CHR. HANSEN
Improving food & health

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August 04, 2022
CAWINN

Re: Chr. Hansen GRAS notice for *Lactobacillus plantarum* NCIMB 30562

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.) plantarum* NCIMB 30562 is GRAS through scientific procedures for its intended use as a microbial ingredient in conventional foods and non-exempt infant formula for term infants, 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 1.0×10^{11} colony-forming units (CFU)/serving to account for loss of viability throughout the shelf-life of the product, and 1.1×10^{8} CFU/g of term, non-exempt infant formula.

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 Use of *Lactobacillus plantarum* NCIMB 30562 in Conventional Foods and Non-Exempt Infant Formula

Prepared by Chr. Hansen A/S

August 2022

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Abbreviations

ADI	Acceptable daily intake
AE	Adverse event
ATCC	American Type Culture Collection
BE	Bioengineering
BIOHAZ	Panel on Biological Hazards
BLAST	Basic Local Alignment Search Tool
BMI	Body mass index
BSL	Biosafety Level
BW	Body weight
CCP	Critical Control Points
CFR	Code of Federal Regulations
CFU	Colony forming units
cGMP	Current good manufacturing practice
CT	Computerized tomography
DNA	Deoxyribonucleic acid
EFFCA	European Food and Feed Cultures Association
EFSA	European Food Safety Authority
EU	European Union
FEEDAP	The Panel on Additives and Products or Substances used in Animal Feed
FDA	Food and Drug Administration
FOS	Fructo-oligosaccharide
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
FSSC	Food Safety System Certification
GM	Genetic modification
GRAS	Generally Recognized as Safe
GRN	GRAS notice
GC-MS	Gas chromatography-mass spectrometry
HACCP	Hazard Analysis and Critical Control Point
IDF	International Dairy Federation
ISO	International Standardization Organization
LAB	Lactic acid bacteria
MIC	Minimum inhibitory concentration
NBFDS	National Bioengineered Food Disclosure Standard
NCBI	National Center for Biotechnology Information
NCIMB	National Collection of Industrial, Food, and Marine Bacteria
NOAEL	No-observed-adverse effect level
OPRP	Operational Prerequisite Program
ONT	Oxford Nanopore MiniON technology
PRP	Prerequisite Program
QPS	Qualified presumption of safety
U.S.	United States
U.S.C	United States Code
USDA	United States Department of Agriculture
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.) plantarum*[1] NCIMB 30562 is GRAS and is not subject to the premarket approval requirements for use as a microbial ingredient in conventional foods and non-exempt infant formula for term infants.

Name and Address of Organization

Chr. Hansen A/S
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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.) plantarum NCIMB 30562 / *Lactiplantibacillus (L.) plantarum* NCIMB 30562

1.3. Intended conditions of use

L. plantarum NCIMB 30562 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 practice (cGMP). *L. plantarum* NCIMB 30562 is also intended for use in non-exempt infant formula for term infants.

The level of inclusion of *L. plantarum* NCIMB 30562 will vary depending on the type of food and application under which it will be used; however, the maximum incorporation level will be 1.0×10^{11} colony-forming units (CFU)/serving to account for loss of viability throughout the shelf-life of the product, and 1.1×10^{8} CFU/g of term, non-exempt infant formula.

L. plantarum NCIMB 30562 is not intended for use in products regulated by the United States Department of Agriculture (USDA).

[1] Scientific ref.: *Lactobacillus plantarum* Bergey et al. 1923 (*Lactiplantibacillus plantarum* comb. nov., as described in Zheng et al. (2020) https://doi.org/10.1099/ijsem.0.004107; University of Alberta http://lactobacillus.ualberta.ca/

1.4. Statutory basis for conclusion of GRAS status

Pursuant to the GRAS rule [81 Fed. Reg. 159 (August 17, 2016)], Chr. Hansen has concluded that the intended use of *L. plantarum* NCIMB 30562 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. plantarum* NCIMB 30562 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on our conclusion that *L. plantarum* NCIMB 30562 is GRAS under the intended conditions of use.

1.6. Availability of information

The data and information that form the basis of Chr. Hansen's conclusion that the intended use of *L. plantarum* NCIMB 30562 is GRAS are available for review and copying by FDA during customary business hours, at the location below, or will be sent 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 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. plantarum* NCIMB 30562 under the intended conditions of use.

1.9. FSIS statement

Not applicable. *L. plantarum* NCIMB 30562 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

August 4, 2022

Winnie Ng
Principal Regulatory Affairs Specialist

Date

August 4, 2022

Katharine Urbain
Head of North America Regulatory Affairs

Date

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

2.1. Identity of the GRAS substance

The subject of this GRAS notice is a strain of the bacterial species *Lactobacillus (L.) plantarum* designated as NCIMB 30562.

Recent taxonomic changes to the genus *Lactobacillus* published by Zheng *et al.* in April of 2020 will effectively change the nomenclature of this organism from *Lactobacillus plantarum* to *Lactiplantibacillus plantarum* moving forward (Zheng et al., 2020).

2.2. Source of the GRAS organism

L. plantarum NCIMB 30562 was originally isolated from a human infant and belongs to the taxa *Lactobacillus plantarum*.

The *L. plantarum* NCIMB 30562 that is the subject of this notice was deposited in the National Collection of Industrial, Food, and Marine Bacteria (NCIMB) culture collection under the NCIMB code 30562.

2.3. Description of the GRAS organism

2.3.1. Lactic acid bacteria

L. plantarum NCIMB 30562 is part of the broader group of bacteria known as lactic acid bacteria (LAB). 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 that produce lactic acid as the primary fermentation product of the metabolism of sugars. This property is utilized broadly in the production of fermented foods. LAB have been used since ancient times in the preservation and production of fermented foods (Mozzi, 2016). In addition to its widespread consumption from food sources, LAB are also a ubiquitous 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. Lactobacillaceae

Lactobacillus spp. are widely used in commercial applications for the fermentation of dairy products, fruits, vegetables, and meats (Aguirre & Collins, 1993; Gasser, 1994) as well as present in food as autochthonous non-starter LAB (Hutkins, 2019). Lactobacilli grow under reduced oxygen conditions in habitats where ample nutrients exist and are found in the gastrointestinal tract of healthy humans of all ages (Bernardeau et al., 2008; Goldin et al., 1992; Saxelin et al., 1996).

While the Lactobacilliaceae family consists of organisms widely used in food and as food supplements, these organisms also colonize the mouth, gastrointestinal tract and female urinary tract of humans. As the human body contains Lactobacilli as normal constituents and is in constant contact from external

sources, it is not unexpected that infection caused by Lactobacilli are rare and usually associated with at risk people with suppressed immune systems (EFSA BIOHAZ Panel, 2021; Rossi et al., 2019). Indeed, a comprehensive review of the safety of Lactobacilli in food determined that the group was safe and suitable for use in food (Bernardeau et al., 2008).

2.3.3. *Lactobacillus plantarum*

L. plantarum is a member of the Phylum Firmicutes in bacterial taxonomy. *L. plantarum* is a well-characterized, non-pathogenic, non-toxigenic, homogeneous species grouping. The species is found in numerous niches including dairy products, meat and vegetable fermentations, including silage (Cogan, 1996; Hutkins, 2019; Maaike et al., 2006; Zheng et al., 2020).

The taxonomic lineage of *L. plantarum* NCIMB 30562 is detailed in Table 1.

Table 1. Taxonomic lineage of *Lactobacillus plantarum* NCIMB 30562

Taxonomy	**Taxonomic Assignment**
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Lactobacillaceae
Genus	*Lactobacillus*
Species	*Lactobacillus plantarum*
Strain	*Lactobacillus plantarum* NCIMB 30562

Recent taxonomic changes to the genus *Lactobacillus* published by Zheng et al. (2020) seek to reduce the confusion surrounding the previously described Lactobacillaceae taxonomy based upon a polyphasic genetic relatedness and phylogeny approach. Criteria for the new ordering of the family is based upon average nucleotide identity, average amino acid identity, core-gene average amino acid identity, core genome phylogeny, signature genes, and metabolic or ecological data. The resulting taxonomic changes have created "new" genera to group like organisms and a prime example is the renaming of *Lactobacillus plantarum* as *Lactiplantibacillus plantarum.* This change groups all the members of the *plantarum* group into a single genus.

Given the comprehensive nature of the proposed taxonomic changes and the fact that the name changes are not part of the current scientific literature, Zheng et al. (2020) suggest that the use of the name "lactobacilli" will remain useful in describing species that have historically been ascribed to the family. With that recommendation in mind, for the purposes of clarity and to avoid undue confusion, we maintain the "old" descriptors for the historical discussion of the family that contains the ingredient *Lactobacillus (L.) plantarum* NCIMB 30562 that is the subject of this notice.

2.3.4. Genotypic classification of *Lactobacillus plantarum* NCIMB 30562

2.3.4.1. Species identification

Analysis of the NCIMB 30562 strain's 16S rDNA sequence was compared to a database of 16S rDNA sequences of type strains (Ludwig et al., 2021). Since 16S rDNA sequence comparison does not resolve between all species of the *Lactobacillus plantarum* group, a comparative sequence analysis of rpoA gene sequences was performed in addition as recommended by Naser et al. (2007). Sequence comparison of the rpoA gene sequence of the NCIMB 30562 strain to a rpoA sequence database of strains of the *Lactobacillus plantarum* group places the NCIMB 30562 strain into the species *Lactobacillus plantarum*. Therefore, the NCIMB 30562 strain is identified as *Lactobacillus plantarum*.

2.3.4.2. Genome sequencing and annotation

To obtain a high-quality genome sequence of *L. plantarum* NCIMB 30562, the strain was genome sequenced in-house using the Illumina MiSeq technology and at an external provider using the Oxford Nanopore MinION technology (ONT). The ONT provide long sequence reads, but with a relatively high error rate, whereas Illumina sequence technology on the other hand performs short reads with few errors. By combining the sequence reads from both technologies in the same assembly a closed genome of high quality can often be obtained.

