CHR. HANSEN
Improving food & health

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April 29, 2022
CAWINN

Re: Chr. Hansen GRAS notice for *Lactobacillus gasseri* BNR17™

Dear Dr. Carlson,

In accordance with the final rule of August 17, 2016 (81 FR 159) and 21 CFR Part 170 Subpart E on the Generally Recognized as Safe (GRAS) notice, Chr. Hansen A/S is notifying the U.S. Food and Drug Administration (FDA) of our conclusion that *Lactobacillus (L.) gasseri* BNR17™ (NCIMB 30370) is GRAS through scientific procedures for its intended use as a microbial ingredient in conventional foods, and is not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetics Act*. The maximum incorporation level in conventional foods will be 10^9 to 10^{11} colony-forming units (CFU)/serving to account for loss of viability throughout the shelf life of the product.

Please do not hesitate to contact us should you require any clarifications regarding this GRAS notice.

Yours sincerely,

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
cawinn@chr-hansen.com

cc: Katharine Urbain, Head of North America Regulatory Affairs (uskaur@chr-hansen.com)

Generally Recognized as Safe (GRAS) Conclusion for the Intended Uses of *Lactobacillus gasseri* BNR17™ in Conventional Foods

Prepared by Chr. Hansen A/S

April, 2022

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Abbreviations

AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
BE	Bioengineering
BIOHAZ	Panel on Biological Hazards
BLAST	Basic Local Alignment Search Tool
CCP	Critical Control Points
CFR	Code of Federal Regulations
CFU	Colony forming units
cGMP	Current good manufacturing practices
CMMEF	Compendium of Methods for the Microbiological Examination of Foods
DNA	Deoxyribonucleic acid
EFFCA	European Food and Feed Cultures Association
EFSA	European Food Safety Authority
EC	European Commission
EU	European Union
FEEDAP	The Panel on Additives and Products or Substances used in Animal Feed
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
FSSC	Food Safety System Certification
GI	Gastrointestinal
GM	Genetic modification
GRAS	Generally Recognized as Safe
GRN	GRAS notice
HACCP	Hazard Analysis and Critical Control Points
ICP-MS	Inductively coupled plasma mass spectrometry
IBS	Irritable bowel syndrome
IDF	International Dairy Federation
ISO	International Standardization Organization
MIC	Minimum inhibitory concentration
NBFDS	National Bioengineered Food Disclosure Standard
NCBI	National Center for Biotechnology Information
NMT	Not more than
OPRP	Operational Prerequisite Program
PCR	Polymerase chain reaction
PRP	Prerequisite Program
QPS	Qualified presumption of safety
rDNA	Recombinant deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SMRT	Single Molecule, Real Time
SOP	Standard operating procedure
TRBA	Technical Rule for Biological Agents
tRNA	Transfer ribonucleic acid
U.S.C.	United States Code
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
VFDB	Virulence Factor Data Base

Part 1. Signed statements and certification

1.1. Statement of intent

In accordance with Title 21 of the Code of Federal Regulations (CFR) Part 170 Subpart E on the Generally Recognized as Safe (GRAS) notice, Chr. Hansen A/S has concluded, through scientific procedures, that *Lactobacillus (L.) gasseri* BNR17™ is Generally Recognized as Safe (GRAS) for use as a microbial ingredient in conventional food and is not subject to the premarket approval requirements.

Name and Address of Organization

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

Chr. Hansen, Inc. (local office)
9015 W Maple St.
Milwaukee, WI 53214
USA

Contact Person:
Kate Urbain
Head of NA Regulatory Affairs
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phone: 414-607-5819
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1.2. Name of GRAS substance

Lactobacillus (L.) gasseri BNR17™ / *Lactobacillus (L.) gasseri* NCIMB 30370

1.3. Intended conditions of use

L. gasseri BNR17™ is intended for use as a microbial ingredient in a variety of conventional foods to be consumed by populations of all ages at levels consistent with current good manufacturing practices (cGMP).

The level of inclusion of *L. gasseri* BNR17™ will vary depending on the type of food and application under which it will be used; however, the maximum incorporation level will be 10^9 to 10^{11} colony-forming units (CFU)/serving.

L. gasseri BNR17™ is not intended for use in infant formula nor products regulated by the United States Department of Agriculture (USDA).

1.4. Statutory basis for GRAS conclusion

Pursuant to the GRAS rule [81 Fed. Reg. 159 (August 17, 2016)], Chr. Hansen has concluded that the intended use of *L. gasseri* BNR17™ is GRAS through scientific procedures in accordance with 21 CFR 170.30 (b).

1.5. Premarket approval status

It is the opinion of Chr. Hansen that *L. gasseri* BNR17™ is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on our conclusion that *L. gasseri* BNR17™ is GRAS under the intended conditions of use.

1.6. Availability of information

The data and information that form the basis for Chr. Hansen's conclusion that the intended use of *L. gasseri* BNR17™ is GRAS are available for review and copying by FDA during customary business hours at the location below or will be sent via electronic submission to FDA upon request made to:

Chr. Hansen, Inc.
Winnie Ng
Principal Regulatory Affairs Specialist
9015 W Maple St., Milwaukee, WI 53214
cawinn@chr-hansen.com

1.7. Freedom of Information Act

It is our opinion that the information contained in this GRAS notification for *L. gasseri* BNR17™ is not exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

1.8. Certification

To the best of our knowledge, this GRAS notification 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 *L. gasseri* BNR17™ under the intended conditions of use.

1.9. FSIS statement

Not applicable. *L. gasseri* BNR17™ is not intended for use in applications under the jurisdiction of the USDA.

1.10. Name, position, and signature of responsible person who signs GRAS notice

April 29, 2022

Winnie Ng
Principal Regulatory Affairs Specialist

Date

April 29, 2022

Katharine Urbain
Head of North America Regulatory Affairs

Date

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

2.1. Name of the GRAS substance

The subject of this GRAS notice is a strain of *Lactobacillus (L.) gasseri* designated as BNR17™.

2.2. Source of the GRAS substance

L. gasseri BNR17™ was first isolated from human breast milk (Jung et al., 2013). The *L. gasseri* BNR17™ strain that is subject of this GRAS notice is deposited in the National Collection of Industrial, Food and Marine Bacteria (NCIMB) culture collection under the NCIMB number 30370.

2.3. Description of the GRAS organism

2.3.1. Lactic acid bacteria

L. gasseri BNR17™ is part of the broader group of bacteria known as lactic acid bacteria (LAB). These bacteria produce lactic acid as the major end-product of carbohydrate fermentation. LAB belong to the order Lactobacillales which include the genera *Lactobacillus, Leuconostoc, Pediococcus*, *Lactococcus, Streptococcus, Carnobacterium*, and others (O'Bryan et al., 2015).

LAB are a functional group defined by organisms whose primary metabolic product from the fermentation of sugars is lactic acid. This property is utilized broadly in the production of fermented foods and LAB have been used since ancient times in the preservation and production of traditional fermented foods (Mozzi, 2016). In addition to its widespread consumption from food sources, LAB are ubiquitous as part of the human microbiota. Use of LAB has thus been increasing in food industries applications (Porto et al., 2017). Given the long history of consumption and human exposure, LAB are considered generally safe by the scientific community (Adams, 1999).

2.3.2. Lactobacillus gasseri

L. gasseri was originally identified as *Lactobacillus acidophilus* based on indistinguishable phenotypic and metabolic characteristics; however, was determined to be a separate organism following the electrophoretic characterization of its lactate dehydrogenases (Gasser, 1970; Gasser et al., 1970). While *L. gasseri* is closely related to and remains a member of the *L. acidophilus* group of organisms (Zheng et al., 2020), it has since been categorized as its own separate species based upon further genetic analyses (Lauer et al., 1980; Lauer & Kandler, 1980).

The taxonomic lineage of *L. gasseri* BNR17™ is detailed in Table 1. Of note, recent taxonomic changes to the genus *Lactobacillus* as published by Zheng et al. (2020) have no effect on the existing nomenclature or taxonomy of the *Lactobacillus gasseri* species.

Table 1 Taxonomic lineage of *L. gasseri* BNR17™

Taxonomy	Taxonomic Assignment
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Lactobacillaceae
Genus	*Lactobacillus*
Species	*Lactobacillus gasseri*
Strain	*Lactobacillus gasseri* BNR17™

The genus *Lactobacillus* is rarely pathogenic and found "*widely distributed in the environment, especially in animal and vegetable food products*" (Bergey and Holt, 1994). *L. gasseri* is one of the predominant species found in the female lower genital tract, is readily present within the oral cavity, and is part of the human microflora as an autochthonous commensal resident of the upper intestinal tract in humans (Lauer et al., 1980; Nardis et al., 2013; Selle & Klaenhammer, 2013; Zheng et al., 2020). Moreover, *L. gasseri* is considered a species of predominance in infants during the early colonization of the gastrointestinal tract (Wall et al., 2007). *L. gasseri* has also been associated with food such as sourdough and fermented milk (Bourdichon et al., 2018; Mogensen et al., 2002).

2.3.3. Genotypic classification of *L. gasseri* BNR17™

The BNR17™ strain has been confirmed as an isolate of *L. gasseri* by the ribotype 5s, 16s, and 23s rDNA homology method. The RiboPrinter® system is an automated southern blotting (ribotyping) platform for microbial identification and characterization.

Additionally, the *L. gasseri* BNR17™ strain has been unambiguously identified by whole genome sequence as a member of the *L. gasseri* species with a sequence that is substantially comparable to the reference *L. gasseri* genome.

The whole genome sequence of *L. gasseri* BNR17™ was determined by combining Illumina HiSeq™ and PacBio™ platforms to ensure high nucleotide sequence fidelity and long-reads-based high assembly completeness, respectively (Walker et al., 2014). Prior to sequencing, culture homogeneity was assessed by two independent polymerase chain reaction (PCR)-based fingerprinting protocols and a batch of high quality and high integrity DNA was purified from the pure culture.

Genome annotation was performed on the assembled Consensus sequences using a pipeline based on the Prokka Prokaryotic Genome Annotation System (Prokaryote gene prediction by Prodigal 2.6; rRNA prediction using barrnap 0.2; tRNA prediction by Aragorn 1.2.36). Annotation statistics of *L. gasseri* BNR17™ revealed a genome size of 2,146,080 bp, 11 contigs (consensus), 2,159 coding sequences, a total of 233 hypothetical proteins, a total gene size of 1,922,262 bp and an average gene size of 867.8 bp (min = 70 bp, max = 8,603 bp). The % GC content was 34.86%. No extra-chromosomal DNA was identified.

