Chr. Hansen representative for assistance.
Packaging	**Primary packaging:** 5 kg per alu pouch **Secondary packaging:** 1 alu pouch per box
Storage and handling	**Temperature:** 2 - 8 °C / 36 - 46 °F **Handling** Store bulk probiotics in the original or tightly closed foil pouch under refrigerated conditions. Allow the product to come to room temperature prior to use. To ensure optimum product quality and enhanced stability of the final product, follow Chr. Hansen's handling and packaging procedure for standardized bulk blends.
Shelf life	In the original sealed packaging, the product has a minimum shelf life of 24 months from date of manufacture when stored according to "Storage and handling". The shelf life of any finished product manufactured from this material will depend on its formulation, packaging and storage conditions and should be established separately.

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Appendix 2

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nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	[redacted]
Date of Manufacture:	04.2018
Best Before Date:	04.2020

Performance	**Result**	**Specification**
Total cell count cfu/g	4.4E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	**Specification**
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

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nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	[redacted]
Date of Manufacture:	08.2018
Best Before Date:	08.2020

Performance	Result	Specification
Total cell count cfu/g	>3.0E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	Specification
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

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nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	
Date of Manufacture:	09.2018
Best Before Date:	09.2020

Performance	Result	Specification
Total cell count cfu/g	8.8E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	Specification
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

Appendix 3

Appendix 1: Pariza et. al. Decision Tree Analysis for Determining the Safety of Microbial Culture for Human Consumption *Bifidobacterium animalis* ssp *lactis* BB-12®

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

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

[ii] Whole Genome Sequencing provides distinct advantages for identification and characterization of microorganisms. In-depth analysis, including functional and comparative genomic studies, is afforded by sequencing the whole genome. This technology can provide a wealth of information that can be used for identification and characterization, including evidence of genetic evolution for adaption of a species to a nutrient-rich environment, such as dairy products or the gastrointestinal tract (Pfeiler, EA, Klaenhammer, TR. 2007. The genomics of lactic acid bacteria. TRENDS in Mibiol, 14(12): 546-553). Less comprehensive molecular analysis, such as RAPD, FISH, and MLST, may also provide adequate information for identification, but the characterization ability is often times limited within a bacterial species (Gosiewski, T, Chnielarczk, A, Strusm M, Brzychczy-Wlock, M, Heczko, PB. 2012. The application of genetics methods to differentiation of three *Lactobacillus* species of human origin. Ann Microbiol62:1437-1445)

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

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

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

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

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

[viii] in this context the tern 'antimicrobial substances' refers to antibiotics that are used in medical or veterinary applications, for example substances that are positive in the JECFA test (FAO. 1981. *FAO Food and Nutrition Paper: 25th Session of the Joint FAO/WHO Expert Committee on Food Additives,* Appendix A, pp. 317-318, FAO/WHO, Geneva, Switzerland).

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

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

[xi] Food fermentations, e.g. Cheddar cheese or yogurt, commonly result in "substantial" microbial food culture populations of $10^6 - 10^8$ colony forming units per gram of the food. Significance should be judged relative to the fermented food, i.e., numbers of different organisms in a microbial population may change during the course of the life of the fermented food, e.g., Lactobacilli counts in Cheddar cheese are routinely low in the initial stages of cheese maturation, but begin to increase in numbers while the Lactococci, responsible for initial acid production, count decreases as the cheese ripens and pH decreases. [Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. N.S. Fitzsimons, T.M. Cogan, S. Condon, T. Beresford. Journal of Applied Microbiology 90(4): 600-608, 2001; Kosikowski, F. V., and V.V. Mistry. Chees and Fermented Milk Foods. 1997. 3rd Ed. F. V. Kosikoski, L.L.D. Westport, CT.].

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

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

[xiv] For example, the Qualified Presumption of Safety list (http://www.efsa.europa.eu/en/topics/topic/qps.htm) prepared and periodically updated by the European Food Safety Authority is the output from a systematic safety review of the included microorganisms by qualified experts.