Output from the MiSeq sequencing (1,457,577 reads) was used for the de novo assembly algorithm of CLC Genomic Workbench (CLC Bio, Qiagen) using published methods (Agersø et al., 2018) and resulted in 71 contigs of 3,312,445 bp with an average coverage of approximately 50. Combing reads from both sequencing technologies, *i.e*., a hybrid assembly, led to a circular chromosome of 3.35 Mb (3,356,372 bp) and a circular plasmid of 60,726 bp. For the chromosome, the coverage of short reads was 93x and 143x for the long reads with a N50 value at 3,295,646. The guanine and cytosine (GC) content is 44.35%. Plasmid profiling by gel electrophoresis verified that the NCIMB 30562 strain contains a plasmid.

The size and the GC content of the NCIMB 30562 genome is in line with *L. plantarum* genomes (n=671) in the NCBI genome database (median length 3.25 Mb and 44.5% GC).

2.3.5. Phenotypic analysis of *Lactobacillus plantarum NCIMB 30562*

L. plantarum NCIMB 30562 is a Gram-positive, non-spore forming, catalase-negative, non-motile bacterium. In terms of cell morphology, *L. plantarum* NCIMB 30562 are straight rods occurring singly, in pairs, or in short chains and can ferment several carbohydrates which include, but are not limited to L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, *etc*.

The full carbohydrate fermentation profile of *L. plantarum* NCIMB 30562, as determined using the API 50 CHL test system, is presented in Table 2 below.

Table 2. Carbohydrate fermentation (API 50 CHL) of the *L. plantarum* NCIMB 30562 strain

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

2.4. Genetic modification status

L. plantarum NCIMB 30562 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. plantarum* NCIMB 30562 does not trigger genetic modification (GM) labeling of the final food product.

2.5. Method of manufacture

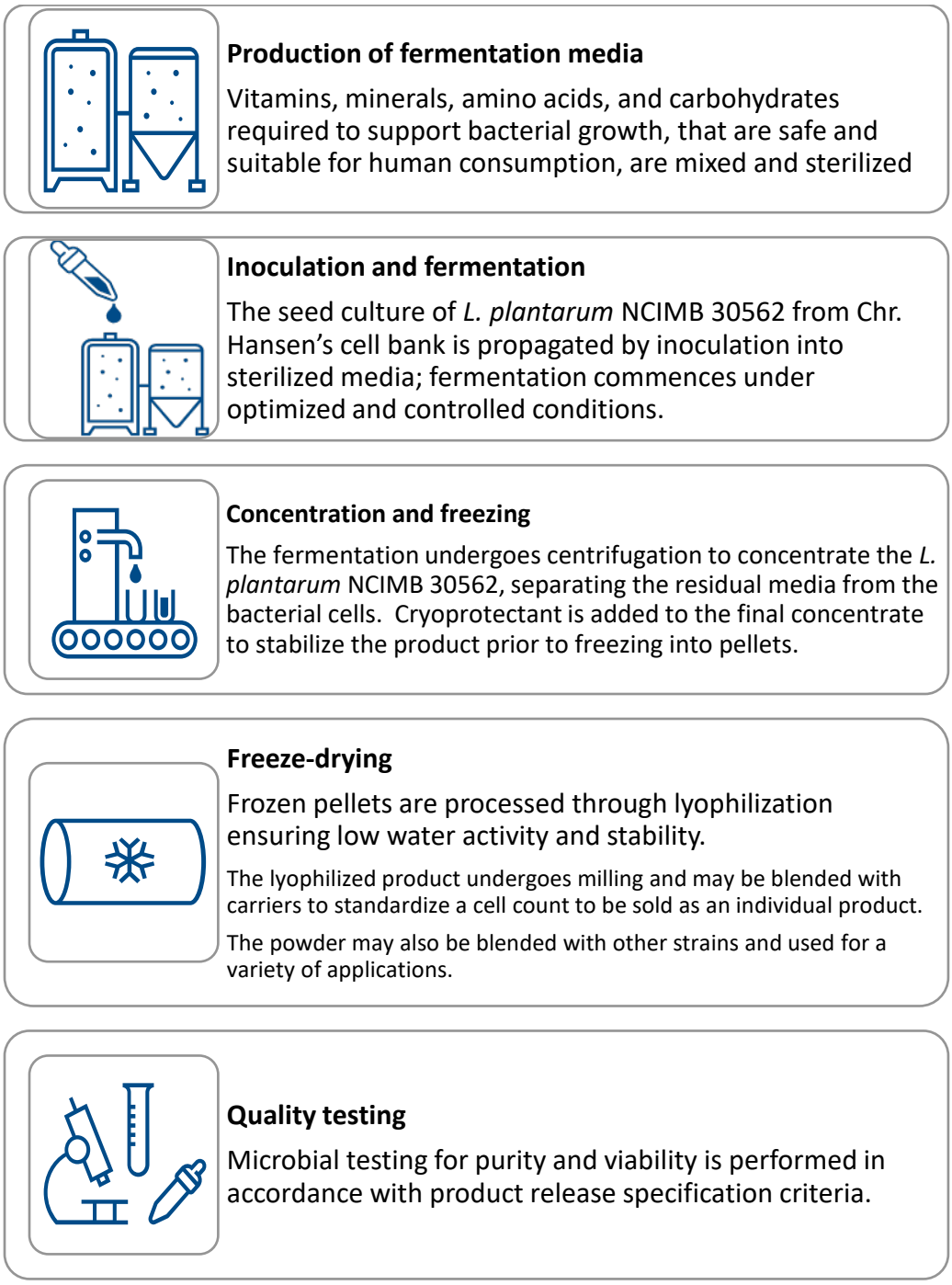
Viable *L. plantarum* NCIMB 30562 is produced by industrial batch fermentation following Chr. Hansen's global protocol for production of cultures which are in accordance with current good manufacturing practices (cGMPs).

Pure strain of the microorganism (*L. plantarum* NCIMB 30562 seed culture) is inoculated into sterilized growth medium specifically designed to meet the nutritional needs of *L. plantarum.* 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 fermentation is subjected to centrifugation for the removal of water-soluble material and to concentrate the desired *L. plantarum* NCIMB 30562. Appropriate food-safe cryoprotectants are added to improve the survival during freeze-drying. The concentrated microorganisms are then frozen into pellets. The raw materials used in the production process primarily consist of carbohydrates, amino acids, vitamins, and minerals that are safe. All substances used in the media are suitable for human consumption.

Frozen pellets are tested for quality and are freeze dried. The resulting lyophilized pellets have a very low water activity which ensures stability of the culture. The lyophilized *L. plantarum* strain is then ground into powder. It can be 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 current good manufacturing practices (cGMPs) consistent with 21 CFR Parts 110 and 117. All Chr. Hansen plants have fully implemented Hazards Analysis and Critical Control Points (HACCP) plans, standard operating procedures, and quality control programs to ensure quality of the product being produced. Each 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 Chr. Hansen facilities manufacturing final products maintain FSSC 22000 certification.

Figure 1. Manufacturing overview of *L. plantarum* NCIMB 30562



2.5.1. Raw materials and processing aids

L. plantarum NCIMB 30562 is produced using standard fermentation techniques. This includes the use of fermentation and standardizing substances that are safe and suitable for use in human food. These substances have no technical function in the finished food product and are all permitted for use in this application.

2.5.2. Quality program

L. plantarum NCIMB 30562 is produced under Chr. Hansen's extensive 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.

2.5.3. Allergen control

Chr. Hansen controls all major food allergens as listed and established in the U.S. Food Allergen Labeling and Consumer Protection Act of 2004, in addition to control of the substances or products causing allergies or intolerances as outlined in Annex II of Regulation (EU) No 1169/2011, as amended. Chr. Hansen communicates the allergen status of its products in accordance with the U.S. and EU regulations.

Allergen control is managed via the company's cGMP and food safety programs that are FSSC 22000 certified at each of the company's production sites. In some cases, dairy or dairy components may be used during the fermentation process. In this case, dairy would be declared as a major allergen on product information sheets and communicated to customers. Allergen communication is managed via our quality management and food safety programs that are ISO 22000 certified.

2.6. Product specifications and product stability

2.6.1. Specifications and batch analyses

The final *L. plantarum* NCIMB 30562 ingredient is in the form of a white to light beige fine powder containing a total viable cell count of at least 4.0 x 10^{11} CFU/g *L. plantarum* NCIMB 30562. The quality control specifications that must be satisfied prior to the commercial release of *L. plantarum* NCIMB 30562 are outlined in Table 3 along with the methods of analysis that are all internationally recognized and/or validated.

Table 3. Product specifications for freeze-dried *L. plantarum* NCIMB 30562

Parameter	Units	Specification	Method of Analysis
Total cell count	CFU/g	≥4.0 x 10^{11}	USP 64, ISO 4833-1
Non-lactic acid bacteria	CFU/g	<500	ISO 13559:2002-M
Total aerobic microbial count	CFU/g	≤2,000	Ph.Eur. 2.6.12 (modified)
Yeast	CFU/g	<10	ISO 6611:2004
Mold	CFU/g	<10	ISO 6611:2004
Enterobacteriaceae	/10 g	Not detected	ISO 21528-1
Cronobacter spp.	/10 g	Not detected	ISO 22964
Coagulase-positive *Staphylococcus*	/1 g	Not detected	NMKL 66:2009
Salmonella spp.	/25 g	Not detected	ISO 6579-1:2017/Amd 1:2020
Listeria spp.	/25 g	Not detected	ISO 11290-1:2017
Abbreviations: CFU, colony forming unit; ISO, International Standardization Organization; NMKL, Nordic Committee on Food Analysis Ph.Eur., European Pharmacopeia; USP, U.S. Pharmacopeia.			

Analyses were conducted on 3 commercially representative batches of *L. plantarum* NCIMB 30562 and the results are summarized in Table 4. The analytical data demonstrate that the final *L. plantarum* NCIMB

30562 ingredient is produced consistently and conforms to the established specifications, and adequate quality control processes are in place.