Genome alignment was carried out by progressive MAUVE with *L. gasseri* ATCC 33323 (GenBank Acc No: NC_008530) type strain of the species as the reference genome and the eleven scaffolds (consensus) of *L. gasseri* BNR17™. With regard to genome size, *L. gasseri* BNR17™ was approximately

250 kbp larger than *L. gasseri* ATCC 33323. This result is consistent with genome size variation reported for other strains of the species, such as *L. gasseri* 4M13 (Oh et al., 2018). The presence of prophage sequences is a characteristic and well-studied feature of *L. gasseri* and may explain the size difference observed (Baugher et al., 2014; Ventura et al., 2006).

2.3.4. Phenotypic analysis of *L. gasseri BNR17™*

The species *L. gasseri* is a member of the Phylum Firmicutes (see Table 1 above). In general, members of the *Lactobacillus* genus, including *L. gasseri*, are gram-positive, rod-shaped, non-spore forming and non-motile. *L. gasseri* are able to ferment several carbohydrates which include, but are not limited to, galactose, glucose, fructose, mannose, etc. The full carbohydrate fermentation profile of *L. gasseri* BNR17™, as determined using the API 50 CHL test system, is presented in Table 2 below.

Table 2 Carbohydrate fermentation profile of *L. gasseri* BNR17™ (API 50 CHL method)

Control	-	Esculin	+
Glycerol	-	Salidn	+
Erythritol	-	Cellobiose	+
D Arabinose	-	Maltose	+
L Arabinose	-	Lactose	-
Ribose	-	Melibiose	-
D Xylose	-	Sucrose	+
L Xylose	-	Trehalose	+
Adonitol	-	Inulin	-
Beta Methyl-D-Xyloside	-	Melezitose	-
Galactose	+	Raffinose	-
Glucose	+	Starch	+
Fructose	+	Glycogen	-
Mannose	+	Xylitol	-
Sorbose	-	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	-
Amygdalin	+	2-Keto-Gluconate	-
Arbutin	+	5-Keto-Gluconate	-

2.3.5. Genetic modification status

L. gasseri BNR17™ is not genetically modified by use of recombinant DNA techniques.

In accordance with U.S. regulations, Chr. Hansen cultures and enzyme products are not subject to bioengineered (BE) labeling under the National Bioengineered Food Disclosure Standard (NBFDS) codified in 7 CFR Part 66.

Further, pursuant with European Union (EU) Regulation (EC) No 1829/2003 and Regulation (EC) No 1830/2003, the use of Chr. Hansen cultures including *L. gasseri* BNR17™ does not trigger genetic modification (GM) labeling of the final food product.

2.4. Method of manufacture

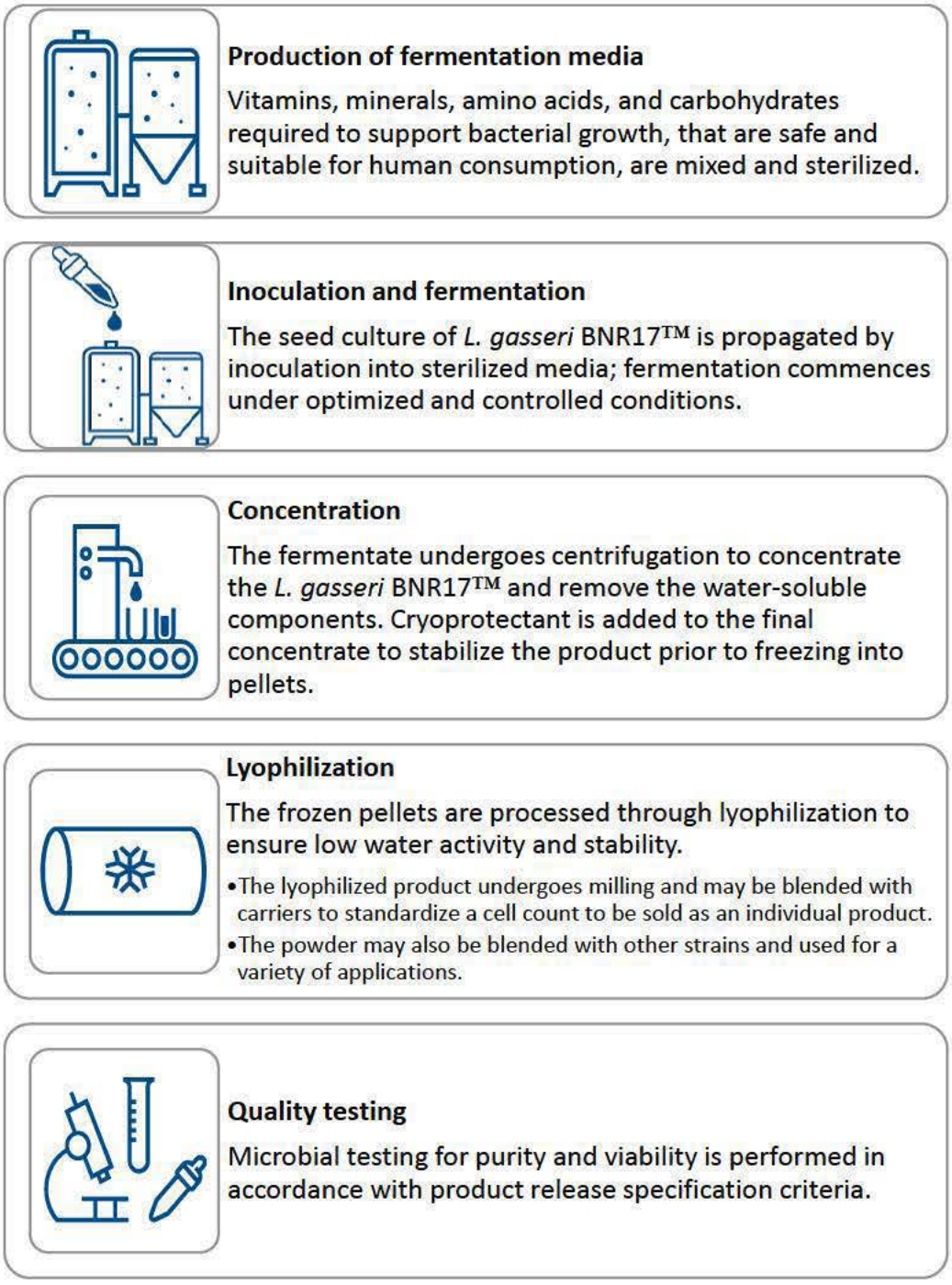
Viable *L. gasseri* BNR17™ is produced by industrial fermentation following Chr. Hansen's global protocol for production of cultures and manufacturing is conducted in accordance with current good manufacturing practices (cGMPs).

Pure strain of the microorganism (*L. gasseri* BNR17™ seed culture) is inoculated into sterilized growth medium specifically designed to meet the nutritional needs of *L. gasseri.* The seed preparation is further scaled up by incubation and fermentation processes until the established fermentation end point is obtained. Strict conditions are maintained throughout the fermentation process to ensure optimal growth. These include maintaining a controlled sterile environment in a closed system and strict control of the temperature and pH. Once the fermentation enters stationary growth, it is cooled to stop the growth process. The fermentate is subjected to centrifugation for the removal of water-soluble material and to concentrate the desired *L. gasseri* BNR17™. Appropriate food-safe cryoprotectants are added to improve survival during freeze-drying. The concentrated microorganisms are then frozen into pellets and undergo lyophilization to remove excess water. The lyophilized pellets are then milled to a powder. The raw materials used in the production process primarily consist of carbohydrates, amino acids, vitamins and minerals that are safe and materials used in the media are suitable for human consumption.

The resulting powder has a very low water activity which ensures stability of the culture. It is tested for quality and sold as-is or blended with other food-grade microbial ingredients, carriers, or food-grade materials appropriate for their intended use. The products are packaged, labeled with necessary information, tested for quality, and sold. A schematic overview of this process is outlined in Figure 1 below.

All manufacturing is done in accordance with cGMP consistent with 21 CFR Parts 110 and 117. A fully implemented Hazards Analysis and Critical Control Points (HACCP) plan, standard operating procedures, and quality control programs are in place to ensure quality of the product being produced. Each Chr. Hansen plant complies with a set of basic GMP rules, also called Pre-Requisite Program (PRP) according to Chr. Hansen's Quality, GMPs and Food Safety Principles, which are publicly available from our website www.chr-hansen.com. As part of the HACCP plan, each manufacturing process has appointed an OPRP (Operational Pre-Requisite Program) and CCPs (Critical Control Points). The OPRP and CCP's are documented and classified as specifically critical for the safety of food ingredients produced in the plant. All facilities manufacturing final products maintain FSSC 22000 certification.

Figure 1 Manufacturing overview of *L. gasseri* BNR17™



2.4.1. Raw materials and processing aids

L. gasseri BNR17™ is produced using standard fermentation techniques. This includes the use of fermentation and standardizing ingredients that are safe and suitable for use in human food. These ingredients have no technical function in the finished food product and are all permitted for the intended application.

2.4.2. Quality program

L. gasseri BNR17™ is produced under a quality program that 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.

2.4.3. Allergen control

Chr. Hansen communicates the allergen status of its products in accordance with the U.S. and EU legislations. Allergen control is managed via cGMP and food safety programs that are FSSC 22000 certified at each of the production sites. Allergen communication is managed via quality management and food safety programs that are ISO 22000 certified.

All major food allergens as listed and established in the U.S. Food Allergen Labeling and Consumer Protection Act of 2004, in addition to the substances or products causing allergies or intolerances as outlined in Annex II of Regulation (EU) No 1169/2011, as amended, are controlled during the production of *L. gasseri* BNR17™.

No major allergens nor substances derived from major allergens are used as raw materials in the fermentation media for the production of *L. gasseri* BNR17™.

2.5. Product specifications and product stability

2.5.1. Specifications

The final *L. gasseri* BNR17™ ingredient is in the form of an off-white to cream powder containing a total viable cell count of at least 200 x 10^9 CFU/g *L. gasseri* BNR17™. The quality control specifications that must be satisfied prior to the commercial release of *L. gasseri* BNR17™ are outlined in Table 3 along with the methods of analysis that are all internationally recognized and/or validated.

Table 3 Product specifications for *L. gasseri* BNR17™

Parameter	Units	Specification	Method of Analysis
Identity	-	*Lactobacillus gasseri* BNR17™	Automated ribotyping or 16s rRNA gene sequence
Total Viable Cell Count	CFU/g	≥ 200 x 10^9	SOP-J32
Microbial Parameters			
Enterococcus	CFU/g	<100	CMMEF Chapter 10
Escherichia coli	/10 g	Negative	USP <2022>
Staphylococcus aureus	/10 g	Negative	USP <2022>
Salmonella	/10 g	Negative	USP <2022>
Listeria species	/25 g	Negative	AOAC 2004.06
Heavy Metal Parameters			
Lead	ppm	NMT 1.0	ICP-MS
Arsenic	ppm	NMT 1.0	
Cadmium	ppm	NMT 0.30	
Mercury	ppm	NMT 0.05	

Parameter	Units	Specification	Method of Analysis
Abbreviations: AOAC, Association of Official Analytical Chemists; CFU, colony forming unit; CMMEF, Compendium of Methods for the Microbiological Examination of Foods; ICP-MS, inductively coupled plasma mass spectrometry; NMT, not more than; SOP, standard operating procedure; USP, U.S. Pharmacopeia.			