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

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

Stice, Szabina

From: Highbarger, Lane A
Sent: Wednesday, May 8, 2019 10:50 AM
To: Emily Gregoire
Cc: Stice, Szabina
Subject: RE: Filing notice L. rhamnosus LGG - GRN 845

Dear Ms. Gregoire,

Thank you for the updated contact information.

I have cc:'ed our office contact who deals with incoming notices and hopefully she can answer your question about the status of your GRAS notice for *Bifidobacteria animalis subs. lactis* BB-12.

Lane A. Highbarger, Ph.D.
Microbiology and Regulatory Review
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
(w) – 240-402-1204

From: Emily Gregoire <USEMGR@chr-hansen.com>
Sent: Wednesday, May 08, 2019 10:26 AM
To: Highbarger, Lane A <Lane.Highbarger@fda.hhs.gov>
Subject: RE: Filing notice L. rhamnosus LGG - GRN 845
Importance: High

Hello Ms. Highbarger,

I am writing to inform you that unfortunately Ms. Kraak-Ripple is no longer with Chr. Hansen. Therefore, I am asking that you kindly communicate with me regarding GRN 845 going forward.

Additionally, Ms. Kraak-Ripple submitted a GRAS notice for *Bifidobacteria animalis subs. lactis* BB-12®. As far as we know we have not received a filing notice, however I do not have access to Ms. Kraak-Ripple's email so, if possible, I was hoping you could help me track down the person at CFSAN that will be reviewing that dossier so that we can divert correspondence to me.

Thank you in advance and have a great day.

Kind Regards / Venlig hilsen

Emily Gregoire

From: Highbarger, Lane A <Lane.Highbarger@fda.hhs.gov>
Sent: Wednesday, April 24, 2019 8:52 AM
To: Sarah Kraak Ripple <ussakr@chr-hansen.com>
Subject: Filing notice L. rhamnosus LGG - GRN 845

Dear Ms. Kraak-Ripple,

I have enclosed the filing letter for your GRAS notice for *Lactobacillus rhamnosus* LGG for use as an ingredient in food, including: milk and dairy products, such as yogurt and other fermented milk products; dairy alternatives (fermented oat milk, fermented soy milk, fermented almond milk, fermented coconut milk); beverages such as juice and protein shakes; shelf-stable products such as bars (granola bars, protein bars, meal replacement bars); confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings); breakfast cereals at a level 10^8 to 10^{10} cfu/ serving throughout the shelf life of the product.

If you have any questions, do not hesitate to contact me.

Lane A. Highbarger, Ph.D.
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Stephanie Hice, PhD

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration

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November 15, 2019
USEMGR

GRN 000856

Dear Stephanie,

Enclosed is Chr. Hansen's response to the questions regarding GRN No. 856 that were received from FDA on behalf of Stephanie Hice, PhD via email on October 30, 2019.

Please contact me with any further questions.

Yours sincerely,

Emily Gregoire

Probiotics Regulatory Affairs Manager – North America

usemgr@chr-hansen.com
Mobile: (b) (6)

Chr. Hansen Response to FDA's Questions/Comments Regarding GRN 000856:

1. **Please state whether any of the raw materials used in the fermentation media and during production of *Bifidobacterium animalis* subsp. *lactis* DSM 15954 are major allergens or derived from major allergens. Please state whether the final ingredient contains any major allergens.**

Chr. Hansen produces BB-12® products for dietary supplements and conventional foods. Due to this, there may be different formulations for different products. Milk allergen is present in both the fermentation media and finished product ingredients for some forms of BB-12®. We have dairy-free products as well which contain no allergens in either the fermentation media or finished product ingredients. Please see the attached statement regarding our allergen management program (Allergen_Management_EN).