Table 4. Analytical data for 3 commercially representative batches of freeze-dried *L. plantarum* NCIMB 30562

Parameter	Units	Specification	Analytical Data		
			Batch 1	Batch 2	Batch 3
Total cell count	CFU/g	≥4.0 x 10^{11}	4.6 x 10^{11}	5.1 x 10^{11}	4.8 x 10^{11}
Non-lactic acid bacteria	CFU/g	<500	<100	<100	<100
Total aerobic microbial count	CFU/g	≤2,000	550	<250	<250
Yeast	CFU/g	<10	<10	<10	<10
Mold	CFU/g	<10	<10	<10	<10
Enterobacteriaceae	/10 g	ND	ND	ND	ND
Cronobacter spp.	/10 g	ND	ND	ND	ND
Coagulase-positive *Staphylococcus*	/1 g	ND	ND	ND	ND
Salmonella spp.	/25 g	ND	ND	ND	ND
Listeria spp.	/25 g	ND	ND	ND	ND
Abbreviations: CFU, colony forming unit; ND, not detected.					

Lead was tested in three batches of *L. plantarum* NCIMB 30562 using inductively coupled plasma mass spectrometry (ICP-MS) following standardized methods for analyzing trace elements in foodstuffs. The analytical results are presented in Table 5 and demonstrate that lead is not a concern in the final *L. plantarum* NCIMB 30562 ingredient.

In the absence of U.S. regulatory limits for lead in foodstuffs, an internal specification of 0.05 ppm lead was established for *L. plantarum* NCIMB 30562, taking into account the typical inclusion rate in finished product applications, since the Codex Alimentarius standards for various foods sets limits according to finished food applications, where our ingredient is typically included at levels below 0.15%.

Table 5. Lead analyses for 3 batches of *L. plantarum* NCIMB 30562

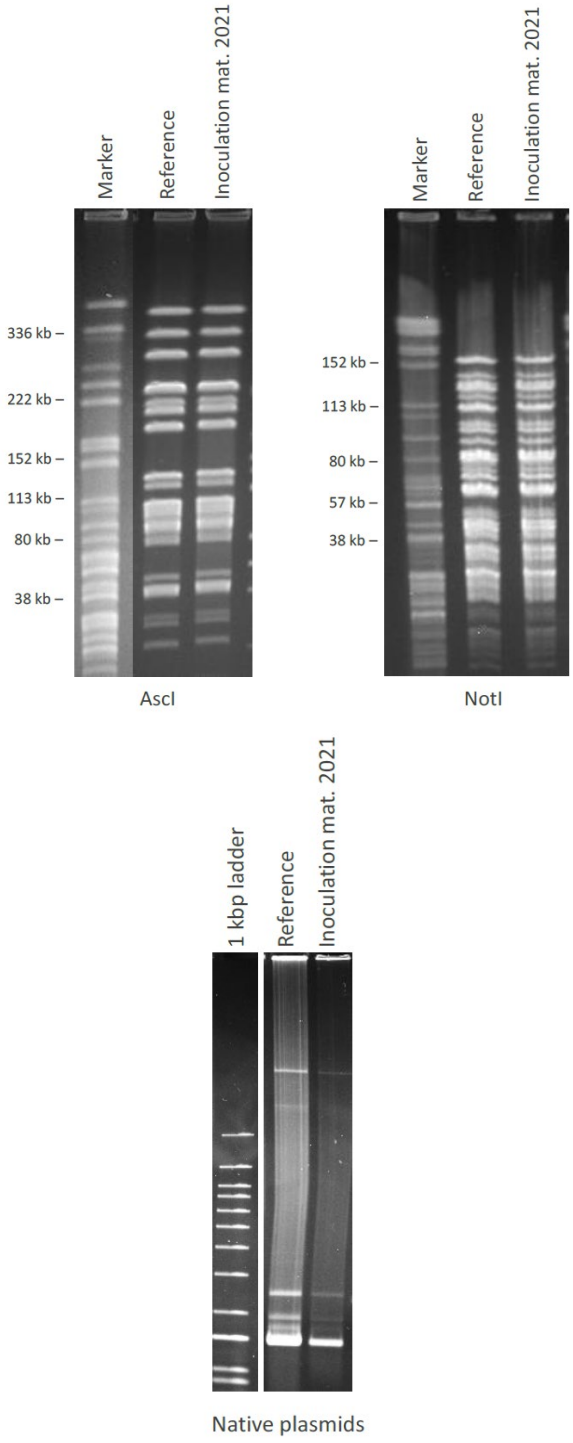
Parameter	Analytical Results (ppm)			Methods of Analysis
	Batch 1	Batch 2	Batch 3	
Lead	<0.005[a]	<0.005[a]	<0.005[a]	DIN EN ISO 15763 (2010)
[a] Limit of quantification.				

2.6.2. Product stability

L. plantarum NCIMB 30562 freeze-dried product has a minimum shelf life of 24 months from the date of manufacture when stored between 2-8°C in original or tightly closed foil pouch under dry conditions protected from direct sunlight.

Furthermore, the genetic stability of *L. plantarum* NCIMB 30562 has been demonstrated by DNA fingerprinting comparing the stock culture in the cell bank and the inoculation material produced in 2021 (Figure 2). The genetic stability of *L. plantarum* NCIMB 30562 demonstrates that the strain safety analysis will hold true over time.

Figure 2. DNA fingerprint and plasmid profiles from the *L. plantarum* NCIMB 30562 reference strain and the inoculation material from 2021



Part 3. Dietary exposure

3.1. Intended use

L. plantarum NCIMB 30562 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. plantarum* NCIMB 30562 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 maximum level of incorporation into conventional foods will be 1.0 x 10^{11} CFU/serving to account for loss of viability throughout the shelf-life of the product.

In addition, *L. plantarum* NCIMB 30562 is intended to be used as an ingredient in protein-based (including but not limited to soy, milk, and whey) non-exempt infant formula for term infants at levels not to exceed 1.1 x 10^{8} CFU/g formula product.

L. plantarum NCIMB 30562 is not intended for use in products regulated by the USDA.

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

Under the intended conditions of use, it is anticipated that level of incorporation of *L. plantarum* NCIMB 30562 for conventional food applications will be up to a maximum of 1.0 x 10^{11} CFU/serving. Typical levels of addition will likely range between 1.0 x 10^{9} to 1.0 x 10^{10} CFU/serving; however, the most likely usage level will be 1.0 x 10^{9} CFU/serving, as this represents an approximate 1% inclusion rate[2]. Higher inclusion rates are possible, but unlikely (see Part 4).

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 the level of 1.0 x 10^{9} CFU/serving, the maximum exposure to *L. plantarum* NCIMB 30562 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 estimated daily intake of *L. plantarum* NCIMB 30562 under the intended conditions of use in conventional food will exceed 1.0 x 10^{11} CFU/day. On a comparative basis, the resultant dietary exposure to *L. plantarum* NCIMB 30562 under the intended conditions of use is consistent with the dietary intake of other *L. plantarum* strains as referenced in GRAS notices that received a letter of "no questions" from the U.S. FDA (GRN No. 685, 722, 847, and 953) which estimates the maximum daily intake ranging between 10^{10} to 10^{11} CFU/day (see Section 6.3.1).

The estimated dietary exposure to *L. plantarum* NCIMB 30562 under the intended use conditions is considered extremely conservative, as it assumes that there is no loss in viability of the strain during shipping and storage. Additionally, there is the assumption that *L. plantarum* NCIMB 30562 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, it is unlikely that individuals would consume even half (10 servings/day) of conventional food products containing *L. plantarum* NCIMB 30562.

[2] 1 g of culture concentrate containing approximately 10^{11} CFU/g to 100 g of food.

3.3. Estimated daily intake from the intended use in infant formula

L. plantarum NCIMB 30562 is intended for use in term, non-exempt infant formula at a maximum incorporation level of 1.1 x 10^8 CFU/g formula product. The daily intake of *L. plantarum* NCIMB 30562 under the intended conditions of use can be estimated on the basis that these formulas will be the sole source of nutrition for infants, assuming an average reconstitution rate of 14.1 g powdered infant formula per 100 mL water wherein commercial infant formulas in the U.S. typically provide an energy content of 0.67 kcal/ml (20 kcal/fl oz) (Martinez & Ballew, 2011). For a 1-month and 6-month old infant with respective caloric requirements of 472 kcal/day and 645 kcal/day (Institute of Medicine, 2005), the estimated daily intake of *L. plantarum* NCIMB 30562 at the maximum use level of 1.1 x 10^8 CFU/g would equate to 1.1 x 10^{10} CFU/day and 1.5 x 10^{10} CFU/day, respectively.

The intended use of the NCIMB 30562 strain is in line with other microbial ingredients that have GRAS status and have received a letter of "no questions" from the FDA for intended use in infant formula at levels ranging from 10^7 to 10^{10} CFU/day. These include LAB species such as *Lactobacillus rhamnosus* (GRN no. 1013), *Lactobacillus acidophilus* (GRN no. 865), *Lactobacillus reuteri* (GRN no. 410), *Lactobacillus paracasei* (GRN no. 810), *Lactobacillus fermentum* (GRN no, 531), as well as *Bifidobacterium* spp. *Bifidobacterium breve* (GRN no. 454, 455), *Bifidobacterium longum* subsp. *infantis* (GRN no. 985, 950), *Bifidobacterium animalis* subsp. *lactis* (GRN no. 952), *Bifidobacterium bifidum* (GRN no. 814), and *Bifidobacterium longum* (GRN no. 813, 877). In addition, a 28-day toxicology study in newborn New Zealand white rabbits supports the safety of the use level of *L. plantarum* NCIMB 30562 in infants based on the no-observed-adverse effect level (NOAEL) established within the study and the corresponding acceptable daily intake (ADI) of 2.5 x 10^{10} CFU/day for a 2.5 kg infant (see Section 6.4). Moreover, in several clinical trials, *L. plantarum* NCIMB 30562 was demonstrated to be well tolerated in infants at levels of 1.0 x 10^9 CFU/day (see Section 6.5.1). Likewise, additional randomized controlled trials in infants/children have demonstrated the *L. plantarum* spp. to be well tolerated at levels of up to 2.3 x 10^{10} CFU/day (see Section 6.5.2).