Analyses were conducted on 3 non-consecutive and commercially representative batches of *L. gasseri* BNR17™ and the analytical results are summarized in Table 4. The analytical data demonstrate that the final *L. gasseri* BNR17™ ingredient is produced consistently and conforms to the established specifications.

Table 4 Analytical data for 3 non-consecutive and representative batches of *L. gasseri* BNR17™

Parameter	Units	Specification	Analytical Data/ Date of Manufacture		
			Batch 1 06/30/2021	Batch 2 09/10/2021	Batch 3 01/02/2022
Identity	-	*Lactobacillus gasseri* BNR17™	Confirmed	Confirmed	Confirmed
Total Viable Cell Count	CFU/g	≥ 200 x 10^9	723 x 10^9	867 x 10^9	777 x 10^9
Microbial Parameters					
Enterococcus	CFU/g	<100	<100	<100	<100
Escherichia coli	/10 g	Negative	Negative	Negative	Negative
Staphylococcus aureus	/10 g	Negative	Negative	Negative	Negative
Salmonella	/10 g	Negative	Negative	Negative	Negative
Listeria species	/25 g	Negative	Negative	Negative	Negative
Heavy Metal Parameters					
Lead	ppm	NMT 1.0	<0.01	<0.01	<0.01
Arsenic	ppm	NMT 1.0	0.02	0.02	0.01
Cadmium	ppm	NMT 0.30	0.039	0.033	0.015
Mercury	ppm	NMT 0.05	0.006	0.005	<0.005
Abbreviations: CFU, colony forming unit; NMT, not more than.					

2.5.2. Product stability

L. gasseri BNR17™ lyophilized powder has a minimum shelf life of 18 months from the date of manufacture when stored at or below 4°C in its original packaging.

The genetic stability of *L. gasseri* BNR17™ has been confirmed by comparing a master seed vial to a working seed vial which demonstrated no changes in single nucleotide polymorphisms (SNPs) based on core genome phylogeny.

Part 3. Dietary exposure

3.1. Intended use

L. gasseri BNR17™ is intended for use as a microbial ingredient in a variety of conventional foods to be consumed by populations of all ages at levels consistent with cGMP.

The level of inclusion of *L. gasseri* BNR17™ will vary depending on the type of food and applications under which it will be used, and if it is to be blended with other microbial ingredients. Under the intended conditions of use, the incorporation level will range between 10^9 to 10^{11}CFU/serving to account for loss of viability throughout the shelf-life of the product.

The intended use of *L. gasseri* BNR17™ subject to this GRAS notice is comparable to the existing GRAS notices for the use of the taxonomically related *L. acidophilus*[1] that have received a letter of "no questions" from the FDA for use in conventional foods at levels ranging from 10^9 to 10^{11} CFU/serving (GRN No. 357, 502, and 871).

3.2. Estimated daily intake from the intended use in conventional foods

Under the intended conditions of use, it is anticipated that level of incorporation of *L. gasseri* BNR17™ for conventional food applications will be up to a maximum of 10^{11} CFU/serving to ensure at least 1.0 x 10^9 CFU/serving through the shelf life of the product.

If it is assumed that the average consumption of a healthy individual is approximately 20 servings of all combined foods per day (Millen et al., 2006), and that all of these foods contain the strain at a level of 1.0 x 10^9 CFU/day, the maximum exposure to *L. gasseri* BNR17™ as attributed to conventional foods is estimated to be 2.0 x 10^{10} CFU/day. Under the most conservative assumptions it is not anticipated that the maximum ingestion based on the intended uses of *L. gasseri* BNR17™ will exceed 1.0 x 10^{11} CFU/day.

Moreover, it is well recognized that the adult microbiome is very stable and only shifts with significant dietary changes or extreme weight loss (Faith et al., 2013). Considering that *Lactobacillus* spp. are ubiquitous and *L. gasseri* is readily present as part of the human microbiome, any exposure to *L. gasseri* BNR17™ in conventional foods under the intended conditions of use in the diet, as subject to this GRAS notice, is not anticipated to significantly alter or contribute to the overall homeostatic nature of the gut microbiota in the general population. Likewise, *L. gasseri* BNR17™ may be an alternative to the taxonomically related *L. acidophilus* for the same existing uses as a microbial ingredient in conventional food (GRN No. 357, 502, and 871), and is therefore not anticipated to increase the existing overall dietary intake of the *L. acidophilus* group of organisms.

Indeed, the estimated dietary exposure to *L. gasseri* BNR17™ under the intended use conditions in the present notice is considered extremely conservative, as it assumes that there is no loss in viability of the cells during shipping and storage. It is also unlikely that individuals would consume even half (10 servings/day) of conventional food products containing *L. gasseri* BNR17™. Additionally, there is the assumption that *L. gasseri* BNR17™ will be incorporated in all foods consumed on a daily basis, which includes foods explicitly excluded from the envisioned uses (*e.g.*, meat and poultry products) and foods that are not compatible with the addition of viable microbial ingredients (*e.g.*, canned foods). In reality, dietary exposure to *L. gasseri* BNR17™ under the intended conditions of use will not increase the existing

[1] Zheng et al. 2020.

exposure to the species given the aforementioned considerations and the transient nature of commensal organisms within the gastrointestinal (GI) tract.

Part 4. Self-limiting levels of use

The intended use levels of *L. gasseri* BNR17™ are not self-limiting; however, the addition of the strain is restricted to applications that can sustain viable *L. gasseri* BNR17™ at the intended use levels throughout the shelf life of the food product.

Part 5. Experience based on common use in food

The conclusion of GRAS status for the intended uses of *L. gasseri* BNR17™ is based on scientific procedures and not common use in food before 1958.

Part 6. Narrative

6.1. Approach to the safety assessment

The data and information providing the basis for our conclusion that the addition of *L. gasseri* BNR17™ under the intended conditions of use is GRAS through scientific procedures are presented in the following sections. The information provided below and throughout this notice is generally available in the public domain and has been properly cited. To demonstrate the safety of *L. gasseri* BNR17™ under the intended conditions of use, Chr. Hansen has rigorously applied the decision tree approach to *"Determining the safety of microbial cultures for human and animal consumption"* as established by Pariza *et al.* (2015), along with the risk assessment conducted by the European Food Safety Authority (EFSA) in accordance with the qualified presumption of safety (QPS) approach (EFSA Scientific Committee, 2005, 2007).

As discussed in Section 2.3.3, the taxonomic identification of the organism has been definitively confirmed as *L. gasseri* by genomic analysis. *In silico* analysis on *L. gasseri* BNR17™ demonstrate the absence of potential virulence factors and genes related to pathogenicity (Section 6.3.2.1). *L. gasseri* BNR17™ was determined to be sensitive to antibiotics and is not a significant concern for antibiotic resistance as confirmed by *in vitro* testing (Section 6.3.3). The BNR17™ strain was also demonstrated to be absent of biogenic amines (Section 6.3.4). Additionally, *L. gasseri* BNR17™ along with other strains of *L. gasseri* have been evaluated in a number of randomized controlled clinical studies and were well tolerated without adverse events attributed to the test article (Section 6.3.5).

6.2. History of safe consumption in foods

LAB such as *L. gasseri* are distributed widely in nature and are most often associated with foods such as dairy, meat, and vegetable products (Carr et al., 2002). Fermentation, one of the oldest food processing technologies is only possible in the presence of microorganisms such as LAB. These microbial cultures may be part of the endogenous flora of the food or may be intentionally added in the case of industrial food fermentation process (Herody et al., 2010).

L. gasseri is commonly associated as a commensal of the human microbiome (Zheng, et al., 2020). Moreover, as mentioned previously, *L. gasseri* BNR17™ was originally isolated from human breast milk (Jung et al., 2013) and, therefore, has an inherent history of consumption in food. Similarly, given that *L. gasseri* belongs to the *L. acidophilus* group of organisms, and because of its relatively recent taxonomic separation from the *L. acidophilus* species based upon genetic analysis, the species is considered to have a similar history of use as *L. acidophilus,* which has been consumed safely with a substantial history of use in yoghurt and fermented milk products.

L. gasseri is presently listed on the International Dairy Federation (IDF)/European Food and Feed Cultures Association (EFFCA)'s "*Inventory of microbial food cultures with safety demonstration in fermented food products"* as having a safe history of use since 1980 in fermented milk, probiotics[2], as well as sourdough (Bourdichon et al., 2018, 2022; Mogensen et al., 2002). The IDF maintains the list using a panel of recognized experts. The source of the organisms in the IDF list may be from addition of commercially prepared starter cultures or from autochthonous organisms present on food raw materials. In either

[2] Note, while there is a history of use of *L. gasseri* as probiotics, the *L. gasseri* BNR17™ strain subject to this GRAS notice is only intended for use as a microbial ingredient in conventional food. The term "probiotics" is used here to convey an accurate representation of the history of use.

case, the organisms must be characterizing and not merely incidental components of the food microflora to be included in the IDF list.

L. gasseri is also on the Danish Veterinary and Food Administration (DVFA)'s *List of notified microbial cultures applied in food* (Danish Veterinary and Food Administration, 2016), which supports a history of use in food.

6.3. Safety of *Lactobacillus gasseri* BNR17™

6.3.1. Recognition of safety by authoritative bodies and qualified experts

Since the initial introduction of the QPS approach in 2007, the EFSA Panel on Biological Hazards (BIOHAZ Panel) has concluded that the species *Lactobacillus gasseri* is suitable for QPS status with no qualifications other than the general requirement for the absence of antibiotic resistance (EFSA Scientific Committee, 2007). The QPS concept was developed to provide a harmonized generic pre-evaluation to support safety risk assessments of microorganisms intentionally introduced into the food chain. Within the QPS approach the four principal considerations for evaluation of the QPS status of a microorganism include: (i) taxonomic identification, (ii) body of knowledge, (iii) safety (including virulence factors causing pathogenicity and antimicrobial resistance of valid taxonomic units), and (iv) intended use. QPS status is granted provided that 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 regularly, wherein the most recent release in 2022 included the monitoring of any new data pertinent to the safety of microorganism species with existing QPS status (EFSA BIOHAZ Panel, 2022). Based on the evaluation by the BIOHAZ Panel in the most recent QPS update, the QPS status of the QPS species within the *Lactobacilli* genus remained unchanged including *Lactobacillus gasseri* (EFSA BIOHAZ Panel, 2022).