2. **Please clarify whether the manufacture of *B. animalis* DSM 15954 begins with a pure culture.**

Yes. The initial inoculation (Found on page 19 of the dossier, #2 in "individual production steps") material is a pure culture sourced from Chr. Hansen's internal culture collection cell bank. The pure culture is tested for genetic stability as well. This is mentioned in Part 2.4.1 of the original dossier: "Genetic stability during storage".

In the development of our bacterial food cultures, both GMP and Food Safety are implemented according to ISO 22000 to secure food safety. This includes measures described below for selection of cultures, as well as characterization, and production and QC release criteria of inoculation material used for production of our commercial cultures. All bacteria for inoculation originate from the Chr. Hansen's Culture Collection in Denmark, and have been characterized using methods, which were up to date at the time of development.

3. **Please specify whether the manufacturing process is monitored for contamination, and if so, how often this is performed.**

Production environment and product facing areas (zone 1) are tested weekly for pathogens and indicator organisms. Results are stored in a Laboratory Information Management System (LIMS). In addition, every batch of product is tested for pathogens and cross-contaminants which further confirms that the manufacturing process meets standards.

4. **Please indicate if the analytical methods used to analyze the batches for conformance with the stated specifications are validated for that particular purpose.**

The analytical methods used for the testing of specifications are all ISO certified or based on ISO methods. All methods are validated.

5. **A specification was provided for *Enterococci*. However, the certificates of analysis for the batch analyses (Appendix 2) do not provide data for *Enterococci*. Please provide the analysis of 3 non-consecutive batches of *B. animalis* DSM 15954 to demonstrate conformance with the stated *Enterococci* specification.**

In addition to analyses reported on our CoAs, Chr. Hansen performs several other internal-only analyses that we use as specifications for release of our products. These specifications are based on our internal risk-assessment program. All results and specifications for each batch are stored in a database. The data gathered from these internal analyses is also trended and evaluated to detect any possible shifts in product quality and/or safety. Products not meeting these specifications are disposed of. Please see attached three non-consecutive QC batch records including results of internal specifications.

6. **Please clarify whether *B. animalis* DSM 15954 is intended to be used in infant formula and/or foods under the jurisdiction of the U.S. Department of Agriculture (USDA).**

B. animalis DSM 15954 is not intended for use in products under the jurisdiction of the USDA. The use of this strain in infant formula is covered by Grn. No. 049. There are no additional infant uses proposed in this GRAS notification.

Statement

May 23, 2018

Valid two years from date of issue

To whom it may concern

Global quality-, safety- and security programs for food application products

Thank you for your inquiry into Chr. Hansen's products. They are produced according to our policies, GMP's and management systems as stated on https://www.chr-hansen.com/en/about-us/policies-and-positions.

We have Policies for:

- Corporate Governance
- Business integrity
- People, Knowledge & Organization
- Finance & IT
- Quality & Product Safety
- Communication

Linked to our Policy for Quality and Product Safety, we have Positions on:

- Allergens
- Product Security
- Product Safety
- Use of Genetic Techniques

Our quality management systems are the back bone for continuously improving our performance and processes, as well as meeting and exceeding our customer's expectations regarding product quality and services.

Strict focus on quality control, product safety and product security at every stage of our activities, and in the entire value chain is the guarantee to our customers - from raw material selection, surveillance of suppliers through production and packaging, to distribution and application at our customers' premises.

Compliance with food safety standards

All Chr. Hansen's production sites are certified according to FSSC 22000 (food safety and food GMP).

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The FSSC 22000 certification scheme has been recognized by the GFSI (Global Food Safety Initiative), and based on the extensive GFSI benchmarking process our FSSC 22000 certifications ensure that our production processes fulfill the main principles of both:

- The International Food Standard (IFS)
- The British Retail Consortium's (BRC's) Global standard for Food Safety

Our quality and food safety management system based on FSSC 22000:2011 includes:

- Focus on raw material, production and customer/finished products
- Risk assessment of the entire value chain - HACCP
- Food GMP (Good Manufacturing Practise = Prerequisite Programs)
- Allergen management
- Audits (internal, customer audits and third-party audits)
- Customer complaint management
- Documented system for corrective action and continuous improvements
- Training and education of employees
- Crisis management
- Product recall
- Active release of products
- Rework
- Production environment
- Infrastructure

In addition, we have a full range of product documentation for all our products.