There is no potential for cumulative exposure to *L. plantarum* NCIMB 30562, as all safe LAB are transient in the gastrointestinal tract. Grimes *et al*. (2017) also demonstrated that as non-formula beverage intake increases, a corresponding decrease in formula occurs, such that it can be reasonably expected that the amount of *L. plantarum* NCIMB 30562 consumed will not significantly increase as the infant ages. Furthermore, *L. plantarum* NCIMB 30562 will not proliferate in the foods for which it is intended for inclusion.

Part 4. Self-limiting levels of use

The intended levels of use for *L. plantarum* NCIMB 30562 are not self-limiting; however, the addition of the strain is restricted to applications that can sustain viable *L. plantarum* NCIMB 30562 at the intended use levels throughout the shelf life of the food and infant formula products.

Part 5. Experience based on common use in food

The conclusion of GRAS status for the intended uses of *L. plantarum* NCIMB 30562 is based on scientific procedures and not common use in food before 1958.

Part 6. Narrative

6.1. Approach of the safety assessment

The data and information providing the basis for our conclusion that the addition of *L. plantarum* NCIMB 30562 under the intended conditions of use is GRAS through scientific procedures are presented in the following sections. The information provided below and elsewhere in this notice is generally available in the public domain and has been properly cited. To determine the safety of *L. plantarum* NCIMB 30562 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), as well as the qualified presumption of safety approach (QPS) implemented by the European Food Safety Authority (EFSA) (EFSA Scientific Committee, 2007).

As discussed in Section 2.3.4.1, the taxonomic identification of strain NCIMB 30562 has been definitively confirmed as *L. plantarum* by genomic analysis. *In silico* analyses on *L. plantarum* NCIMB 30562 demonstrate the absence of potential virulence factors and genes related to pathogenicity (see Section 6.3.2.1), as well as the absence of antibiotic resistance genes (see Section 6.3.3.1), which were further confirmed by *in vitro* assays (see Section 6.3.3.2). The strain does not exhibit undesirable metabolic activities (*e.g.*, cytotoxicity, hemolysis, or production of biogenic amines and D-lactate) (see Section 6.3.2 and 6.3.4). *L. plantarum* NCIMB 30562 was demonstrated to be safe in a 28-day toxicology study in newborn rabbits (see Section 6.4). Additionally, *L. plantarum* NCIMB 30562 has been evaluated in several randomized controlled clinical studies without adverse events (see Section 6.5.1) and this was consistent with the findings from a comprehensive search of the scientific literature through to May 2022 to identify other published studies pertinent to the safety of *L. plantarum* as a species (see Section 6.5.2).

6.2. History of safe consumption in foods

L. plantarum is generally regarded and documented in the scientific literature as non-pathogenic, non-toxigenic and safe for use in food. This general recognition of safety of the species, and, thereby, all isolates thereof, is reflected in the regulatory approvals and documentation of the safe history of use on the species level. General recognition therefore extends to all isolates (strains) that are correctly identified as *L. plantarum*.

Consumption of lactic acid bacteria including *L. plantarum* has occurred for longer than recorded history. *L. plantarum* can be found in high numbers in most foods that have been fermented with LAB, especially when the fermented food is plant based such as brined olives, sauerkraut, salted gherkins, sourdough, Nigerian ogi (made from maize or sorghum), Ethiopian Kocho, Ethiopian sourdough made from teff, and cassava. People who are eating food that has been fermented with LAB are subsequently consuming large numbers of *L. plantarum* (Molin, 2003).

L. plantarum is 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 in a variety of fermented foods such as dairy, meat, fish, vegetables, wine, and beer (Bourdichon et al., 2012, 2018, 2022; Mogensen et al., 2002). 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 case, the organisms must be characterizing and not merely incidental components of the food microflora to be included in the IDF list.

L. plantarum 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) and is commonly used in dietary supplement products as well (Maaike et al., 2006).

6.3. Safety of *L. plantarum* NCIMB 30562

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 plantarum* 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 species with existing QPS status (EFSA BIOHAZ Panel, 2022). From the 2 new publications identified by the BIOHAZ evaluation on the *Lactobacilli* genus, the QPS status of the QPS species within this genus remained unchanged including *Lactobacillus plantarum* (EFSA BIOHAZ Panel, 2022).

To date, there have been five GRAS notices for *L. plantarum* filed with the FDA of which all have received "no questions" letters and the details are summarized in Table 6. While the strains of *L. plantarum* previously notified to the FDA differ from the NCIMB 30562 strain as subject to this GRAS notice, these demonstrate that the species *L. plantarum* is safe for human consumption in a variety of food applications at levels in the region of 10^{10} to 10^{11} CFU/day.

Table 6. GRAS notices for *L. plantarum* that received "no questions" from the FDA

GRN No.	Species/Strain	Intended Use	Use Level/Dietary Exposure
685	*Lactobacillus plantarum* strain 299v	For use as an ingredient in conventional foods	Up to 1.0 x 10^{10} CFU/serving Maximum of 1.0 x 10^{11} CFU/day
722	*Lactobacillus plantarum* Lp-115	For use as an ingredient in yogurt and other dairy products; soy products; beverages; chewing gum, confectionary snacks and other foods	Up to 1.0 x 10^{10} CFU/serving Estimate of 1.0 x 10^{10} CFU/day
847	*Lactobacillus plantarum* ECGC 13110402	For use as an ingredient in general foods.	Up to 1.0 x 10^{10} CFU/serving Estimate of 9.1 x 10^{10} to 1.82 x 10^{11} CFU/day
946	*Lactobacillus plantarum* strain DSM 33452	Intended for use in the production of wine and musts[a]	1.0 x 10^{7}CFU/g[b]

GRN No.	Species/Strain	Intended Use	Use Level/Dietary Exposure
953	Lactobacillus plantarum strain CECT 7527, CECT 7528, and CECT 7529	For use individually or in combination, as an ingredient in conventional foods,	Up to 4 x 10^9 CFU/serving, when used individually and at a maximum level of 1.2 x 10^{10} CFU/serving, when used in combination Maximum of 1.0 x 10^{10} CFU/day
[a] To increase the rate of malolactic fermentation (turning malic acid into lactic acid) and to prevent the growth of microorganisms that could cause off flavors in the finished product. [b] Under this application, *L. plantarum* is not viable in the finished product and does not contribute to lactic acid bacteria in dietary exposure.			

6.3.2. Pathogenicity/Toxigenicity

Lactobacillus spp. are classified as Risk Group 1 (Public Health Agency of Canada, 2018), with no specific special hazards identified. The genus *Lactobacillus* is rarely pathogenic and found "*widely distributed in the environment, especially in animal and vegetable food products*" (Bergey & Holt, 1994). Likewise, *Lactobacillus plantarum* is classified as Risk Group 1 by the German Federal Institute for Occupational Health and Safety under their Technical Rule for Biological Agents (Committee on Biological Agents, 2015). Risk Group 1 is defined as organisms that are highly unlikely to cause an infectious disease in humans with no specific special hazards identified. In the U.S., the American Type Culture Collection (ATCC) classifies the *Lactobacillus plantarum* sp. as Biosafety Level (BSL) 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 EFSA's QPS evaluation, where the following statements were made regarding *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).

6.3.2.1. *In silico* and *in vitro* analyses

To confirm the safety of *L. plantarum* NCIMB 30562, *in silico* genome screening for potential virulence factors (genes encoding for or enhancing pathogenicity, virulence, or toxigenicity) was performed as recommended by EFSA (EFSA FEEDAP Panel, 2018). The genome was screened for virulence factors by a

BlastN analysis against the curated virulence factor database (VFDB) (Chen et al., 2016). Phenotypic tests for cytotoxicity and hemolysis were also conducted.

I*n silico* genome screening for potential virulence factors and other genes related to pathogenicity, virulence, or toxicity in the *L. plantarum* NCIMB 30562 strain did not reveal any virulence or toxicity genes or other genes of safety concern. This was further supported by phenotypic tests which found the *L. plantarum* NCIMB 30562 strain to be non-hemolytic when grown on blood agar plates and non-cytotoxic in a Vero cell assay.

6.3.2.2. Case reports

Chr. Hansen has conducted a comprehensive review of the literature through May 2022 to identify publications pertinent to the safety evaluation of *L. plantarum* 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. plantarum*) (EFSA BIOHAZ Panel, 2022); the details of the search criteria and identified studies are outlined in Table 7. 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, for completeness, covered publications following June 2020 to the present.

Table 7. Search strategy for *L. plantarum* studies related to pathogenicity and toxigenicity

Source	Outcome	Search String	Number of hits
PubMed Date filter: June 2020 to May 2022	Antimicrobial/ antibiotic/antimycotic	*Lactobacillus plantarum* AND antibiotic resistan* OR antimicrobial resistan* OR antimicrobial susceptibil*	117
	Infection/bacteremia/ fungemia/sepsis	*Lactobacillus plantarum* AND infection* OR abscess* OR septic* OR bacteremia OR toxin*	152
	Type of disease	*Lactobacillus plantarum* AND endocarditis OR abscess OR meningitis	4
	Disease risk	*Lactobacillus plantarum* AND opportunistic OR virulen*	37

The search results were then screened for relevance in terms of safety concerns where *L. plantarum* 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 7.

Table 8. Study selection criteria

Inclusion criteria: • The subject of the study is *Lactobacillus plantarum*. • The study pertains to safety concerns of *Lactobacillus plantarum* • The study was conducted in humans. • Effects must be able to be attributable to *Lactobacillus plantarum*. • The study was published from June 2020 to May 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 plantarum*.

- The study does not assess or describe safety concerns.
- The study was not conducted in humans.
- Effects are not attributable to *Lactobacillus plantarum*.
- The study was not published from June 2020 to May 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, no relevant studies or case reports were identified concerning the pathogenicity or toxigenicity of *L. plantarum* in humans following oral consumption. Conversely, one report of *Lactobacillus plantarum* bioprosthetic aortic valve endocarditis was identified (Tavernese et al., 2020); however, this occurred in a 48-year-old male with an existing aortic surgical bioprosthesis and the subject responded well following conventional medical treatment. While the source of the *L. plantarum* is unknown in this report, this is consistent with the observation that *Lactobacillus* spp. can present as opportunistic pathogens under rare circumstances.