In the US, prior sanctions granted by the FDA permit the use of harmless lactic acid-producing bacteria, including *L. gasseri* species, as optional ingredients in certain specified standardized foods. LAB are permitted for use in cultured milk (including buttermilk) (21 CFR 131.112), sour cream (21 CFR 131.160), cottage cheese (21 CFR 133.128), and yogurt (21 CFR 131.200) (U.S. Food & Drug Administration, 2018). The safe and suitable LAB were evaluated for safety by the FDA at the request of the dairy industry and the letters from FDA in response that are the basis of the prior sanctions are the equivalent to GRAS notice "no objection" letters. Likewise, a number of GRAS notices for the use of the taxonomically related *L. acidophilus,* as an ingredient in conventional foods at levels ranging from 10^9 to 10^{11} CFU/serving, have received a letter of "no questions" from the Agency (GRN No. 357, 502, and 871).

6.3.2. Pathogenicity/Toxigenicity

6.3.2.1. *In silico* analyses

The genome of *L. gasseri* BNR17™ was assessed for virulence factors and/or toxins associated with bacterial pathogenicity, via a Basic Local Alignment Search Tool (BLAST)-based tool against the Virulence Factor Data Base (VFDB) (Chen et al., 2016). The VFDB is an integrated comprehensive online resource for curating information about virulence factors of bacterial pathogens and includes 30,178 genes related to 1,796 virulence factors of 74 pathogenic genera. Screening of the whole genome sequence of strain BNR17™ against the VFDB did not identify any meaningful hits. Thus, the genome of *L. gasseri* BNR17™ was confirmed to be free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity.

6.3.2.2. Case reports

Lactobacillus gasseri is classified as Risk Group 1 by the German Federal Institute for Occupational Health and Safety under their Technical Rule for Biological Agents (TRBA) (Committee on Biological Agents, 2015). Risk Group 1 is defined as organisms that are highly unlikely to cause an infectious disease in with no specific special hazards identified; however, for *L. gasseri*, the TRBA did additionally note that the species was "*identified as or suspected as a pathogen in individual cases, predominantly in the case of significant immune-compromised people*". The American Type Culture Collection (ATCC) classifies the *Lactobacillus gasseri* sp. as Biosafety Level 1 which is defined as "*well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment*" (Centers for Disease Control and Prevention, 2020). This is consistent with the conclusions of EFSA's BIOHAZ Panel during the development and evaluation of the QPS list, where it was concluded that for *Lactobacillus* spp.*:*

> "*Many of the referred microorganisms falling within this grouping are normal inhabitants of the digestive tract of humans and livestock or are commonly used in the preparation of foods and feed. Consequently, there has been a long history of human exposure with only very occasional reports of adverse effects and then only amongst compromised individuals… The second issue highlighted the debate about the distinction between opportunistic infections, of which almost all microorganisms that humans commonly encounter are capable, and pathogenicity. Many Lactobacillus species have been occasionally encountered in clinical specimens, the clinical significance of which is not always clear. Such occurrences have almost invariably been associated with immunocompromised patients, those who had suffered surgical or accidental insult or who had a serious underlying illness, and remain rare. As such, these infections can be considered opportunistic and beyond the capacity of any safety assessment to exclude*" (EFSA Scientific Committee, 2007).

For completeness, Chr. Hansen has also conducted a comprehensive review of the literature through March 2022 to identify publications pertinent to the safety evaluation of *L. gasseri* with respect to pathogenicity and toxigenicity in humans. The literature search followed the same search strategy as EFSA's QPS approach for *Lactobacillus* (more specifically, *L. gasseri*) (EFSA BIOHAZ Panel, 2021). The details of the search criteria and identified studies are outlined in Table 5. Considering that EFSA monitors new data pertinent to the safety of species with existing QPS status, the literature search was an update to the existing information and covered publications following June 2020 to the present.

Table 5 Search strategy for *L. gasseri* studies related to pathogenicity and toxigenicity

Source	Outcome	Search string	Number of hits
PubMed Date filter: June 2020 to March 2022	Antimicrobial/ antibiotic/antimycotic	*Lactobacillus gasseri* AND antimicrobial resistan* OR antibiotic resistan* OR antimicrobial susceptibil*	18
	Infection/bacteremia/ fungemia/sepsis	*Lactobacillus gasseri* AND infection* OR abscess* OR sepsis* OR septic* OR bacteremia OR bacteraemia OR toxin*	47
	Type of disease	*Lactobacillus gasseri* AND endocarditis OR abscess OR meningitis	2
	Mortality/morbidity	Not applied	-
	Disease risk	*Lactobacillus gasseri* AND opportunistic OR virulen*	5

The search results were then screened for relevance in terms of safety concerns where *L. gasseri* acted as a human pathogen. Results were screened at the title and abstract level for relevance based on a select set of selection criteria as outlined in Table 6.

Table 6 Study selection criteria for relevance to *L. gasseri* pathogenicity and toxigenicity

Screening Strategy
Inclusion criteria: • The subject of the study is *Lactobacillus gasseri.* • The study pertains to safety concerns of *Lactobacillus gasseri.* • The study was conducted in humans. • Effects must be attributable to *Lactobacillus gasseri.* • The study was published from June 2020 to March 2022. • The study is derived from primary research or a case report. • The publication is not a review, conference proceeding, etc. • The full-text of the article is available. • The publication is in English.
Exclusion criteria: • The subject of the study is not *Lactobacillus gasseri.* • The study does not assess or describe safety concerns. • The study was not conducted in humans. • Effects are not attributable to *Lactobacillus gasseri*. • The study was not published from June 2020 to March 2022. • The study is not derived from primary research or a case report. • The publication is a review, conference proceeding, etc. • The full-text of the article is not available. • The publication is not in English.

On the basis of the above literature search strategy and selection criteria, one publication pertinent to the safety of *L. gasseri* was identified since the last published QPS evaluation and is described in greater detail below.

In a case of liver abscess and bacteremia, a 59-year-old male was admitted to the hospital with weakness, fever, and abdominal pain (Ramos-Coria et al., 2021). The patient had an extensive history of gastrointestinal conditions including open cholecystectomy, acute necrotizing pancreatitis, complicated with pancreatic fistula, distal pancreatectomy, splenectomy, and pancreaticojejunal anastomosis causing Type 3C diabetes mellitus and adhesive small bowel occlusion. Upon further assessment, the patient was diagnosed with septic shock secondary to liver abscesses and blood cultures identified lactic acid-forming bacteria, specifically *Lactobacillus gasseri*; however, the patient did not report consuming any oral products containing LAB. The patient was given intravenous antibiotics and responded well, with a decrease in the size of the abscesses. The patient was discharged after 24 days and given oral antibiotic for a subsequent 4-week period wherein total clinical recovery was reported. While the exact source of the *L. gasseri* in the etiology of the bacteremia and liver abscesses is unclear, this report demonstrates that *L. gasseri* may act as an opportunistic organism causing pathology in individuals with extensive medical history and underlying conditions.

The newly identified case report is consistent with a handful of other reports which suggest that *L. gasseri* may be involved in rare and uncommon opportunistic infections such as septic urinary infection (Dickgießer et al., 1984), empyema (Esquibel et al., 2017), endocarditis (Elikowski et al., 2017), and

peritonitis (Ramachandran et al., 2020); however, in all cases, these infections have been associated with co-morbidities or additional underlying health anomalies. Furthermore, with the exception of the case report on peritonitis (Ramachandran et al., 2020), it is unclear whether *L. gasseri* had been orally ingested, and all cases have fully resolved with antibiotic treatment.

To summarize, *L. gasseri* has QPS status as evaluated by EFSA and has been documented as having safe and technological benefits when used in food (Bourdichon et al., 2018, 2022; Danish Veterinary and Food Administration, 2016; Mogensen et al., 2002). While there have been case reports of opportunistic infections attributed to *L. gasseri*, there is overwhelming evidence to show that *L. gasseri* is safe to consume as a microbial ingredient in conventional foods with a low-virulence risk. The literature also suggests that, except in cases of underlying conditions, the concern for pathogenicity from *L. gasseri* is low. This is consistent with EFSA's conclusion that the pathogenicity of *L. gasseri* may be considered opportunistic in nature, similar to other *Lactobacillus* spp. that are commonly used in the food supply. Additionally, as described in Section 6.3.2.1, *in silico* analyses have demonstrated that *L. gasseri* BNR17™ does not exhibit pathogenic nor virulent traits. Based on this, along with other data presented, it can be concluded that *L. gasseri* is safe under the intended use in food.

6.3.3. Antibiotic resistance

The minimum inhibitory concentration (MIC) of 9 antibiotics were determined for the *L. gasseri* BNR17™ strain according to the ISO10932/IDF223. The strain *Lactobacillus paracasei* subsp. *paracasei* DSM 5622, type strain homologue to *L. paracasei* subsp. *paracasei* ATCC 334, was applied as a positive control and expected MIC values were confirmed to support the validity of the test. The range of antibiotics tested complies with the EFSA *"Guidance on the characterization of microorganisms used as feed additives or as production organisms"* (EFSA FEEDAP Panel, 2018). The analytical results outlining the MIC values for *L. gasseri* BNR17™ are summarized in Table 7.

Table 7 MIC values for *L. gasseri* BNR17™ from two independent experiments

Antibiotic type	Antibiotic	EFSA cut-off values[a] (mg/L)	MIC Value (mg/L) Replicate A	Replicate B	Resistance Profile
Aminoglycoside	Gentamicin	16	2	1	Sensitive
	Kanamycin	64	32	16	Sensitive
	Streptomycin	16	4	2	Sensitive
Tetracycline	Tetracycline	4	2	1	Sensitive
Macrolide	Erythromycin	1	0.063	0.125	Sensitive
Lincosamide	Clindamycin	4	1	1	Sensitive
Chloramphenicol	Chloramphenicol	4	2	4	Sensitive
β-lactam	Ampicillin	1	0.25	0.5	Sensitive
Glycopeptide	Vancomycin	2	1	1	Sensitive

Abbreviations: MIC = minimum inhibitory concentration

[a] EFSA microbiological cut-off values for *Lactobacillus acidophilus* group as listed in EFSA's *Guidance on the characterisation of microorganisms used as feed additives or as production organisms* (EFSA FEEDAP Panel, 2018). Taxonomically, *L. gasseri* is closely related to and a member of the *L. acidophilus* group of organisms. *L. gasseri* was initially identified as *L. acidophilus* and was subsequently categorized in a separate species based upon genetic analysis (Lauer et al., 1980; Lauer & Kandler, 1980).