Quality assurance and control in Chr. Hansen's production

The quality system in place at all production plants combines food GMP (Good Manufacturing Practice), HACCP (Hazard Analysis of Critical Control Points) and contaminants testing, which is included in the release criteria, as well as parameters related to the performance of our products.

Prevention of microbial contamination of products focuses on the use of HACCP principles to proactively secure food safety, rather than solely on final testing of products. Sampling points, test schedules, and specifications are managed item per item.

The HACCP principles are based upon:

- Identification and control of potential hazard
- Definition and mapping of critical control points (CCPs) and Operational Pre-Requisite Programs (OPRPs)
- Definition of critical limits for CCPs
- Scheduling and reporting of measurements and observations

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Statement

- Procedures for corrective actions, when monitoring deviations from critical limits
- Procedures to verify the effectiveness of the HACCP plan
- Documentation and record keeping

Food security/defense

Chr. Hansen complies with requirements for Food Fraud in FSSC 22000 and thereby GFSI requirements. We are mindful of our obligation to protect and ensure the authenticity of our products. All our production plants have established food defense, traceability, withdrawal, recall, crisis management, vulnerability assessments and facility access controls, and we hold a C-TPAT certificate (against bioterrorism). Furthermore, our Vendor Management Program incorporates food defense and food fraud principles. Our food defense program includes a food fraud vulnerability assessment using the SSAFE tool and a specific vulnerability assessment to prevent intentional adulteration in compliance with both PAS 96:2017 and the Food Safety Modernization Act.

Chr. Hansen's global food GMP governance is described in "Quality, GMP's & Food Safety Principles", which is available on https://www.chr-hansen.com/en/about-us/policies-and-positions. (quality & product safety)

Furthermore, we offer relevant support material such as FSSC certificates, and summary third party audit reports for all our production plants. Full audit reports are considered confidential.

If you have further questions, please contact us.

Yours sincerely
Chr. Hansen A/S
Quality Management

Birte Skov Larsen
QA Specialist

Electronically generated, therefore not signed

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Statement

November 20, 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'.

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The information contained herein is presented in good faith and is, to the best of our knowledge and belief, true and reliable. It is offered solely for your consideration, testing and evaluation, and is subject to change without prior and further notice unless otherwise required by law or agreed upon in writing. There is no warranty being extended as to its accuracy, completeness, currentness, non-infringement, merchantability or fitness for a particular purpose. To the best of our knowledge and belief, the product(s) mentioned herein do(es) not infringe the intellectual property rights of any third party. The product(s) may be covered by pending or issued patents, registered or unregistered trademarks, or similar intellectual property rights. All rights reserved.

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
Global Business Support

Chr. Hansen A/S – Food Cultures & Enzymes
Chr. Hansen Natural Colors A/S

Electronically generated, therefore not signed

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nu-trish® BB-12®

Product Information

Version: 7 PI EU EN 11-08-2019

Description

Thermophilic culture.

The culture is a defined single strain with a long history of safe use. Substantial clinical documentation on possible health benefits are available upon request and likewise are certificates of identification and certificates of safety and origin.

BB-12® is a registered trademark of Chr. Hansen.

Culture composition:
Bifidobacterium

Material No:	706146	Color:	Off-white to slightly reddish or brown
Size	20X25 g	Format:	FD-DVS
Type	Pouch(es) in box	Form:	Granulate

Storage and handling

< -18 °C / < 0 °F

Shelf life

At least 24 months from date of manufacture when stored according to recommendations.
At +5°C (41°F) the shelf life is at least 6 weeks.