Opportunistic infections by *Lactobacillus* spp. have been reported; however, are extremely rare and restricted to severely immuno-compromised individuals or individuals with predisposed conditions (Bernardeau et al., 2008; Dani et al., 2015; Saarela et al., 2002; Salminen et al., 2004; Sullivan & Erik Nord, 2006). Infection or pathology linked to *L. plantarum* species is even more rare. In a review of 89 cases of patients with *Lactobacillus* bacteremia, *L.* plantarum was only found as the infecting organism in one incidence of a case of endocarditis stemming from poor oral hygiene (Salminen et al., 2004). In the same study, it was noted that 82% of patients had severe or fatal comorbidities. A second review followed 45 cases of *Lactobacillus* bacteremia over 15 years (Husni et al., 1997). The conclusions mirrored the results of the Salminen et al. (2004) study, wherein the investigators concluded that *Lactobacilli* are relatively avirulent organisms that produce bacteremia in patients with serious underlying conditions. In both studies, *L. plantarum* infection was only found as an opportunistic infection and was not linked to its consumption in food or as a food ingredient.

Thus, the pathogenicity of *L. plantarum 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* and *in vitro* analyses have demonstrated that *L. plantarum* NCIMB 30562 does not exhibit pathogenic/virulent traits.

6.3.3. Antibiotic resistance

6.3.3.1. Genome search

The genome sequence of the *L. plantarum* NCIMB 30562 strain was analyzed *in silico* for the presence of known antibiotic resistance genes by BlastN analysis against the ResFinder database (Zankari et al., 2012) and BlastX analysis against the National Center for Biotechnology Information (NCBI) Bacterial Antimicrobial Resistance Reference Gene Database. The genome of *L. plantarum* NCIMB 30562 did not contain any antibiotic resistance genes, therefore, any phenotypic resistance observed in the strain is intrinsic and not due to acquired antibiotic resistance genes.

6.3.3.2. *In vitro* assay

The minimum inhibitory concentration (MIC) values of 9 antibiotics were determined for *L. plantarum* NCIMB 30562 according to the ISO 10932 | IDF 223 international standard with two biological duplicates. The medium was controlled as recommended in the ISO standard by the use of *Lactobacillus plantarum* ATCC 14917, which was tested in parallel and had MIC values within the ranges given in the ISO standard. 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 are summarised in Table 9.

Table 9. MIC Values for *L. plantarum* NCIMB 30562

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

Abbreviations: MIC, minimum inhibitory concentration; n.r., not required to be tested by EFSA.
[a] For *Lactobacillus plantarum* group as established by EFSA (EFSA FEEDAP Panel, 2018).

L. plantarum NCIMB 30562 is sensitive to all of the antibiotics tested, with MIC values that are at or below the EFSA cut-off values for the *Lactobacillus plantarum* group (EFSA FEEDAP Panel, 2018). The MIC values for kanamycin are one two-fold dilution above the EFSA cut-off value in both duplicates; however, it is considered acceptable due to the technical variation of the phenotypic method as also recognized by EFSA in several published opinions. This is consistent with the results of the *in silico* analysis of the genome of *L. plantarum* NCIMB 30562 where no antibiotic resistance genes were detected (see Section 6.3.3.1).

6.3.4. Metabolic activities

6.3.4.1. Biogenic amines production

Many LAB 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 deamination 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 are 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).

L. plantarum NCIMB 30562 was tested for biogenic amine production by use of an in-house standard operating procedure (SOP) modified from Bover Cid et al. (2008). Detection of histamine and tyramine was analyzed by use of gas chromatography-mass spectrometry (GC-MS) (in-house SOP modified from Smart et al., 2010). The method was optimized and validated for both qualitative and quantitative detection of the two biogenic amines. Positive and negative controls as well as an internal standard were included. *L. plantarum* NCIMB 30562 did not produce any of the two biogenic amine compounds tested when grown in the presence of specific amino acid precursors known to induce production.

6.3.4.2. D-/L-lactate production

L. plantarum NCIMB 30562 was tested for production of D-lactate/L-lactate by the use of an in-house method modified based on (Dunlop & Neidle, 1987) and was optimized and validated for determination of the ratio between D- and L-lactic acid. The NCIMB 30562 strain was found to produce a ratio of 62% D-lactate and 38% L-lactate and is thereby characterized as a DL-lactate producing strain in line with Bergey's Manual for the *Lactobacillus plantarum* species (Hammes & Hertel, 2015).

During the fermentation of carbohydrates, species of LAB produce either exclusively L-lactic acid, exclusively D-lactic acid, a racemic mixture of both L- and D- lactic acid, or predominantly one form of lactic acid over the other (Axelsson, 2004). *Lactobacilliaceae* that have a long-history of safe use in the food supply include species that are known to produce D-lactic acid in predominance, or racemic mixtures of D- and L-lactic acid. For example, *Lactobacillus delbrueckii* is an obligatory homofermentive organism which produces D-lactic acid from hexose sugars in predominance and is one of the most economically important and widely consumed lactic acid bacteria used by the food industry in the manufacturing of a variety of fermented dairy products. *L. plantarum* NCIMB 30562 produces a racemic mixture of roughly equivalent amounts of D-lactic and L-lactic acid similar to other *L. plantarum* strains.

Human cells contain L-lactate dehydrogenase and therefore only produces L-lactic acid as a normal metabolic intermediary, while it is believed that the tiny amount of D-lactic acid detected in circulation originates from colonic carbohydrate-fermenting bacteria (Bianchetti et al., 2018a; Pariza et al., 2015). Despite the long-history of use in food and widespread consumption of D-lactic acid-producing LAB, the overgrowth of human gut commensal microorganisms capable of producing D-lactate during chronic antibiotic use by individuals with short-bowel syndrome and intestinal failure has resulted in cases of D-lactic acidosis and encephalopathy (Hudson et al., 1990; Karton et al., 1987; Oh et al., 1979; Scully, et al., 1989). Since the phenomenon was described in 1979, there have been fewer than 96 cases reported in the scientific literature (Bianchetti et al., 2018b; Htyte et al., 2011; Oh et al., 1979). The rare observations in individuals with short-bowel syndrome have raised concerns about the ingestion of D-lactic acid producing strains and the misconception that D-lactic acid is poorly metabolized in humans. Based upon these early observations that indicated the risk of D-lactate acidosis in infants resulting from the feeding of D-lactic acid acidified formula, Codex Alimentarius standards state that only L-lactic acid-producing bacteria be used in infant formula[3].

The Codex recommendations have propagated concerns with the use of D-lactate producing organisms, however, these concerns are not supported by more recent scientific evidence related to the

[3] Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants Codex Stan 72-1981; Formerly CAC/RS 72-1972. Adopted as a worldwide Standard in 1981. Amendment: 1983, 1985, 1987, 2011, 2015 and 2016. Revision: 2007.

commercial use of D-lactate producing organisms in food. While early observations raised safety concerns with the use of D-lactate producing organisms in infant formula, more recent studies indicate that D-lactic acid is, in-fact, readily metabolized in humans (Ewaschuk et al., 2005), and toxicity only occurs in rare instances where levels of the acid saturate metabolic and elimination pathways such as may occur to those inflicted with short bowl syndrome. D-lactic acidosis and encephalopathy, attributed to D-lactic acid production by intestinal organisms, have never been reported in normal individuals with functional small intestines.

Thus, as recognized by Pariza et al. (2015) in the "*Decision Tree for Determining the Safety of Microbial Cultures to be Consumed by Humans or Animals*", there is no scientific evidence to suggest that healthy individuals would be affected detrimentally by the addition of viable D-lactic acid producing bacteria to foods, and that the production of D-lactic acid, in and of itself, does not pose a significant safety concern to healthy humans.

The safety of D-lactic acid-producing strains of lactic acid bacteria consumed by infants is further supported by a large body of clinical evidence obtained with *L. plantarum* NCIMB 30562, notably in term newborn infants (see Section 6.5.1). Additionally, Łukasik et al. (2018) identified five randomized controlled clinical trials designed to assess the risk of infant consumption of D-lactate-producing organisms from 2005 to 2017 covering 544 healthy infants and identified no clinically relevant adverse events associated with D-lactic acid-producing *Lactobacillus* and *Bifidobacterium* strains in fermented infant formulas when consumed by healthy infants. The studies focus on the safety and tolerance of feeding known D-lactate-producing organisms to healthy infants in dosages ranging from 1.0×10^8 to 1.0×10^{10} CFU/day. The investigators concluded that "*probiotics*[4] *and fermented formulas did not cause D-lactic acidosis in healthy children*"; however, "*a harmless, subclinical accumulation of D-lactate was theoretically possible*" since blood levels were not measured in all studies.

More recently, Food Standards Australia New Zealand (FSANZ) assessed the risk of LAB to the health and safety of infants as part of a review of their regulation of infant formula products (Food Standards Australia New Zealand, 2021). As part of the assessment, a comprehensive literature review was conducted up to 2019 to identify clinical trials, case reports, and other relevant epidemiological studies pertinent to the safety of viable LAB when supplemented into infant formula. The studies assessed included those conducted on both D- and L-lactate producing species of the genus *Lactobacillus*, *Bifidobacterium*, and *Propionibacterium*. On the basis of this assessment, FSANZ concluded:

> "*The published clinical trials on the safety of a number of DL-lactic acid producing bacteria—alone or in combination with L-lactic acid producing bacteria—did not identify any risks for healthy full term and preterm infants. Infant formulas supplemented with DL-lactic acid producing bacteria were well tolerated, and no adverse events associated with the lactic acid producing bacteria were noted in the clinical trials assessed. FSANZ concludes that infant formula supplemented with non-pathogenic, non-toxigenic DL-lactic acid producing microorganisms does not present a risk to public health and safety for healthy, full term and preterm infants*."

[4] Note the term "probiotic" is used in this instance to accurately represent the scientific literature; however, *L. plantarum* NCIMB 30562, as subject of this GRAS notice, is only intended for use as a microbial ingredient in conventional foods and non-exempt infant formula.