The results obtained for the MIC values demonstrated that the strain *L. gasseri* BNR17™ is sensitive to all antibiotics tested according to the EFSA (2018) guidance, with MIC values at or below the reported

species characteristics (cut-off values). As the cut-off values are derived from an analysis of a representative sample of strains of the species, the values are an indicator of the innate antibiotic resistance traits of the species. Values at or below the cut-off values indicate that the strain *L. gasseri* BNR17™ has not acquired antibiotic resistance factors independent of the innate characteristics of the species. Thus, it is reasonable to conclude that horizonal gene transfer is not a concern for this strain.

A follow-up analysis assessed the annotated genome sequence of *L. gasseri* BNR17™ against the Comprehensive Antibiotic Resistance Database (CARD version 3.0.4) via RGI software version 5.1.0 (Alcock et al., 2019) for the identification of known antibiotic determinants involved in the resistance to critically important, highly important and important antibiotics used in human medicine according to the World Health Organization (WHO, 2017) and EFSA (EFSA FEEDAP Panel, 2018). No known antibiotic resistance genes were identified in the *L. gasseri* BNR17™ genome by RGI analysis against the CARD database.

6.3.4. Biogenic amines

Biogenic amines in foods can produce adverse reactions, especially in susceptible individuals (EFSA BIOHAZ Panel, 2011; Omer et al., 2021; Özogul & Özogul, 2019). Many lactic acid bacteria exhibit amino acid decarboxylase activity. Histamine, tyramine, putrescine, and cadaverine are generated by decarboxylation of histidine, tyrosine, ornithine and lysine, respectively (Diaz et al., 2015; Gardini et al., 2016; Landete et al., 2007; Romano et al., 2013). Moreover, the deimination of agmatine can also form putrescine via N-carbymoyl putrescine (Garai et al., 2007). Reports of toxicity from the consumption of biogenic amines are rare, and when they occur are usually associated with histamine, and to a lesser extent tyramine exposure. It should be emphasized however, that exposure to these compounds is expected on a daily basis as the gastrointestinal tract contains numerous microorganisms with active amine degradation enzymatic capacity, and the presence of biogenic amines in wine, cider, cheeses, and cured meats due to the presence of lactic acid fermenting bacteria is common (Ferreira & Pinho, 2006; Garai et al., 2006; Landete et al., 2007; Suzzi & Gardini, 2003)

An *in silico* analysis was performed to identify possible genetic determinants for the synthesis of biogenic amines within the genome of *L. gasseri* BNR17™. The bioinformatics analysis was adapted from a PCR based genetic screen reported by Li et al. (2018). The nucleotide sequence of biogenic amine related genes, from the NCBI database, were aligned against the genome of *L. gasseri* BNR17™ using BLASTx. No relevant matches were detected within the *L. gasseri* BNR17™ genome for histidine decarboxylase, tyrosine decarboxylase, lysine decarboxylase, or agmatine deiminase, which are involved in the generation of histamine, tyramine, cadaverine, or N-carbamoyl putrescine, respectively. Some genetic homology was identified between the BNR17™ genome and a reference ornithine decarboxylase gene; however, phenotypically, the BNR17™ strain did not produce detectable amounts of putrescine, as confirmed through a biochemical assay according to Bover-Cid & Holzapfel (1999) using appropriate positive controls for the production of histamine, tyramine, cadaverine, and putrescine. The assay is based on a chromogenic change linked to the production of biogenic amines and allows for the detection of metabolic products regardless of the biosynthetic pathway. *L. gasseri* BNR17™ was confirmed to not yield a positive reaction for any of the tested biogenic amines.

6.3.5. Clinical studies

6.3.5.1. *L. gasseri* BNR17™

A number of human clinical studies have been conducted on *L. gasseri* BNR17™, namely, in obese yet otherwise healthy subjects (Jung et al., 2013; Kim et al., 2018), as well as subjects with diarrhea-dominant irritable bowel syndrome (IBS) (Shin et al., 2018). A summary of these studies is presented in Table 8. While not standard Phase I safety studies, these efficacy studies do include some safety parameters and demonstrate that *L. gasseri* BNR17™ is well tolerated in healthy adults at levels of up to 6.0×10^{10} CFU/day for 12 weeks and in subjects with IBS at levels of 10^{10} CFU/day for 8 weeks, with no reports of significant test article-related adverse events on any of the study participants. Thus, these studies support the safety of *L. gasseri* BNR17™ subject to this GRAS notice wherein the intended conditions of use (see Part 3, Section 3.2).

Table 8 Summary of clinical studies conducted with *L. gasseri* BNR17™

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Kim et al., 2018	Randomized, double-blind, placebo-controlled	Healthy obese adults (aged 20-75 years; BMI of 25 to 35 kg/m^2) N=90 (27 male; 63 female)	Oral, capsule Control: placebo Intervention: 1. *Lactobacillus gasseri* BNR17™ at 10^9 CFU/day 2. *Lactobacillus gasseri* BNR17™ at 10^{10} CFU/day	12 weeks	Vital signs and routine battery of blood biochemistry and urine tests were not significantly different between groups. No AEs reported through the duration of the study.
Jung et al., 2013	Randomized, double-blind, placebo-controlled	Healthy obese adults (aged 19-60 years; BMI ≥ 23 kg/m^2 and fasting blood sugar ≥ 100 mg/dL) N=57 (22 male; 35 female)	Oral, capsule Control: placebo Intervention: *Lactobacillus gasseri* BNR17™ at 6.0×10^{10} CFU/day	12 weeks	No difference in BP or pulse rate between groups. Hematology and blood biochemistry were unchanged between groups. No AEs were related to the test-article (1 case each of diarrhea and nausea in the BNR17™ and placebo group, respectively) – no serious AEs were noted through the duration of the study.
Shin et al., 2018	Randomized, double-blind, placebo-controlled	Subjects with diarrhea dominant IBS via the Rome III criteria with no concurrent medication	Oral, capsule Control: placebo Intervention: *Lactobacillus gasseri* BNR17™ at 10^{10} CFU/day	8 weeks	No changes in vital signs, hematology, blood biochemistry, and urinalysis between groups. No AEs noted through the duration of the study.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
		(20-55 years of age) N=51 (22 male; 29 females)			

Abbreviations: AE, adverse event; BMI, body mass index; BP, blood pressure; CFU, colony forming unit; IBS, irritable bowel syndrome.

6.3.5.2. Studies on other *L. gasseri* strains

L. gasseri is a well-established and commonly used microbial ingredient consumed world-wide and the safety of the species for use in food has been confirmed by a number of authoritative bodies (Bourdichon et al., 2012, 2018, 2022; EFSA Scientific Committee, 2007; Mogensen et al., 2002).

Strain level safety is supported by the strain-specific clinical studies (as detailed in Section 6.3.5.1), along with the biosafety analyses conducted on *L. gasseri* BNR17™ to demonstrate the absence of virulence and toxigenic factors associated with pathogenicity (Section 6.3.2), the absence of antimicrobial resistance (Section 6.3.3), and no production of biogenic amines (Section 6.3.4). This approach to safety substantiation is consistent with the Pariza et al. (2015) decision tree to evaluate and demonstrate the safety of microbial cultures used for consumption in humans and animals.

In addition to strain-specific clinical studies on the *L. gasseri* BNR17™, studies on other *L. gasseri* strains are pivotal to support safety at the species level. To identify randomized controlled human clinical studies relevant to the tolerability of *L. gasseri* as a species, Chr. Hansen conducted a comprehensive search of the literature using the PubMed® database for relevant publications through to March 2022. A summary of the pertinent studies is detailed in Table 9.

While the majority of the studies were conducted to test the efficacy of *L. gasseri*, overall, the species was well tolerated in all identified studies. Specifically, no toxicologically relevant changes nor significant adverse events were reported in healthy children consuming *L. gasseri* up to 1.0 x 10^9CFU/day for 12 months (Boonyaritichaikij et al., 2009) and healthy adults with obese tendencies at 1.0 x 10^{11} CFU/day for 12 weeks (Kadooka et al., 2010). Thus, these studies corroborate the safety of *L. gasseri* BNR17™ under the intended conditions of use where the estimated daily exposure is well within the levels evaluated in the existing literature that demonstrate the tolerability of the *L. gasseri* species.

Table 9 Summary of clinical studies on other *L. gasseri* strains

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Boonyaritichaikij et al., 2009	Randomized, single/double-blind, placebo-controlled	Healthy children (aged 5-7 years; asymptomatic *Heliobacter pylori* positive and negative) N=400 (201 male; 199 female)1	Oral, cheese Control: placebo Intervention: *Lactobacillus gasseri* OLL2716 (LG21) at approximately 1.0 x 10^9 CFU/day	12 months	No AEs reported in the study.
Chen et al., 2010	Randomized, double-blind, placebo-controlled	Children with mild to moderate persistent asthma and allergic rhinitis (aged 6-12 years) N=105 (60 male; 45 female)	Oral, capsule Control: placebo Intervention: *Lactobacillus gasseri* A5 at 4.0 x 10^9 CFU/day	8 weeks	No AEs (major or minor) occurring through the study period.
Ogawa et al., 2015	Randomized, double-blind, placebo-controlled	Healthy adults (27-69 years of age; BMI of ca. 21 kg/m^2) N=37 (12 male; 18 female)	Oral, fermented milk Control: placebo Intervention: *Lactobacillus gasseri* SBT2055 at 5.0 x 10^7 CFU/g fermented milk (or 5.0 x 10^9 CFU/day when consuming 100 g test article per day)	7 days	Physical and biochemical blood parameters were not significantly different. No AEs were reported for the duration of the study and no irregularities in daily life.
Yamanaka et al., 2019	Randomized, double-blind, placebo-controlled	Male adults with hyperuricemia; discontinued urate lowering drugs for 4 weeks prior to and through the duration of 8-week study period (aged 63.3±7.6 years; BMI of 25±2.1	Oral, yogurt Control: placebo Intervention: *Lactobacillus gasseri* PA-3Y at ≥8.5 x 10^7 CFU/g (or 1.7 x 10^{10}	8 weeks	No significant difference in BW, heart rate, BP, and blood biochemistry between groups through the duration of the intervention period.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
		kg/m^2; SUA levels ≥7.0 mg/dL) N=25	CFU/day when consuming a 200 g of yoghurt beverage daily)		
Kadooka et al., 2010	Multi-center, randomized, double-blind, placebo-controlled	Healthy adults with obese tendencies (aged 33-63 years; BMI of 24.2–30.7 kg/m^2, abdominal visceral fat area of 81.2–178.5 cm^2) N=87 subjects (59 male; 28 female)	Oral, fermented milk Control: placebo Intervention: *Lactobacillus gasseri* SBT2055 at 5.0 x 10^{10} CFU/100 g fermented milk (or 1.0 x 10^{11} CFU/day when consuming 200 g test article per day)	12 weeks	No irregularity in daily life nor were any AEs observed in the subjects through the duration of the study.
Kadooka et al., 2013	Multi-center, randomized, double-blind, placebo-controlled	Healthy adults with obese tendencies (aged 35-60 years; large visceral fat area of 80.2 – 187.8 cm^2) N=210 subjects (105 male; 105 female)	Oral, fermented milk Control: placebo Intervention: 1. *Lactobacillus gasseri* SBT2055 at 1.0 x 10^6 CFU/g fermented milk (or 2.0 x 10^8 CFU/day when consuming 200 g test article per day) 2. *Lactobacillus gasseri* SBT2055 at 1.0 x 10^7 CFU/g fermented milk (or 2.0 x 10^9 CFU/day when consuming 200 g test article per day)	12 weeks	Clinical chemistry measures in all groups remained within normal ranges. Beneficial effects in abdominal adiposity and other measures of obesity (BMI, waist and hip circumferences, body fat mass) were attenuated during the 4-week follow up period following intervention. No irregularity in daily life nor were any AEs observed in the subjects through the duration of the study.
Ohtsu et al., 2017	Randomized, double-blind, placebo-controlled	Healthy adults with functional dyspepsia as defined by the Rome III criteria (42.8±9.0 years of	Oral, yogurt Control: placebo	12 weeks	AEs reported for 5 subjects in the placebo group and 2 subjects in the intervention group – no significant differences in incidence and the

