CHR. HANSEN
Improving food & health

Division of Biotechnology and GRAS Notice Review
Center for Food Safety & Applied Nutrition (HFS-255)
U.S. Food & Drug Administration
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May 26, 2020
USEMGR

Reference: Chr. Hansen GRAS Notification for
Bifidobacterium longum subsp. infantis DSM 33361

To Whom it May Concern,

In accordance with the 21 CFR 170 Subpart E, regulations for Generally Recognized as Safe (GRAS) notifications, Chr. Hansen, Inc. has concluded, through scientific procedures, that *Bifidobacterium longum subsp. infantis* DSM 33361 is Generally Recognized As Safe (GRAS) and is not subject to the premarket approval requirements for use as an ingredient in conventional foods including (but not limited to) dairy products and other fermented milk products, fermented plant-based products, beverages, shelf stable products, confectionery, and breakfast cereals. *Bifidobacterium longum subsp. infantis* DSM 33361 is also intended as an ingredient in non-exempt infant formula (including cow-milk, soy, and protein hydrolysate based formulas). The addition level may be as high as 2.8×10^{10} CFU/serving to account for loss of viability throughout the shelf of the product for conventional foods, and 1×10^{10} cfu/g for infant formula.

Yours sincerely,

Emily Gregoire
Probiotics Regulatory Affairs Manager – North America

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Generally Recognized As Safe (GRAS) Determination for

Bifidobacterium longum subsp. infantis DSM 33361

Prepared by

Chr. Hansen, Inc.

February, 2020

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Allergen Management in Chr. Hansen

Abbreviations

BA: Biogenic Amine
BIOHAZ: Panel on Biological Hazards
CFU: Colony forming units
CFR: Code of federal regulations
EFFCA: European Food and Feed Cultures Association
EFSA: European Food Safety Authority
FDA: Food and Drug Administration
FSIS: Food Safety and Inspection Service
FSSC: Food safety system certification
GMP: Good Manufacturing Practices
GRAS: Generally Recognized As Safe
IDF: International Dairy Federation
ISO: International Organization for Standardization
LAB: Lactic Acid Bacteria
MIC: Minimum Inhibitory Concentration
QPS: Qualified Presumption of Safety
SSU: Small subunit
TU: Taxonomic unit
USDA: United States Department of Agriculture

Part 1: Signed Statements and Certification

1.1 Basis of GRAS Conclusion

In accordance with the 21 CFR 170 Subpart E, regulations for Generally Recognized as Safe (GRAS) notifications, Chr. Hansen, Inc. has concluded, through scientific procedures, that *Bifidobacterium longum subsp. infantis* DSM 33361 is Generally Recognized As Safe (GRAS) and is not subject to the premarket approval requirements for use as a bacterial ingredient in conventional foods including (but not limited to) dairy products and other fermented milk products, fermented plant-based products, beverages, shelf stable products, confectionery, and breakfast cereals. *Bifidobacterium longum subsp. infantis* DSM 33361 is also intended as an ingredient in non-exempt infant formula (including cow-milk, soy, and protein hydrolysate based formulas). The addition level may be as high as $2.8x10^{10}$ CFU/serving to account for loss of viability throughout the shelf of the product for conventional foods, and $1x10^{10}$ cfu/g for infant formula.

Name and Address of Organization

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

Contact Person:
Emily Gregoire
Regulatory Affairs Manager
Human Health North America
Chr. Hansen, Inc.
9015 West Maple Street
Milwaukee, WI 53214

1.2 Name of GRAS Organism

Bifidobacterium longum subsp*. infantis* DSM 33361

Currently commercially sold as "*B. infantis* BB-02"

1.3 Intended Conditions of Use

Bifidobacterium longum subsp. infantis DSM 33361 intended to be consumed by the general population as well as term infants. Intended applications include but are not limited to the following: milk and dairy products such as yogurt and other fermented milk products; dairy alternatives (plant-based (oat, soy, almond, coconut, pea, etc.) fermented milk and yogurt products); beverages such as juice and protein shakes; shelf-stable products such as bars (granola, protein, meal replacement bars), confectionery (gummy candy, hard candy, soft chew candy, chewing gum, coatings), cereals (RTE and hot), and non-exempt infant formula (including

cow-milk, soy, and protein hydrolysate based formulas). The addition level may be as high as $2.8x10^{10}$ CFU/serving to account for loss of viability throughout the shelf of the product for conventional foods, and $1x10^{10}$ cfu/g for infant formula.