The outcome of FSANZ's assessment is consistent to the previous findings on D-/L-lactate producing bacteria when consumed by infants. Conversely, FSANZ did indicate that,

> "*For infants with underlying clinical complications—including preterm, low birth weight and immunocompromised infants—there are case reports of sepsis and bloodstream infections associated with dietary supplementation with non-pathogenic L- and DL-lactic acid producing bacteria. However, due to a lack of sufficient data on infectivity and exposure, FSANZ is unable to assess the level of the risk in these circumstances*."

Nonetheless, D-lactate-producing bacteria have been concluded to be GRAS for infant formula uses in notifications that received "no questions" from the FDA. These include use of *Lactobacillus reuteri* strain DSM 17938 at levels of up to 1.0 x 10^8 CFU/g powdered whey-based term infant formula (GRN No. 410) and *Lactobacillus fermentum* CECT5716 for use in powdered milk-based infant formula at 1.0 x 10^7 CFU/g powdered (GRN No. 531).

In summary, concerns of health risk arising from ingesting D-lactic acid-producing bacteria by healthy infants, based upon early studies of infants fed D-lactic acid acidified formula and rare clinical reports of D-lactic acid acidosis in infants resulting from small intestinal overgrowth of gut commensal organisms in infants with intestinal disorders, have largely been dispelled by more recent robust, well-controlled, sufficiently powered clinical trials where D-lactate-producing bacteria were fed to healthy infants without reports of treatment-related adverse events.

6.4. Toxicology Testing

A 28-day toxicity study was conducted in newborn New Zealand white rabbits (n=8/group), wherein each group was orally administered either i) control, ii) *L. plantarum* NCIMB 30562 (5.0 x 10^{10} CFU/50 g body weight/day or 1.0 x 10^{12}CFU/kg body weight/day), iii) L-glutamine (30 mg/50 g body weight/day or 600 mg/kg body weight/day), iv) fructo-oligosaccharide (FOS) (10 mg/50 g body weight/day or 200 mg/kg body weight/day), or v) a combination of *L. plantarum* PPLP217, L-glutamine, and FOS at the levels as outlined (DeTolla, 2004). The testing was performed in an Association of Assessment and Accreditation of Laboratory Animal Care International-accredited facility and all experimental procedures were carried out in compliance with good laboratory practices. On Day 29, the rabbits were sacrificed, and samples were collected for hematology, clinical chemistry, and histopathology. The organs examined included the heart, lung, liver, kidney, small and large intestine, and spleen.

Through the duration of the study, animals in all groups were healthy and gained weight accordingly. While mortalities occurred in all test groups, they were not attributed to a test article effect. Clinical chemistry and hematology were within historical levels and no toxicologically relevant changes were reported in any of the groups. Histopathological changes were unremarkable with no acute or chronic toxicologic changes noted. On the basis of this study, the investigators concluded "*no changes that could be attributed to Lactobacillus, L-glutamine, FOS, or the combination (all three elements)*".

These data are, in all respects, expected given that *L. plantarum*, L- glutamine, and FOS are common in the human diet and not expected to elicit adverse effects even in newborn animals. The data are also consistent with safety observations in well-controlled clinical trials discussed in detail in Section 6.5.

Based on the results of the 28-day study, the no-observed-adverse effect level (NOAEL) can be determined to be 5.0 x 10^{10} CFU/50 g body weight/day (or 1.0 x 10^{12} CFU/kg body weight/day). In

consideration of a 100-fold safety factor, this would equate to an acceptable daily intake (ADI) of $1x10^{10}$ CFU/kg body weight/day or 2.5×10^{10} CFU/day for a 2.5 kg body weight infant. This level is well above the estimated daily intake in infants of up to 1.5×10^{10} CFU/day based on the intended conditions of use and therefore is not expected to be a significant toxicological concern.

6.5. Human studies

6.5.1. *L. plantarum* NCIMB 30562

A number of human studies have been conducted on *L. plantarum* NCIMB 30562 in healthy term newborn populations (Chandel et al., 2017; Panigrahi et al., 2008, 2017). A summary of these studies is presented in Table 10. While not standard Phase I safety studies and conducted primarily as efficacy studies in conjunction with FOS, the results demonstrate that *L. plantarum* NCIMB 30562 is well tolerated in infants at levels of up to 1.0×10^9 CFU/day and there are no reports of significant treatment-related adverse events on study participants. This is unsurprising given the normal exposure of humans to *L. plantarum* and FOS in diet.

Table 10. Summary of clinical studies conducted with *L. plantarum* NCIMB 30562

Reference	Study Design	Study Population	Intervention[a]	Duration of Intervention	Safety-Related Outcomes
Panigrahi et al., 2017	Randomized, double-blind, placebo-controlled trial	Healthy newborn 1-4 days old (≥2 kg BW at birth; ≥35 weeks of gestation) 4,456 newborns enrolled	Oral synbiotic preparation Control: 250 mg maltodextrin Intervention: *Lactobacillus plantarum* ATCC 202195 at 1.0 x 10^9 CFU/ day + 150 mg FOS/day (dissolved in dextrose saline)	7 days	• Several AEs were reported but were determined to be unrelated to the test article (hydrocephalus, biliary atresia and laryngomalacia, and non-fatal cases of neonatal malaria) • Six cases of abdominal distension (5 control, 1 intervention) • Otherwise, the test substance was well tolerated.
Panigrahi et al., 2008 Chandel et al., 2017[b]	Randomized, double-blind, controlled trial	Healthy newborn 1-3 days old (≥1.8 kg BW at birth; ≥35 weeks of gestation); 33 newborns enrolled (12	Oral synbiotic preparation Control: 2 mL dextrose saline Intervention: *Lactobacillus plantarum* ATCC 202195 at 1.0 x 10^9 CFU/ day + 150 mg FOS/day (dissolved in 2	7 days	• No changes in body weight during the intervention period. • No serous AEs were reported.

Reference	Study Design	Study Population	Intervention[a]	Duration of Intervention	Safety-Related Outcomes
		control, 19 intervention)	mL of 5% dextrose saline)		

Abbreviations: AE, adverse event; BW, body weight; CFU, colony forming units; FOS, fructo-oligosaccharide.
[a] In these publications, the intervention was *L. plantarum* ATCC 202195. The ATCC 202195 strain was deposited as *L. plantarum* NCIMB 30562 following acquisition by Chr. Hansen. Both ATCC 202195 and NCIMB 30562 are the same strain.
[b] Frozen stool samples were taken from the Panigrahi et al., 2008 study for 16 rRNA gene sequencing of fecal microbes.

6.5.2. Other *L. plantarum* strains

In addition to strain specific studies in healthy neonates, the demographic consumer group for infant formula (as previously described in Table 10), a comprehensive review of the literature through to May 2022 was also conducted to identify clinical studies as relevant to the tolerability of *L. plantarum* as a species. A summary of the identified studies is presented in Table 11. Overall, the additional clinical evidence corroborates the safety of the *L. plantarum* species in general when fed to various age groups, including infants, children and adults at levels ranging in the region of 10^8 to 10^{10} CFU/day. Additionally, no significant adverse events have been reported with *L. plantarum* spp. when given to undernourished children at levels of 9.0 x 10^8-1.2 x 10^9 CFU/day (Kamil et al., 2022) and to adults with irritable bowel syndrome at levels of 1.0 x 10^{10} CFU/day (Ducrotté et al., 2012) or 2.0 x 10^{10} CFU/day (Niedzielin et al., 2001; Nobaek et al., 2000).

Of importance, for the purpose of this notice, is that the available studies demonstrate the safety of *L. plantarum* species when fed at dosages of up to 10^{10} CFU/day to the general population including infants, while safety for the NCIMB 30562 strain is demonstrated by the strain specific-studies (see Sections 6.4 and 6.5.1) and biosafety analyses on the specific strain (see Sections 6.3.2 to 6.3.4) following the Pariza et al. (2015) decision tree approach.