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
		age; BMI of 20.3±2.7 kg/m^2) N=106 (27 male; 79 female)	Intervention: *Lactobacillus gasseri* OLL2716 at ≥10^9 CFU/serving/day		AEs were determined to be unrelated to the test article.
Ohtsu et al., 2021	Randomized, double-blind, placebo-controlled	Healthy adults with mild to moderate stomach upset without treatment (41.1±11.0 years of age) N=27 (4 male; 23 female)	Oral, yogurt Control: placebo Intervention: *Lactobacillus gasseri* OLL2716 at ≥10^9 CFU/serving/day	12 weeks	AEs reported in placebo group included cold (6), GI symptoms (3), sleep disorders (3) and arthralgia (1), while AEs reported in intervention group included cold (6), GI symptoms (6), sleep disorder (4), headache (2), arthralgia (1). None of the AE were serious nor related to the test article – all AEs were transient and resolved and did not limit participation in the study of any subjects.

Abbreviations: AE, adverse event; BMI, body mass index; BP, blood pressure; BW, body weight; CFU, colony forming unit; GI, gastrointestinal; SUA, serum uric acid

6.4. Pariza decision tree analysis

As indicated above, in assessing the safety of *L. gasseri* BNR17™ under the intended conditions of use, Chr. Hansen has consulted the "*Decision Tree for Determining the Safety of Microbial Cultures to be Consumed by Humans or Animals*" by Pariza et al., (2015). The decision tree is composed of thirteen questions, and their responses as they apply to *L. gasseri* BNR17™ are described below:

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

   YES (go to 2)

2. Has the strain genome been sequenced?

   YES (go to 3)

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

   YES (go to 4)

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

   YES (go to 5)

5. Does the strain produce antimicrobial substances (used in human or veterinary medicine)?

   NO (go to 6)

6. Has the strain been genetically modified using rDNA techniques?

   NO (go to 8a)

8a. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component?

   YES (go to proceed to 9a)

9a: Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for use by an authoritative group of qualified scientific experts?

   YES (go to 10a)

10a: Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9a continue to support the conclusion that the species, to which the strain belongs, is safe for use in food?

   YES (go to 11a)

11a: 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?

NO (go to 12a)

12a: Will the intended use of the strain expand intake of the species?

No (go to 14a)

13a. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies?

NO (go to 14a)

14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

6.5. Conclusion of GRAS status

Chr. Hansen concludes that the intended uses of *Lactobacillus (L.) gasseri* BNR17™ are GRAS based on scientific procedures.

Chr. Hansen has applied the framework of the Pariza *et al*. (2015) decision tree and elements of the EFSA QPS approach (EFSA Scientific Committee, 2007) to demonstrate the safety of *L. gasseri* BNR17™ for use as a microbial food ingredient. The data presented in this GRAS notice fully support the conclusion that *L. gasseri* BNR17™ is GRAS under the intended uses as described. The basis of the GRAS conclusion for the use of *L. gasseri* BNR17™ are summarized by the following pivotal considerations:

- *L. gasseri* has a history of safe consumption from traditional fermented foods – *Lactobacillus gasseri* is presently included in EFSA's QPS list (EFSA BIOHAZ Panel, 2021) and the IDF/EFFCA's *Inventory of microbial food cultures with safety demonstration in fermented food products* (Bourdichon et al., 2012, 2018; Mogensen et al., 2002), as well as the DVFA's *List of notified microbial cultures applied in food (Danish Veterinary and Food Administration, 2016)*

- Chr. Hansen's manufacturing and quality control programs (cGMP, HACCP, FSSC 22000) ensure the safety and quality of the final *L. gasseri* BNR17™ ingredient.

- *L. gasseri* BNR17™ is not genetically modified, is not pathogenic or toxigenic, is not able to produce biogenic amines, and does not carry any transferable genes conferring antibiotic resistance.

- *L. gasseri* BNR17™ has been evaluated in a number of human clinical studies in which the strain was safely consumed without adverse events.

Based on the above considerations, the safety of *L. gasseri* BNR17™ is supported with a reasonable certainty of no harm under the intended conditions of use.

Part 7. List of supporting data and information

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March 22, 2023
CAWINN

Response to Questions/Comments Regarding GRN 001089

Dear Marissa Santos,

In regard to the questions/comments on GRN 001089 for the intended use of *Lactobacillus gasseri* NCIMB 30370 received from the U.S. FDA on March 8, 2023, please find Chr. Hansen's responses attached.

We trust that this meets with your immediate needs and remain available for any other questions you may have.

Yours sincerely,

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RESPONSE TO FDA QUESTIONS ON GRN 001089 FOR *LACTOBACILLUS GASSERI* NCIMB 30370 RECEIVED ON MARCH 8, 2023

The following is Chr. Hansen's response to the questions/comments on GRN 001089 for the intended use of *Lactobacillus gasseri* NCIMB 30370 as received from the U.S. FDA on March 8, 2023.

RESPONSES:

1. **Please provide a statement that all processing aids used in the manufacture of *Lactobacillus gasseri* NCIMB 30370 (*L. gasseri* strain NCIMB 30370) are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses, or are subjects of effective food contact notifications.**

   Chr. Hansen confirms that all processing aids used in the manufacture of *L. gasseri* NCIMB 30370 are used in accordance with applicable U.S. regulations and/or are GRAS for their intended uses. All processing aids are safe and suitable for human consumption under the intended conditions of use.

2. **In Table 4 you provide the specifications for *L. gasseri* strain NCIMB 30370 and the results from the analyses of three nonconsecutive batches. We note that the specifications for lead, arsenic, and cadmium are significantly higher than the results from your batch analyses. Please lower your specifications for these heavy metals to reflect the results of your batch analyses and to be as low as possible.**

   In the U.S., Chr. Hansen is not aware of any regulatory limits or action levels for heavy metals specific to microbial cultures for use as food ingredients. That said, in the absence of U.S. legislated heavy metal limits for this ingredient category, Chr. Hansen justifies its heavy metal specifications based on the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995, as amended) as an international standard, in conjunction with our extensive Quality Program which includes risk assessment and management procedures.

   Notably, our heavy metal specification takes into account that the Codex limits are set at the commodity[1] or product level, *i.e.*, based on finished food applications, and are not set at the ingredient level. With this in consideration, and the fact that our microbial ingredients are included in the finished food at very low levels (typically less than 0.15% of the finished food), our current heavy metal specifications would result in exposure levels that are well below the heavy metal limits of representative foods under the most conservative (worst-case) scenarios (see Table below). Thus, the *L. gasseri* NCIMB 30370 ingredient does not significantly contribute to heavy metals in the final food applications, and we are reasonably certain that our existing heavy metal specification will be sufficient to mitigate any risk in the finished food product under the intended conditions of use.

[1] Note that microbial cultures are not listed as a commodity in the Codex Standard.

Heavy Metals	Units	Chr. Hansen's *L. gasseri* NCIMB 30370 ingredient		Codex Alimentarius[a]
		Specification in GRN 001089	Estimated Contribution to Finished Product[b]	Maximum Level in Representative Food Products[c]
Lead	ppm (or mg/kg)	NMT 1.0	0.0015	0.02[d]
Arsenic	ppm (or mg/kg)	NMT 1.0	0.0015	0.1[e]
Cadmium	ppm (or mg/kg)	NMT 0.30	0.00045	0.8[f]
Mercury	ppm (or mg/kg)	NMT 0.05	0.000075	-[g]

Abbreviations: GRN, GRAS notice; NMT, not more than.
[a] Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995, as amended).
[b] In consideration of a worst-case scenario, at the maximum specification limit and typical inclusion level of 0.15% in the final food. Calculated as: heavy metal specification limit x 0.0015
[c] Most conservative limit, *i.e.*, representative food with the lowest maximum limit excluding drinking water. Note that representative food products with Codex maximum limits are limited, thus the food products illustrated are only for purposes of an example and do not necessarily represent the actual proposed intended use.
[d] For secondary milk products.
[e] For fat spreads and blended spreads.
[f] For chocolate containing or declaring ≥50% to < 70% total cocoa solids on a dry matter basis.
[g] There are no limits for mercury in representative foods that are relevant to the intended uses of Chr. Hansen's *L. gasseri* NCIMB 30370 ingredient; however, for comparative purposes, the limit for mercury in natural mineral waters is 0.001 mg/kg.

Furthermore, the Codex Standard states that "*MLs [maximum levels] should only be set for food in which the contaminant may be found in amounts that are significant for the total exposure of the consumer…*". Heavy metals are not a reasonable hazard in Chr. Hansen's production of microbial cultures. Heavy metal contamination is not a potential hazard in our process or products. The only potential source of heavy metals comes from raw materials used during the fermentation process and cryoprotectants added post fermentation. To mitigate risk of contamination through these materials, risk assessments are conducted during implementation of new raw materials and cryoprotectants. Supplier approval includes a statement of compliance to the legislation in force on compliance to heavy metal limits. Potable water is used in our process, which complies with heavy metal limits for drinking water. Seed materials (pre-inoculation material-PIM and direct inoculation material-DIM) are grown with those same standards. PIM and DIM are inoculated into the fermentations at extremely low levels which suggests that their contribution to heavy metal contamination of a production batch is exceedingly small.