Application

Usage

The culture is primarily used in production of probiotic dairy products. The culture can be applied in combination with other lactic acid cultures such as yogurt or mesophilic aromatic cultures (type LD).
A HACCP risk assessment has been carried out for fermented dairy products. For all other applications a risk assessment should be completed before the product is released for sale as food safety hazards will differ from fermented products.

Suggested dosage

It is recommended that BB-12® is inoculated according to the desired probiotic cell count in the final product. This is influenced by the shelf life, the pH and storage temperature of the final product. For fermented products the interaction with other strains as well as fermentation time and temperature may also affect the final probiotic cell count.

Directions for Use

Remove cultures from the freezer just prior to use. Do not thaw Disinfect the package prior to opening. Open the pouch and pour the freeze-dried granules directly into the pasteurized product using slow agitation. Agitate the mixture for 10-15 minutes to distribute the culture evenly. The recommended incubation temperature is dependent on the application in which the culture is used. For more information on specific applications see our technical brochures and suggested recipes.

Range

Single strain BB-12® is available in frozen and freeze-dried form. Blends with BB-12® for production of probiotic fermented milk are also available. They have all been composed to provide a high cell count of BB-12® in the final product when applied according to our recommendations.

Technical Data

The information contained herein is to the best of our knowledge and belief, true and accurate and the product(s) mentioned herein do(es) not infringe the intellectual property rights of any third party. The product(s) may be covered by pending or issued patents, registered or unregistered trademarks, or similar intellectual property rights. All rights reserved.

Other Information
BB-12® is anaerobic (slightly oxygen tolerant). Growth of BB-12® is dependent on a good interaction with other fermenting strains. The culture will not grow in milk by it self, but can grow slowly in milk at temperatures between 37-43°C (98 - 109°F) in synergy with the fermenting culture. BB-12® converts lactose to L+ lactic acid and acetic acid.

BB-12® is very stable and has a high resistance towards acids in fermented milk products.

Analytical Methods
References and analytical methods are available upon request.

Dietary information

Kosher:	Kosher Dairy Excl. Passover
Halal:	Certified
VLOG:	Conform

Legislation
Chr. Hansen's cultures comply with the general requirements on food safety laid down in Regulation 178/2002/EC. Lactic acid bacteria are generally recognized as safe and can be used in food, however, for specific applications we recommend to consult national legislation.

The product is intended for use in food.

Food Safety
No guarantee of food safety is implied or inferred should this product be used in applications other than those stated in the Usage section. Should you wish to use this product in another application, please contact your Chr. Hansen representative for assistance.

Labeling
Suggested labeling "lactic acid culture" or "starter culture", however, as legislation may vary, please consult national legislation.
Labeling with probiotic strain names is possible if a trademark license agreement is in place. Please Contact your local Chr. Hansen representative for further information.

Trademarks
Product names, names of concepts, logos, brands and other trademarks referred to in this document, whether or not appearing in large print, bold or with the ® or TM symbol are the property of Chr. Hansen A/S or an affiliate thereof or used under license. Trademarks appearing in this document may not be registered in your country, even if they are marked with an ®.

Technical support
Chr. Hansen's Application and Product Development Laboratories and personnel are available if you need further information.

GMO Information
In accordance with the below mentioned legislation of the European Union we can inform that:

nu-trish® BB-12® is not a GM (genetically modified) food *.
It does not contain or consist of GMOs and is not produced from GMOs in accordance with Regulation 1829/2003* on GM food and feed.
As such GM labelling is not required for nu-trish® BB-12® or the food it is used to produce**. Moreover, the product does not contain any GM labelled raw materials.

The information contained herein is to the best of our knowledge and belief, true and accurate and the product(s) mentioned herein do(es) not infringe the intellectual property rights of any third party. The product(s) may be covered by pending or issued patents, registered or unregistered trademarks, or similar intellectual property rights. All rights reserved.

** Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.*
*** Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.*

Please note the information presented here does not imply that the product can either be used in, or is externally certified to be used in, food or feed labelled as 'organic' or 'GMO free'. Requirements to make these claims vary per country, please contact us for more information.

Allergen Information

List of common allergens in accordance with the US Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and EU Regulation 1169/2011/EC with later amendments	Present as an ingredient in the product
Cereals containing gluten* and products thereof	No
Crustaceans and products thereof	No
Eggs and products thereof	No
Fish and products thereof	No
Peanuts and products thereof	No
Soybeans and products thereof	No
Milk and products thereof (including lactose)	Yes
Nuts* and products thereof	No
List of allergens in accordance with EU Regulation 1169/2011/EC only	
Celery and products thereof	No
Mustard and products thereof	No
Sesame seeds and products thereof	No
Lupine and products thereof	No
Mollusks and products thereof	No
Sulphur dioxide and sulphites (added) at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO_2	No

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

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MKE QC Lab Requisition Result Report

For Internal Use Only

Date: 13/11/2019
Time generated: 22:08
Generated by: USJHS

Job Name: (b) (4)
Experiment ID:
Link to CINAC Report: CINAC: 706146

Place of Production: AVE
Date First Authorized: 25.03.2019

Sample ID:	Sampling Point:	Sample Comment:
(b) (4)	FD-PK	

(b) (4)	A	Deviation from theoretical Dose Inoc %	2	%			<=10
	A	pH-12h	5.9	pH		>=5.9	
	A	Total Bacillus	<10	cfu/g		<100	
	A	B. cereus	<10	cfu/g		<10	
	A	Verification coagulase-positive staphylococci	Not Applicable				Not Applicable
	A	Coagulase-positive staphylococci	<10	cfu/g	<10	<10	
	A	Microscopy and verification on BEA	Not Applicable				Not Applicable
	A	Enterococci	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Mesophilic lactic acid bacteria	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Thermophilic streptococci	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Lactobacilli/Pediococci	<10	cfu/g		<100	
	A	Non lactic acid bacteria	<10	cfu/g	<500	<500	
	A	Enterobacteriaceae	<10	cfu/g	<10	<10	
	A	Yeasts and moulds	<10	cfu/g	<10	<10	
	A	**Verification of gas producing bacteria (MRS tube)**	**Not applicable**				**Not applicable**
	A	**Gas producing vancomycin-resistent lactic acid bacteria**	**<10**	**cfu/g**		**<10**	
	A	Total cell count	>3.0E+11	cfu/g	>=1E+11	>=3E+11	

Sample ID:	Sampling Point:	Sample Comment:
(b) (4)	FD-PK-AW_E	

(b) (4)	A	Aw	0.03		<0.15

MKE QC Lab Requisition Result Report

For Internal Use Only

Date: 13/11/2019
Time generated: 22:05
Generated by: USJHS

Job Name: 706146/3475308 **Place of Production:** AVE

Experiment ID: **Date First Authorized:** 17.07.2019

Link to CINAC Report: CINAC: 706146

Sample ID: **Sampling Point:** **Sample Comment:**

(b) (4) FD-PK

(b) (4)	A	Deviation from theoretical Dose Inoc %	0	%			<=10
	A	pH-12h	6.0	pH		>=5.9	
	A	Total Bacillus	<10	cfu/g		<100	
	A	B. cereus	<10	cfu/g		<10	
	A	Verification coagulase-positive staphylococci	Not Applicable				Not Applicable
	A	Coagulase-positive staphylococci	<10	cfu/g	<10	<10	
	A	Microscopy and verification on BEA	Not Applicable				Not Applicable
	A	Enterococci	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Mesophilic lactic acid bacteria	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Thermophilic streptococci	<10	cfu/g		<100	
	A	Verification by microscopy	Not applicable				Suspected culture growth
	A	Lactobacilli/Pediococci	<10	cfu/g		<100	
	A	Non lactic acid bacteria	<10	cfu/g	<500	<500	
	A	Enterobacteriaceae	<10	cfu/g	<10	<10	
	A	Yeasts and moulds	<10	cfu/g	<10	<10	
	A	Total cell count	>3.0E+11	cfu/g	>=1E+11	>=3E+11	