Table 11. Summary of clinical studies conducted on *L. plantarum*

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Surono et al., 2014	Randomized, double-blind, placebo-controlled	Healthy infants/ children (12 to 24 months old) N=48 subjects	Oral, microencapsulated Control: placebo Intervention: 1. *Lactobacillus plantarum* IS-10506 at 2.3×10^{10} CFU/ day 2. Zinc at 8 mg/day 3. *Lactobacillus plantarum* IS-10506 and zinc at 2.3×10^{10} CFU/ day and 8 mg/day, respectively	90 days	• No AEs were observed in the subjects.
Kusumo et al., 2019	Randomized, double-blind, placebo-controlled	Healthy infants/children (12 to 24 months old) N=38 subjects	Oral, capsule Control: placebo Intervention: 1. *Lactobacillus plantarum* IS-10506 at 1.6×10^{10} CFU/day 2. Zinc at 8 mg/day (as zinc sulfate) 3. *Lactobacillus plantarum* IS-10506 and zinc at 1.6×10^{10} CFU/day and 8 mg/day (as zinc sulfate), respectively	90 days	• BW remained unchanged between the probiotic groups and the control. • No significant GI events were observed. • Blood lysis, non-compliance and sickness were reported; however, no other details on subjects/etiology were provided in the publication.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Han et al., 2012	Randomized, double-blind, placebo-controlled	Healthy infants/children (12 months to 13 years old with atopic dermatitis and excluded use of systemic or topical corticosteroids) N=83 subjects	Oral preparation (no other details) Control: placebo Intervention: *Lactobacillus plantarum* CJLP133 at 1.0 x 10^{10} CFU/day	12 weeks	• No AEs reported.
Ho et al., 2021	Randomized, double-blind, placebo-controlled	Healthy subjects (25.47 ± 4.64 years of age; with self-reported insomnia) N=40 subjects	Oral, capsules Control: placebo Intervention: *Lactobacillus plantarum* PS128 at 6.0 x 10^{10} CFU/day	30 days	• No AEs reported.
Huang et al., 2019	Randomized, double-blind, placebo-controlled	Healthy adults (20 to 30 years of age) N=54 (27 male, 27 female)	Oral, capsule Control: placebo Intervention: *Lactobacillus plantarum* TWK10 at 3.0 x 10^{10} or 9.0 x 10^{10} CFU/day	6 weeks	• No significant differences in hematology between groups. • No differences between groups in biochemistry parameters related to safety. • No AEs reported.
Culpepper et al., 2019	Randomized, double-blind crossover study	Healthy adults (53±8 years of age; with high waist circumference) N= 114 subjects	Oral, capsule Control: placebo Intervention: 1. *Lactobacillus plantarum* HA-119 at 5.0 x 10^{9} CFU/day 2. *Bacillus subtilis* RO179 at 2.5x 10^{9} CFU/day	6 weeks (per intervention)	• No changes in hematology in any of the test groups. • Minor GI effects (nausea, vomiting, or stomach upset) reported at comparable incidence between placebo and intervention groups. • No serious AEs reported.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
			3. *Bifidobacterium animalis* subsp. *lactis* B94 at 5.0 x 10^9 CFU/day		
Oh et al., 2021	Randomized, double-blind, placebo-controlled	Healthy subjects (19 -70 years of age; prediabetic [isolated impaired glucose intolerance]) N=40 subjects	Oral, capsule Control: placebo Intervention: *Lactobacillus plantarum* HAC01 at 4.0 x 10^9 CFU/day	8 weeks	• No serious AEs were reported (proportion of AE comparable between groups. • No significant differences in any other safety parameters (vital signs, ECG, and laboratory data).
Jung et al., 2022	Randomized, double-blind, placebo-controlled	Healthy subjects (mean of 50 to 51 years of age; with functional diarrhea) N=22 subjects	Oral, powder Control: placebo Intervention: *Lactobacillus plantarum* CJLP243 (KCCM11045) at 1.0 x 10^{10} CFU/day	2 months	• No significant differences in hematology and blood chemistry parameters tested between groups. • No AEs reported.
Rahayu et al., 2021	Randomized, double-blind, placebo-controlled	Healthy subjects (35 to 56 years of age, BMI ≥25) N=60 subjects	Oral, supplemented skimmed milk powder (sachet) Control: un-supplemented Intervention: *Lactobacillus plantarum* Dad-13 at 2.0 x 10^9 CFU/day	90 days	• No AEs reported.
Chong et al., 2019a	Randomized, double-blind, placebo-controlled	Healthy adults (18-60 years of age; moderate stress levels based on Cohen's Perceived Stress Scale) N=111 subjects	Oral, sachet Control: placebo Intervention: *Lactobacillus plantarum* DR7 at 1.0 x 10^9 CFU/day	12 weeks	• No AEs reported.

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Chong et al., 2019b	Randomized, double-blind, placebo-controlled	Healthy adults (18-60 years of age) N=124 subjects	Oral, sachet Control: placebo Intervention: *Lactobacillus plantarum* DR7 at 1.0 x 10^9 CFU/sachet[a]	12 weeks	• No significant differences in hematology. • No AEs reported.
Lew et al., 2019	Randomized, double-blind, placebo-controlled	Healthy adults (31.7±11.1 years of age; moderate stress levels based on Cohen's Perceived Stress Scale) N=103 subjects	Oral, sachet Control: placebo Intervention: *Lactobacillus plantarum* P8 at 2.0 x 10^{10} CFU/day	12 weeks	• No AEs reported.
Park et al., 2020	Randomized, double-blind, placebo-controlled	Healthy adults (48.3 ± 1.5 years of age, TG levels <200 mg/dL) N=70 subjects	Oral, capsule Control: placebo Intervention: *Lactobacillus plantarum* LPQ180 at 1.0 x 10^9 CFU/day	12 weeks	• No AEs reported.
Kim et al., 2021	Randomized, double-blind, placebo-controlled	Healthy subjects (19 to 39 years of age; with mild to moderate acne vulgaris) N=30 subjects	Oral, bag (sachet) Control: placebo Intervention: *Lactobacillus plantarum* CJLP55 at 1.0 x 10^{10} CFU/day	12 weeks	• Hematology, serum and urine analyses were normal in all subjects through the duration of the study. • No differences in tolerability between groups. • No AEs reported.
Sohn et al., 2022	Randomized, double -blind, placebo-controlled	Healthy subjects (20-60 years of age; BMI of 25-30 kg/m^2) N=81 subjects	Oral, capsule Control: placebo	12 weeks	• No significant differences in blood biochemistry parameters between groups, with the exception of significant decrease in total cholesterol

Reference	Study Design	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
			Intervention: *Lactobacillus plantarum* LPK at 4.0 x 10^9 CFU/day		and triglyceride levels (efficacy parameters) in the L. plantarum group. • No differences in AEs – all reported were mild and were not related to the test article (pruritus, facial laceration, low back pain, insomnia, vasovagal syncope).
Bosch et al., 2012	Randomized, double-blind, placebo-controlled	Elderly subjects (institutionalized; 65-85 years of age[b]) N=60 subjects	Oral, supplemented in powdered skim milk Control: placebo Intervention: 1. *Lactobacillus plantarum* CECT7315/7316 at 5 x 10^9 CFU/day 2. *Lactobacillus plantarum* CECT7315/7316 at 5 x 10^8 CFU/day	3 months	• No AEs reported.

Abbreviations: AEs, adverse events; BMI, body mass index; BW, body weight; CFU, colony forming units.
[a] Intervention regimen (i.e., form and daily intake) unclear from publication.
[b] All subjects were vaccinated with a trivalent influenza vaccine at least 3 to 4 months prior to starting the interventional period.

6.6. Pariza decision tree analysis

As indicated above, in assessing the safety of *L. plantarum* NCIMB 30562, 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. plantarum* NCIMB 30562 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?

   NO (go to 13 a). However, the NCIMB 30562 strain is a human commensal, and *L. plantarum* as a species has a history of safe consumption from foods. Thus, it is considered appropriate 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.

6.7. Conclusion of GRAS status

Chr. Hansen concludes that the intended uses of *Lactobacillus (L.) plantarum* NCIMB 30562 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 to demonstrate the safety of *L. plantarum* NCIMB 30562 for use as a microbial ingredient in conventional foods and non-exempt, term infant formula. The data presented in this GRAS notice fully support the conclusion that *L. plantarum* NCIMB 30562 is GRAS under the intended uses as described. The basis of the GRAS conclusion for the use of *L. plantarum* NCIMB 30562 are summarized by the following pivotal considerations:

- *L. plantarum* has a history of safe consumption from traditional fermented foods – *L. plantarum* is presently included in EFSA's QPS list (EFSA BIOHAZ Panel, 2022), the IDF/EFFCA's *Inventory of microbial food cultures with safety demonstration in fermented food products* (Bourdichon et al., 2012, 2018, 2022; Mogensen et al., 2002), and 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. plantarum* NCIMB 30562 ingredient.

- *L. plantarum* NCIMB 30562 is not genetically modified, is not pathogenic nor toxigenic, is not able to produce biogenic amines, and does not carry any transferable genes conferring antibiotic resistance.

- *L. plantarum* NCIMB 30562 has been evaluated in several clinical studies in which the strain was safely consumed without adverse events in infants (Chandel et al., 2017; Panigrahi et al., 2008, 2017) and additional clinical studies on *L. plantarum* support safety at the species level.

Based on the above considerations, the safety of *L. plantarum* NCIMB 30562 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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From: Winnie Ng
To: Morissette, Rachel
Cc: Kate Urbain
Subject: [EXTERNAL] RE: questions for GRN 001113
Date: Friday, May 26, 2023 3:11:28 PM
Attachments: image009.png
image010.png
image011.png
image012.png
image013.png
image014.png
Chr. Hansen Response to GRN1113 Questions_L. plantarum NCIMB 30562_2023.05.26.pdf

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Dear Dr. Morissette,

Please find attached our response to the questions for GRN 001113.

If you have any questions, please let me know.

Kindest Regards,
Winnie

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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, May 15, 2023 9:21 AM
To: Winnie Ng <CAWINN@chr-hansen.com>
Subject: questions for GRN 001113

Dear Dr. Ng,

Please see attached our questions for GRN 001113.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

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May 26, 2023
CAWINN

Response to Questions Regarding GRN 001113

Dear Dr. Morissette,

In regard to the questions on GRN 001113 for the intended use of *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) NCIMB 30562 received from the U.S. FDA on May 15, 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,

Winnie Ng
Principal Regulatory Affairs Specialist

E-mail: cawinn@chr-hansen.com
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RESPONSE TO FDA QUESTIONS ON GRN 001113 FOR *LACTIPLANTIBACILLUS PLANTARUM* (FORMERLY *LACTOBACILLUS PLANTARUM)* NCIMB 30562 RECEIVED ON MAY 15, 2023

The following is Chr. Hansen's response to the questions on GRN 001113 for the intended use of *Lactiplantibacillus (L.) plantarum* (formerly *Lactobacillus plantarum)* NCIMB 30562 as received from the U.S. FDA on May 15, 2023.

RESPONSES:

1. **Chr. Hansen states that a literature search was performed through May 2022. Please confirm that no new information that may appear counter to your GRAS conclusion has been published since then.**

   An updated literature search was conducted to identify any publications pertinent to the safety of *L. plantarum* and the NCIMB 30562 strain published since the original GRAS notice submission. The literature search followed the same method and search strategy as the European Food Safety Authority (EFSA)'s qualified presumption of safety (QPS) approach for *Lactobacillus* (specifically *L. plantarum*), and as outlined in Section 6.3.2.2 of the original GRAS notice, with the date filter ranging from May 2022 to May 2023.

   The search results were then screened for relevance in terms of safety concerns related to pathogenicity and toxigenicity in humans. Based on this literature search strategy, no new publications pertinent to the safety of *L. plantarum* were identified since the submission of the original GRAS notice.

   In addition, an updated search was conducted to identify randomized controlled human clinical studies published since the submission of the original GRAS notice. The updated literature search was conducted using the PubMed database to identify pertinent publications from May 2022 through to May 2023. While there were no new studies conducted specifically on the NCIMB 30562 strain, the search identified 9 additional studies relevant to the tolerability of *L. plantarum* as a species. A summary of the studies is presented in Table 1 below.

   Notably, although all of the identified studies were conducted to investigate the efficacy of the *L. plantarum* species, no significant adverse events were attributable to the test articles within these studies. *L. plantarum* was well tolerated at levels of up to 1.6×10^{10} CFU/day in children and 3.0×10^{11} CFU/day in adults when consumed over a 4-week period.