In consideration of the above, it is reasonable to conclude that our current heavy metal specifications will pose no risk under the intended conditions of use of *L. gasseri* NCIMB 30370. Moreover, as detailed by our analytical data in the original GRAS notice, Chr. Hansen can meet and exceed the heavy metal specifications put in place with our current Quality Program.

3. **You state that *L. gasseri* strain NCIMB 30370 has a shelf life of a minimum of 18 months from the date of manufacture. Please clarify that the ingredient is stable for at least 18 months.**

Chr. Hansen confirms that the ingredient *L. gasseri* NCIMB 30370 is stable for the entirety of its shelf life of at least 18 months.

4. You estimate the dietary exposure to *L. gasseri* strain NCIMB 30370 to be 2.0 x 10^{10} colony forming units (CFU)/day presuming that a healthy individual consumes approximately 20 servings of food per day and that all these foods contain *L. gasseri* strain NCIMB 30370 at a level of 1 x 10^{9} CFU/serving. We note that the maximum use level for your ingredient is stated to be 10^{11} CFU/serving. Please provide a dietary exposure estimate based on the maximum use level for your ingredient.

Under the intended conditions of use, the incorporation level of the notified substance will range between 10^{9} to 10^{11} CFU/serving to account for loss of viability throughout the shelf-life of the product. If it is assumed that an average healthy individual consumes approximately 20 servings of food per day (Millen et al. 2006), and a "worst-case" scenario where all servings of food would contain the maximum level of *L. gasseri* NCIMB 30370 at 10^{11} CFU/serving, then the estimated dietary intake at the maximum proposed use level would be approximately 2.0 x 10^{12} CFU/day.

In reality, the estimated maximum daily intake is extremely conservative, and it is highly unlikely that even half of the conventional food consumed in a day would contain *L. gasseri* NCIMB 30370. Likewise, *Lactobacillus* spp. are ubiquitous and lactic acid bacteria are transient in the gut (Faith et al., 2013). The *L. gasseri* species is part of the human microbiome and any additional exposure to the notified substance under the intended conditions of use is not anticipated to significantly alter or contribute to the overall homeostatic nature of the gut microbiota in the general population.

Furthermore, *L. gasseri* NCIMB 30370 may be considered an alternative to the taxonomically related *L. acidophilus* for the same existing uses as a microbial ingredient in conventional food (GRN No. 357, 502, and 871). These notified *L. acidophilus* strains are intended for use at levels ranging between 10^{9} to 10^{11} CFU/serving, which is the same use level proposed for *L. gasseri* NCIMB 30370 subject to GRN 1089. Therefore, the intended use of *L. gasseri* NCIMB 30370 is not anticipated to increase the existing overall dietary intake of the *L. acidophilus* group of organisms.

References:

Faith, J. J., Guruge, J. L., Charbonneau, M., Subramanian, S., Seedorf, H., & Goodman, A. L. (2013). The Long-Term Stability of the Human Gut Microbiota. *Science*. Retrieved from Science: http://science.sciencemag.org/content/341/6141/1237439

Millen, A. E., Midthune, D., Thompson, F. E., Kipnis, V., & Subar, A. F. (2006). The National Cancer Institute Diet History Questionnaire: Validation of Pyramid. American Journal of Epidemiology, 279-288.

5. On page 30 of the notice, question 4 of the Pariza decision tree analysis states, "Does the strain produce antimicrobial substances (used in human or veterinary medicine)?" You provided the answer "NO." For the administrative record, please provide a brief discussion on *L. gasseri* strain NCIMB 30370's inability to produce antimicrobial substances.

L. gasseri is not known to produce antimicrobial substances of relevance for use in human or animals. Specifically, *L. gasseri* is considered suitable for qualified presumption of safety (QPS) status by the European Food Safety Authority (EFSA). As part of this approach, the ability of the species to produce

antimicrobial substances is taken into account as part of the four principal considerations for evaluation of the QPS status of a microorganism. Safety concerns for a taxonomic unit on the QPS list are, where possible, reflected as "qualifications" which, if identified, should then be assessed at the strain level (EFSA BIOHAZ Panel, 2023). At present, the only EFSA qualification for *L. gasseri* is, as described in the GRAS notice, the generic qualification for all QPS bacterial taxonomic units – that is, individual strains should not harbor any acquired antimicrobial resistance genes to clinically relevant antimicrobials (EFSA BIOHAZ Panel, 2023). Thus, production of antimicrobials relevant to use in human and veterinary medicine is not regarded a trait that is a significant concern for lactobacilli that qualifies for the QPS approach including *L. gasseri*. Moreover, based on systematic and extensive literature searches EFSA reassesses all species on the QPS list for whether the species can sustain its QPS status twice yearly. EFSA did not find any safety concern with the species *L. gasseri,* and the QPS status of *L. gasseri* has not changed through the latest update of the list of QPS recommended microorganisms from January 2023. On this basis, Chr. Hansen concludes with reasonable certainty that *L. gasseri* NCIMB 30370 does not produce antimicrobial substances used in human or veterinary medicine.

Reference: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2023. Scientific Opinion on the update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. EFSA Journal 2023;21(1):7747, 23 pp. https://doi.org/10.2903/j.efsa.2023.7747ISSN:1831-4732

6. **For the administrative record, please briefly specify how the purity of *L. gasseri* strain NCIMB 30370 inoculum is ensured.**

   As indicated in the GRAS notice, *L. gasseri* NCIMB 30370 is produced following Chr. Hansen's global protocol for production of food cultures which includes an extensive Quality Program with a fully implemented Hazards Analysis and Critical Control Points (HACCP) plan, and all facilities maintain FSSC 22000 certification. The Quality Program serves to verify the control of the production facility, where the purity of the *L. gasseri* NCIMB 30370 inoculum is maintained as part of our HACCP plan.

   Specifically, Chr. Hansen's Culture Collection is maintained and propagated under a strictly controlled aseptic environment. The inoculum is handled through aseptic technique by qualified technical personnel and automated machinery is used wherever possible. Throughout the production of the inoculation material, samples are sent for extensive quality control (QC) testing for contamination and cross contamination check as well as strain identification. Each batch is controlled and approved according to an established purity specification. The inoculation material is not released to production unless the QC results comply with the specification. During production, QC testing is performed at 2 intermediate stages and prior to release of the finished material.

   Production batches of *L. gasseri* NCIMB 30370 are thoroughly tested throughout the production process. All finished materials are tested and released according to the product release specification to guarantee the identity, total count, and purity of the microorganisms.

7. **On page 13, you state, "No major allergens nor substances derived from major allergens are used as raw materials in the fermentation media for the production of *L. gasseri* BNR17™." As of January**

1, 2023, sesame is considered a major food allergen. (https://www.fda.gov/food/cfsan-constituent-updates/faster-act-video-food-industry-andother-stakeholders).
For the administrative record, please state whether sesame or substances derived from sesame are used in your manufacturing process and whether this poses a safety concern.

Chr. Hansen complies with the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) and the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act.

Chr. Hansen confirms that sesame and substances derived thereof are not used in the manufacturing process of any of our strains. Therefore, sesame and substances derived from sesame do not pose a safety concern in the *L. gasseri* NCIMB 30370 ingredient.

8. **On page 16, you state that you conducted a literature search through March 2022. Please confirm that no new information that may appear counter to your GRAS conclusion has been published since March 2022.**

An updated literature search was conducted from March 2022 through March 2023 to identify any publications pertinent to the safety of *L. gasseri* published since the original GRAS notice submission. The same methodology was used for the search strategy as outlined in the original GRAS notice and as defined by EFSA's QPS approach for *Lactobacillus* (specifically *Lactobacillus gasseri*). The details of the search criteria and identified studies are outlined in the Table below.

Source	Outcome	Search string	Number of hits
PubMed Date filter: March 2022 to March 2023	Antimicrobial/ antibiotic/antimycotic	*Lactobacillus gasseri* AND antimicrobial resistan* OR antibiotic resistan* OR antimicrobial susceptibil*	11
	Infection/bacteremia/ fungemia/sepsis	*Lactobacillus gasseri* AND infection* OR abscess* OR sepsis* OR septic* OR bacteremia OR bacteraemia OR toxin*	32
	Type of disease	*Lactobacillus gasseri* AND endocarditis OR abscess OR meningitis	1
	Mortality/morbidity	Not applied	-
	Disease risk	*Lactobacillus gasseri* AND opportunistic OR virulen*	8

The search results were then screened for relevance in terms of safety concerns related to pathogenicity and toxigenicity in humans. Based on the above literature search strategy, one new publication pertinent to the safety of *L. gasseri* was identified since the original GRAS notice.

The publication was a review of case reports on lactobacilli infections published from 2019 to 2021 (Rossi et al., 2022). Within this review article 1 case of infection was identified for *L. gasseri* in a 59-year-old male that presented with liver abscesses; however, the subject had a predisposing risk factor of multiple abdominal surgeries with modified bio-digestive anatomy and the underlying condition of diabetes mellitus (Ramos-Coria et al., 2021). Indeed, this case report had previously been identified and extensively discussed as part of the original GRAS notice for *L. gasseri* NCIMB 30370 and is consistent with the findings of a low concern for pathogenicity of *L. gasseri* except in rare cases of opportunistic infections where subjects are predisposed with underlying risk factors and conditions.

In addition, an updated search was conducted to identify randomized controlled human clinical studies published since the original GRAS notice. The updated literature search was conducted using the PubMed database for publications through to March 2023. The search identified 1 additional study relevant to the tolerability of *L. gasseri* as a species. A summary of the details of the study are included in the Table below. While the study was conducted to investigate the efficacy of *L. gasseri*, no adverse events were reported during the study period, and therefore it supports the tolerability of the species in healthy adults at levels of 1.0 x 10^{10} CFU/day.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Sawada et al. 2022	Randomized, double blind, placebo controlled, parallel group	Healthy premenopausal women (aged 40-60 years) N=80	Oral, tablet Control: placebo Intervention: *Lactobacillus gasseri* CP2305 at 1.0 x 10^{10} CFU/day	6 consecutive menstrual cycles	No adverse events were observed through the duration of the study.

Abbreviations: AE, adverse event; CFU, colony forming unit.

Thus, to the best of our knowledge, there are no new scientific data published since March 2022 that would counter our GRAS conclusion for *L. gasseri* NCIMB 30370 under the intended conditions of use. This is consistent with the most recent QPS update in January 2023, where EFSA concluded the QPS status of the QPS species within the *Lactobacilli* genus remained unchanged including *Lactobacillus gasseri* (EFSA BIOHAZ Panel, 2023).