1.4 Statutory Basis for GRAS Determination

Pursuant to the GRAS rule [81 Fed. Reg. 159 (17 August 2016)], Chr. Hansen has concluded that *Bifidobacterium longum subsp. infantis* DSM 33361 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 *Bifidobacterium longum subsp. infantis* DSM 33361 is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetics Act based on our conclusion that *Bifidobacterium longum subsp. infantis* DSM 33361 is GRAS under the intended use conditions.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that *Bifidobacterium longum subsp. infantis* DSM 33361, when used as described in this dossier, is GRAS based on scientific procedures.

1.6 Availability of Information

The data and information that are the basis for Chr. Hansen's conclusion that *Bifidobacterium longum subsp. infantis* DSM 33361 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.
Attn: Emily Gregoire
9015 W Maple St., Milwaukee, WI 53214
usemgr@chr-hansen.com

1.7 Freedom of Information Act

It is our opinion that the information contained in this dossier is not exempt from disclosure under the Freedom of Information Act.

1.8 Certification

To the best of our knowledge, this GRAS conclusion is a complete, representative, and balanced dossier that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of *Bifidobacterium longum subsp. infantis* DSM 33361.

1.9 FSIS Statement

Not Applicable.

1.10 Name, Position and Signature of responsible person who signs dossier and signature

Emily Gregoire

Regulatory Manager, Human Health North America
Chr. Hansen, Inc.
9015 West Maple Street
Milwaukee, WI 53214

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

2.1 Name of GRAS Organism

The subject of this Generally Recognized as Safe (GRAS) assessment is a strain of the bacterial species *Bifidobacterium infantis* designated as DSM 33361.

2.2 Source, Description and Identity of GRAS Organism

2.2.1 Source of the GRAS Organism

Bifidobacterium longum subsp. infantis DSM 33361 was originally isolated from the intestine of a healthy infant.

2.2.2 Description of the GRAS Organism

The organism that is the subject of this GRAS notice, *Bifidobacterium longum* subsp*. infantis* DSM 33361, is a thoroughly characterized strain.

In 2008, three subspecies of *B. longum* (subsp. *suis*, *longum*, *infantis*) were designated by Mattarelli et al. (2008) and same year pan-genome sequence of *B. longum* subsp. *infantis* clarified the unique position of the subspecies *infantis* (Sela et al., 2008). In recent years, it is common to use the name "*B. infantis*" only after prior elaboration of the full species and subspecies name; however, despite this taxonomic acceptance and resolution nearly a decade ago, the inaccurate use of the subspecies designation *infantis* remains (Lewis et al., 2016). Given this, in reviewing the available studies, an attempt has been made to identify the correct strain.

Bifidobacterium is one of the most important organisms for human gut microflora (O'Sullivan et al. 1992; Fuller and Gibson 1997). The name Bifidobacterium derives from the observation that they can exist in a Y-shaped or bifid form. These organisms have been used in food products and dietary supplements for decades, with a compelling record of safe consumption (Reid, 2002; Kocian et al., 1994; FAO/WHO, 2002). The available information suggests that shortly after birth Bifidobacteria predominate in the intestinal tract of new born infant. Bifidobacteria are important and normal constituents of the human gastrointestinal microflora and occur at levels ranging from 10^9 to 10^{10} cells/g of feces (Tanaka et al., 2000). Among the Bifidobacteria genera found in intestinal tract microbiota, *Bifidobacterium infantis* is a natural inhabitant and is a lactic acid bacterium (LAB) which has been used for many years in fermented food.

2.2.3 Phenotypic Characteristics

Bifidobacterium longum subsp. infantis DSM 33361 is a Gram positive with following characteristics: round with a smooth edge; Elevation: convex; Surface: smooth and shiny; Consistency: soft; Appearance: white (on MRS, after 3 days at 37°C, anaerobic incubation); Catalase reaction: negative. As regards cell morphology, this microorganism is long,

curved/irregular, single and pairs, and non-motile. The strain is fructose-6-phosphate phosphoketolase positive and bile tolerant. The cell size of *B. infantis* is reported as 1 to 1.5 µm width x 4 µm length. The carbohydrate fermentation and enzyme activity (rapid ID32 A) of the DSM 33361 strain (Table 2).

Genetic stability during storage and production as confirmed by DNA fingerprinting of Chr. Hansen reference stock material and inoculation material produced since 1992 show identical fingerprints. (See section 2.5.3, Figure 2)

The taxonomic identification of *Bifidobacterium longum subsp. infantis* DSM 33361 was confirmed in-house at Chr. Hansen using the references Mattarelli et al. (2008) and Sakata et al. (2002). The strain was deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) under the accession number DSM 33361 (deposit according to the Budapest Treaty). The taxonomic lineage of *Bifidobacterium longum subsp. infantis* DSM 33361 is provided in Table 1.