   Thus, to the best of our knowledge, there are no new scientific data published since May 2022 that would counter our GRAS conclusion for *L. plantarum* NCIMB 30562 under the intended conditions of use. This is consistent with the most recent QPS update in January 2023, where EFSA concluded that the QPS status of the QPS species within the *Lactobacilli* genus remained unchanged including *Lactiplantibacillus plantarum* (EFSA BIOHAZ Panel, 2023).

Table 1. Summary of randomized controlled clinical studies on *L. plantarum* published May 2022 to May 2023.

Reference	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Zhang et al., 2023	Healthy children (3-6 years of age; with early childhood dental caries) N=79	Oral, powder sachet Control: placebo Intervention: *Lactobacillus plantarum* CCFM8724 at 1.6 x 10^{10} CFU/day	4 weeks	No AEs were reported through the duration of the study.
Zhu et al., 2023	Healthy adults (mean age of 22 years; college students with exam-induced stress and anxiety) N=60	Oral, powder sachet Control: placebo Intervention: *Lactobacillus plantarum* JYLP-326 at 3.0 x 10^{10} CFU/day	3 weeks	No AEs reported.
Watanabe et al., 2022	Healthy adults (mean of 36-38 years of age) N=66	Oral, powder sachet Control: placebo Intervention: *Lactiplantibacillus plantarum* SNK12 at 1.0 x 10^{11} or 3.0 x 10^{11} CFU/day	4 weeks	No clinically relevant changes in hematology and urinalysis. No AEs were reported through the duration of the study and the test article was well tolerated.
Jung et al., 2022	Healthy adults (mean of 39 years of age; with IBS [Rome IV criteria]) N=27	Oral, capsule Control: placebo Intervention: *Lactiplantibacillus plantarum* APsulloc 331261 (KCCM11179P, GTB1™) at 1.0 x 10^{10} CFU/day	4 weeks	Mild GI symptoms (abdominal bloating, heartburn, constipation) were noted in the study subjects; however, no serious AEs were reported through the duration of the study.
Ma et al., 2023	Healthy adults (18-65 years of age; with chronic constipation [Rome IV criteria]) N=163	Oral, powder sachet Control: placebo Intervention: *Lactiplantibacillus* plantarum P9 at 1.0 x 10^{11} CFU/day	4 weeks	No AEs were reported through the duration of the study.

Reference	Study Population	Intervention	Duration of Intervention	Safety-Related Outcomes
Štšepetova et al., 2023	Healthy adults (18-65 years of age; elevated cholesterol [≥5.0 mmol/l] and/or LDL-cholesterol [≥3.0 mmol/l] and/or triglyceride [≥1.7 mmol/l]) N=285	Oral, yoghurt Control: no added culture Interventions: *Lactiplantibacillus plantarum* Inducia at 5.9 x 10^9 or 2 x 10^9 CFU/day	8 weeks	No AEs reported.
Prakoeswa et al., 2022	Healthy adults (mean of 37.87 ± 14.21 years of age; mild to moderate atopic dermatitis) N=30	Oral, capsule Control: placebo Intervention: *Lactobacillus plantarum* IS-10506 at 2.0 x 10^{10} CFU/day	8 weeks	No AEs reported.
Sohn et al., 2023	Healthy adults (mean of 40 years of age; BMI of 25 to 30 kg/m^2) N=100	Oral, capsule Control: placebo Intervention: *Lactobacillus plantarum* LMT1-48 at 2.0 x 10^{10} CFU/day	12 weeks	No serious AE were reported through the duration of the study nor any differences in AEs between groups. Vital signs upon physical examination were not clinically significant.
OjiNjideka Hemphill et al., 2023	Healthy pregnant women (mean of 28.9 ± 6.5 years of age; 13.4 ± 4.1 weeks of gestation) N=20	Oral, capsule taken with beverage Control: placebo Intervention: *Lactobacillus plantarum* Lp299v at 10^{10} CFU/capsule[a] All subjects also consumed a prenatal vitamin with iron.	To delivery (overall mean 38.8 ± 0.7 weeks gestational age at delivery)	The test article was well tolerated during pregnancy. All AEs were minor and no significant differences in AE reported through the duration of the study. No significant difference in gestational age at delivery, neonatal weight at delivery or infant sex.

Abbreviations: AE, adverse event; BMI, body mass index; CFU, colony forming unit; IBS, Irritable Bowel Syndrome.
[a] Daily dosage is unclear in publication.

References:

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cocconcelli, P. S., Escámez, P. S. F., Maradona, M. P., … Herman, L. (2023). Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 17: suitability of taxonomic units notified to EFSA until September 2022. *EFSA Journal. European Food Safety Authority*, *21*(1), e07746. https://doi.org/10.2903/j.efsa.2023.7746

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2. Chr. Hansen provides estimates of dietary exposure for male infants 1 and 6 months of age; however, the notice does not include a discussion of the estimated dietary exposures to *L. plantarum* NCIMB 30562 for other infant age groups or female infants that are within the expected consumer population (e.g., infants up to 12 months of age). Please provide an appropriate narrative and dietary exposure estimates for all infants expected to consume infant formula containing *L. plantarum* NCIMB 30562 at the maximum intended use level.

The estimated dietary exposure to *L. plantarum* NCIMB 30562 for male and female infants 1 to 12 months of age are summarized in the Table below. The estimates were calculated using the intended use of *L. plantarum* NCIMB 30562 as subject to GRN 001113 (*i.e.*, 1.1×10^8 CFU/g infant formula), the estimated caloric intake requirements of infants as outlined by the Institute of Medicine (2005), and assuming an average reconstitution rate of 14.1 g powdered infant formula per 100 mL water, wherein commercial infant formulas in the U.S. typically provide an energy content of 0.67 kcal/mL (20 kcal/fl oz) (Martinez & Ballew, 2011).

Table 2. Estimated dietary intake of *L. plantarum* NCIMB 30562 under the intended use in infant formula.

Age (months)	Male Estimated Caloric Intake[a] (kcal/day)	Maximum Intended Use of *L. plantarum* NCIMB 30562[b] (CFU/g infant formula)	Estimated Daily Intake[c] (CFU/day)	Female Estimated Caloric Intake[a] (kcal/day)	Maximum Intended Use of *L. plantarum* NCIMB 30562[b] (CFU/g infant formula)	Estimated Daily Intake[c] (CFU/day)
1	472	1.1×10^8	1.1×10^{10}	438	1.1×10^8	1.0×10^{10}
2	567		1.3×10^{10}	500		1.2×10^{10}
3	572		1.3×10^{10}	521		1.2×10^{10}
4	548		1.3×10^{10}	508		1.2×10^{10}
5	596		1.4×10^{10}	553		1.3×10^{10}
6	645		1.5×10^{10}	593		1.4×10^{10}
7	668		1.5×10^{10}	608		1.4×10^{10}
8	710		1.6×10^{10}	643		1.5×10^{10}
9	746		1.7×10^{10}	678		1.6×10^{10}
10	793		1.8×10^{10}	717		1.7×10^{10}
11	817		1.9×10^{10}	742		1.7×10^{10}
12	844		2.0×10^{10}	768		1.8×10^{10}

Age (months)	Male Estimated Caloric Intake[a] (kcal/day)	Maximum Intended Use of *L. plantarum* NCIMB 30562[b] (CFU/g infant formula)	Estimated Daily Intake[c] (CFU/day)	Female Estimated Caloric Intake[a] (kcal/day)	Maximum Intended Use of *L. plantarum* NCIMB 30562[b] (CFU/g infant formula)	Estimated Daily Intake[c] (CFU/day)

Abbreviations: CFU, colony forming units.

[a] As estimated energy requirement (equivalent to total energy expenditure plus energy deposition) (IOM, 2005).

[b] Subject to the maximum intended use level in infant formula as outlined in GRN 001113.

[c] Calculated as: (caloric intake [kcal/day] x reconstitution rate [14.1 g powdered infant formula per 100 mL water] x intended use [CFU/g powdered infant formula]) / energy content (0.67 kcal/mL infant formula).

Under the intended conditions of use at a maximum incorporation level of 1.1×10^8 CFU/g infant formula, the estimated intake of *L. plantarum* NCIMB 30562 will range from 1.1×10^{10} to 2.0×10^{10} CFU/day in male infants at 1 and 12 months, respectively. In female infants, the estimated daily intake will range from 1.0×10^{10} to 1.8×10^{10} CFU/day at 1 and 12 months, respectively.

As indicated in the original GRAS notice, the estimated dietary exposure of the NCIMB 30562 strain is consistent with other *Lactobacillus* spp. intended for use as microbial ingredients in infant formula that have received a letter of "no questions" from the FDA, where estimated daily intake ranges from 10^7 to 10^{10} CFU/day (GRN 410, 531, 810, 865, and 1013). Collectively, with the QPS status of the *L. plantarum* species and the extensive history of safe use in food, the dietary exposure to *L. plantarum* NCIMB 30562 under its intended use in infant formula is not expected to be a significant concern.

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From: Winnie Ng
To: Morissette, Rachel
Cc: Arie Carpenter
Subject: [EXTERNAL] RE: GRN 1113
Date: Friday, June 30, 2023 12:13:23 PM
Attachments: image009.png
image010.png
image011.png
image012.png
image013.png
image014.png
GRAS Notice_Chr. Hansen_L. plantarum NCIMB 30562_Aug 2022.pdf
Chr. Hansen Response to GRN1113 Questions_L. plantarum NCIMB 30562_2023.05.26.pdf

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Dear Dr. Morissette,

As requested, please find attached unsecured copies of the original GRAS notice and the recent response to FDA questions.

Kindest Regards,

Winnie Ng, Ph.D., DABT
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Monday, March 20, 2023 12:09 PM
To: Winnie Ng <CAWINN@chr-hansen.com>
Subject: GRN 1113 filing letter

Dear Dr. Ng,

Please see attached the filing letter for GRN 1113. Let me know if you have questions at this time.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist/Biologist

Division of Food Ingredients
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