References:

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2023. Scientific Opinion on the update of the list of qualified presumption of safety (QPS) recommended microorganisms intentionally added to food or feed as notified to EFSA. EFSA Journal 2023;21(1):7747, 23 pp. https://doi.org/10.2903/j.efsa.2023.7747ISSN:1831-4732

Ramos-Coria, D., Canto-Losa, J., Carrillo-Vázquez, D., Carbajal-Morelos, L., Estrada-León, R., & Corona-Rodarte, E. (2021). *Lactobacillus gasseri* liver abscess and bacteremia: a case report. *BMC Infectious Diseases*, *21*(1), 518. https://doi.org/10.1186/s12879-021-06181-w

Rossi, F., Amadoro, C., Gasperi, M., Colavita, G. (2022). Lactobacilli Infection Case Reports in the Last Three Years and Safety Implications. *Nutrients*, 14, 1178. https://doi.org/10.3390/nu14061178

Sawada, D., Sugawara, T., Hirota, T., Nakamura, Y. (2022). Effects of *Lactobacillus gasseri* CP2305 on Mild Menopausal Symptoms in Middle-Aged Women. *Nutrients*, 14, 1695. https://doi.org/10.3390/nu14091695

Santos, Marissa

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Wednesday, April 19, 2023 9:04 AM
To: Santos, Marissa
Cc: Kate Urbain
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Marissa,

Thank you for the opportunity to address the FDA's question on GRAS Notice No. 001089 dated April 06, 2023. Please find our response as follows:

Chr. Hansen agrees with the FDA's request to lower the specification for lead to be consistent with our analytical data. Our Quality Assurance confirms that the specification for lead can be reduced from not more than (NMT) 1 ppm to NMT 0.3 ppm.

Please let me know if you have any further questions.

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
Human Health & Nutrition - Probiotics
Chr. Hansen Inc.

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Email: cawinn@chr-hansen.com
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From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Thursday, April 6, 2023 2:21 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

During our review of GRAS Notice No. 001089 and the responses to our questions that you provided on March 22, 2023, we note an additional question that needs to be addressed and I've included it below.

1) In the amendment dated March 22, 2023, you note in your response to question 2 the absence of US regulatory limits or action levels for heavy metals specific to microbial cultures used as food ingredients to justify your heavy metal specifications. You also explain that your heavy metal limits were established based on the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995) and the inclusion of the ingredient in the final food product at levels less than 0.15%. We note that specifications help to ensure that the ingredient is being manufactured in accordance with good manufacturing practices. In addition, we further note that *Lactobacillus gasserii* NCIMB 30370 is intended for use in conventional foods that may be consumed by young children, and that your specification for lead is two orders of magnitude higher than the levels reported from your batch analyses. We would also like to remind you that FDA's recent "Closer to Zero" initiative focuses on reducing dietary exposure to lead, arsenic, cadmium and mercury from foods consumed by infants and young children. Therefore, we request that you lower the specification for lead so that it is consistent with the results from your batch analyses and is as low as possible. For more information on our Closer to Zero initiative, please access the following link: Closer to Zero: Reducing Childhood Exposure to Contaminants from Foods.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have any questions or need further clarification, please feel free to reach out to me.

Regards,
Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

From: Santos, Marissa
Sent: Wednesday, March 22, 2023 2:57 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

Thank you for these responses. Our team is reviewing them and we will reach out should we have any additional questions.

Regards,
Marissa

Marissa Santos, M.S.

Regulatory Review Scientist and Microbiology Reviewer
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Wednesday, March 22, 2023 2:43 PM
To: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Marissa,

Please find attached our response to the questions on GRAS Notice No. 001089.

If you have any questions, please let us know.

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
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From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Wednesday, March 8, 2023 8:34 AM
To: Winnie Ng <CAWINN@chr-hansen.com>
Subject: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

During our review of GRAS Notice No. 001089, we noted several questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have any questions or need further clarification, please feel free to reach out to me.

Regards,
Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
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Santos, Marissa

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Monday, May 8, 2023 4:06 PM
To: Santos, Marissa
Cc: Kate Urbain; Arie Carpenter
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Marissa,

Thank you to you and your team for the additional clarity in the call last week.

We can confirm that a lead specification of not more than 0.10 ppm can be established for *Lactobacillus gasseri* NCIMB 30370.

If you have any other questions, please feel free to contact us.

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
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From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Friday, April 21, 2023 12:18 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Hi Dr. Ng,

Thank you! I've confirmed our call for Thursday, May 4th from 12:00 pm to 12:30 pm. Please see the Zoom call-in information below. If you have any questions, please don't hesitate to reach out.

Have a great weekend,

Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

Hi there,

Marissa Santos is inviting you to a scheduled ZoomGov meeting.

Join Zoom Meeting

One tap mobile: US: +16692545252,,1611069657#,,,,*494841# or +16469641167,,1611069657#,,,,*494841#

Meeting URL: https://fda.zoomgov.com/j/1611069657?pwd=WnpOd1FHbnY5S2xtUE5QYjNnVmozQT09

Meeting ID: 161 106 9657

Passcode:PKY%7b

Join by Telephone

For higher quality, dial a number based on your current location.

Dial:
+1 669 254 5252 US (San Jose)
+1 646 964 1167 US (US Spanish Line)
+1 646 828 7666 US (New York)
+1 415 449 4000 US (US Spanish Line)
+1 551 285 1373 US
+1 669 216 1590 US (San Jose)
833 568 8864 US Toll-free
833 435 1820 US Toll-free

Meeting ID: 161 106 9657

Passcode:494841

International numbers

Join from an H.323/SIP room system

H.323: 161.199.138.10 (US West)
161.199.136.10 (US East)

Meeting ID: 161 106 9657

Passcode:494841

SIP: 1611069657@sip.zoomgov.com

Passcode:494841

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Friday, April 21, 2023 12:11 PM
To: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Hi Marissa,

Thank you for being so accommodating. We are available Thursday May 4 at 12:00 pm - 1:00 pm.

Please send the details of the call and thanks again!

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
Human Health & Nutrition - Probiotics
Chr. Hansen Inc.

From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Friday, April 21, 2023 11:44 AM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Hi Dr. Ng,

Unfortunately that time doesn't work on our end. I've listed more availabilities below:

- Monday, May 1: 11:30 am – 1 pm
- Wednesday, May 3: 10 am – 11 am; 2 pm – 3 pm
- Thursday, May 4: 11:30 am – 1 pm

Please let me know if any of these dates and times works better.

Thank you!
Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Friday, April 21, 2023 11:34 AM
To: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>; Arie Carpenter <USARBR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Hi Marissa,

Thank you for the opportunity to meet with you.

Next week is a bit busy with business travel. Can we propose an alternate time, Thursday April 27th between 1:00 pm to 3:00 pm? Does this work for you?

Thanks again!

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
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Chr. Hansen Inc.

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From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Thursday, April 20, 2023 3:17 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Hi Dr. Ng,

We would like to meet with you to discuss the specifications for heavy metals in your notice. We have the following times available:

- Tuesday, April 25: 10:00 am – 11:00 am
- Wednesday, April 26: 2:00 pm – 3:00 pm
- Thursday, April 27: 9:00 am – 10:00 am

Please let me know if any of the dates and times above work for you.

Regards,
Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Wednesday, April 19, 2023 9:04 AM
To: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Marissa,

Thank you for the opportunity to address the FDA's question on GRAS Notice No. 001089 dated April 06, 2023. Please find our response as follows:

Chr. Hansen agrees with the FDA's request to lower the specification for lead to be consistent with our analytical data. Our Quality Assurance confirms that the specification for lead can be reduced from not more than (NMT) 1 ppm to NMT 0.3 ppm.

Please let me know if you have any further questions.

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
Human Health & Nutrition - Probiotics
Chr. Hansen Inc.

Mobile: +1 705 746 0491
Email: cawinn@chr-hansen.com
www.chr-hansen.com

From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Thursday, April 6, 2023 2:21 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

During our review of GRAS Notice No. 001089 and the responses to our questions that you provided on March 22, 2023, we note an additional question that needs to be addressed and I've included it below.

1) In the amendment dated March 22, 2023, you note in your response to question 2 the absence of US regulatory limits or action levels for heavy metals specific to microbial cultures used as food ingredients to justify your heavy metal specifications. You also explain that your heavy metal limits were established based on the Codex Alimentarius General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995) and the inclusion of the ingredient in the final food product at levels less than 0.15%. We note that specifications help to ensure that the ingredient is being manufactured in accordance with good manufacturing practices. In addition, we further note that *Lactobacillus gasserii* NCIMB 30370 is intended for use in conventional foods that may be consumed by young children, and that your specification for lead is two orders of magnitude higher than the levels reported from your batch analyses. We would also like to remind you that FDA's recent "Closer to Zero" initiative focuses on reducing dietary exposure to lead, arsenic, cadmium and mercury from foods consumed by infants and young children. Therefore, we request that you lower the specification for lead so that it is consistent with the results from your batch analyses and is as low as possible. For more information on our Closer to Zero initiative, please access the following link: Closer to Zero: Reducing Childhood Exposure to Contaminants from Foods.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have any questions or need further clarification, please feel free to reach out to me.

Regards,
Marissa

Marissa Santos, M.S.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Tel: 240.402.8160
marissa.santos@fda.hhs.gov

From: Santos, Marissa
Sent: Wednesday, March 22, 2023 2:57 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: RE: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

Thank you for these responses. Our team is reviewing them and we will reach out should we have any additional questions.

Regards,
Marissa

Marissa Santos, M.S.
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marissa.santos@fda.hhs.gov

From: Winnie Ng <CAWINN@chr-hansen.com>
Sent: Wednesday, March 22, 2023 2:43 PM
To: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Cc: Kate Urbain <USKAUR@chr-hansen.com>
Subject: [EXTERNAL] [WARNING : MESSAGE ENCRYPTED] [WARNING : MESSAGE ENCRYPTED] RE: GRN 1089 - Questions for the Notifier

Dear Marissa,

Please find attached our response to the questions on GRAS Notice No. 001089.

If you have any questions, please let us know.

Kindest Regards,
Winnie

Winnie Ng, Ph.D., DABT
Principal Regulatory Affairs Specialist
Human Health & Nutrition - Probiotics
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From: Santos, Marissa <Marissa.Santos@fda.hhs.gov>
Sent: Wednesday, March 8, 2023 8:34 AM
To: Winnie Ng <CAWINN@chr-hansen.com>
Subject: GRN 1089 - Questions for the Notifier

Dear Dr. Ng,

During our review of GRAS Notice No. 001089, we noted several questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your responses.

If you have any questions or need further clarification, please feel free to reach out to me.

Regards,
Marissa

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