Sample ID: **Sampling Point:** **Sample Comment:**

(b) (4) FD-PK-AW_E

(b) (4)	A	Aw	0.08		<0.15

MKE QC Lab Requisition Result Report

For Internal Use Only

Date: 13/11/2019
Time generated: 22:11
Generated by: USJHS

Job Name: (b) (4) **Place of Production:** AVE

Experiment ID: **Date First Authorized:** 08.10.2019

Link to CINAC Report: CINAC: 706146

Sample ID: **Sampling Point:** **Sample Comment:**

(b) (4) FD-PK

(b) (4)	A	Deviation from theoretical Dose Inoc %	0	%			<=10
	A	pH-12h	5.9	pH		>=5.9	
	A	Total Bacillus	<10	cfu/g		<100	
	A	B. cereus	<10	cfu/g		<10	
	A	Verification coagulase-positive staphylococci	Not Applicable				Not Applicable
	A	Coagulase-positive staphylococci	<10	cfu/g	<10	<10	
	A	Microscopy and verification on BEA	Not Applicable				Not Applicable
	A	Enterococci	<10	cfu/g		<100	
	A	Verification by microscopy	No cross contaminat ion				Suspected culture growth
	A	Mesophilic lactic acid bacteria	<10	cfu/g		<100	
	A	Verification by microscopy	No cross contaminat ion				Suspected culture growth
	A	Thermophilic streptococci	<10	cfu/g		<100	
	A	Verification by microscopy	No cross contaminat ion				Suspected culture growth
	A	Lactobacilli/Pediococci	<10	cfu/g		<100	
	A	Non lactic acid bacteria	<10	cfu/g	<500	<500	
	A	Enterobacteriaceae	<10	cfu/g	<10	<10	
	A	Yeasts and moulds	<10	cfu/g	<10	<10	
	A	Total cell count	>3.0E+11	cfu/g	>=1E+11	>=3E+11	

Sample ID: **Sampling Point:** **Sample Comment:**

(b) (4) FD-PK-AW_E

(b) (4)	A	Aw	0.03		<0.15

nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	
Date of Manufacture:	04.2018
Best Before Date:	04.2020

Performance	**Result**	**Specification**
Total cell count cfu/g	4.4E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	**Specification**
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	
Date of Manufacture:	08.2018
Best Before Date:	08.2020

Performance	Result	Specification
Total cell count cfu/g	>3.0E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	Specification
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

nu-trish® BB-12®

Certificate of Analysis

Form:	Freeze-dried DVS
Material No:	706146
Batch no:	
Date of Manufacture:	09.2018
Best Before Date:	09.2020

Performance	Result	Specification
Total cell count cfu/g	8.8E+11	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	Specification
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Electronically generated - signature not required

nu-trish® BB-12®

Product Specification

Form:	Freeze-dried DVS
Material No:	706146
Culture Composition:	Bifidobacterium

Performance	**Specification**
Total cell count cfu/g	>=1E+11

Contaminants are tested and controlled in a relevant combination of samples from the environment, process or products. The set-up is based on HACCP principles as stated in the ISO 27205 I IDF 149:2010 to guarantee that the product fulfills the following specifications

Purity	**Specification**
Coagulase-positive staphylococci cfu/g	<10
Non lactic acid bacteria cfu/g	<500
Enterobacteriaceae cfu/g	<10
Yeasts and moulds cfu/g	<10
Listeria monocytogenes	Absent in 25 g
Salmonella spp.	Absent in 25 g

Storage and shelf life: See labels and product packaging

Electronically generated - signature not required