Table 1: Taxonomic lineage of *Bifidobacterium longum subsp. infantis* DSM 33361

Taxonomy	**Taxonomic Assignment**
Kingdom	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Bifidobacteriales
Family	Bifidobacteriacea
Genus	*Bifidobacterium*
Species	*Bifidobacterium longum* subsp*. infantis*
Strain	*Bifidobacterium longum subsp. infantis* DSM 33361

Table 2: carbohydrate fermentation and enzyme activity (rapid ID32 A) of the DSM 33361 strain

Control	-	Nitrate reduction	-
Urease	-	Production of indol	-
Arginine dehydrolase	-	Alcaline phosphatase	-
α - galactosidase	+	Arginine arylamidase	+
β - galactosidase	+	Proline arylamidase	+
β – galactosidase-6 phosphate	-	Leucyl-glycine arylamidase	-
α - glucosidase	+	Phenylalanine arylamidase	+
β - glucosidase	-	Leucine arylamidase	+
α- arabinosidase	-	Pyroglutamic acid arylamidase	+
β – glucuronidase	-	Tyrosine arylamidase	+
N-acetyl-β-glucosaminidase	+	Alanine arylamidase	-
Mannose	+	Glycine arylamidase	+
Raffinose	+	Histidine arylamidase	+
Glutamic acid decarboxylase	-	Glutamyl-glutamic acid arylamidase	-
α - fucosidase	-	Serine arylamidase	+

2.2.4 Genotypic Characteristics

In an attempt to further characterize the identity of strain DSM 33361, the sequence analysis of the strain's 16S rDNA sequence was compared to a database of 16S rDNA sequences of type strains and multi locus sequence typing (Yanokura et al., 2015). The 16S rDNA sequence (*E. coli* pos. 85-1541, 1443 basepairs) of the DSM 33361 strain is found to be identical to the sequence of the type strain of *B. longum* subsp. *infantis* (GenBank acc. No. D86184). Given this, the DSM 33361 strain is thereby placed in the species *Bifidobacterium longum*. In the MLST scheme used in Yanokura et al. (2015) for differentiation of the four subspecies of *B. longum,* the DSM 33361 strain falls into the sequence type 14, which belongs to the *B. longum* subsp. *infantis* cluster.

2.2.5 Genomic Analysis

In order to obtain a high-quality genome sequence of the *Bifidobacterium longum* subsp. *infantis* DSM 33361 strain, the strain was genome sequenced using the Illumine MiSeq Technology and Oxford Nanopore Technology. Output from the MiSeq sequencing (2,521,691 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 25 contigs of 2,733,909

bp with a GC content of 58.9% and an average coverage of 104. Combing reads from both sequencing technologies lead to a circular genome of 2,757,834 bp. No plasmid was observed in the genome assembly and plasmid profiling verifies the lack of plasmids.

The Oxford Nanopore Technology (ONT)/MiSeq combined genome sequence of the *Bifidobacterium longum subsp. infantis* DSM 33361 was subjected to annotation using RAST (Aziz et al., 2008). RAST (Rapid Annotation using Subsystem Technology) is an annotation tool for bacterial and archaeal genomes and provides a high-quality annotation. The RAST annotation of the combined genome sequence for the DSM 33361 strain contained 2623 coding sequences (CDS), 67 RNAs and 15 repeats. The genome size and number of CDSs in the DSM 33361 strain was comparable to *B. longum* subsp. *infantis* in the NCBI genome database. These studies support the identity of *Bifidobacterium longum subsp. infantis* DSM 33361.

2.2.5.1 Antibiotic Resistance Genes

In an attempt to identify genes with high homology to previously published antibiotic resistance genes, the genome of *Bifidobacterium longum* subsp. *infantis* DSM 33361 was analyzed against a curated published database of antibiotic resistance genes from various bacteria and also a published database focusing on antimicrobial resistance genes of relevance to Gram-positive bacteria and in particular to lactic acid bacteria and Bifidobacteria. The genome of the DSM 33361 strain was analyzed and no antibiotic resistance genes were detected. Similarly, no phenotypic antibiotic resistance was observed in *Bifidobacterium longum subsp. infantis* DSM 33361.

Bifidobacteria are not known to be antibiotic producers. *B. infantis* has not been reported in the literature as able to produce antibiotics. Moreover, whole genome sequencing of *Bifidobacterium longum subsp. infantis* DSM 33361 has not revealed any open-reading frames encoding genes for antibiotic production.

2.2.5.2 Virulence Factor Genes

The genome of *Bifidobacterium longum* subsp. *infantis* DSM 33361 was analyzed against a published database of virulence factors containing virulence factors from 30 different pathogens including Gram positive pathogens such as *Enterococcus, Staphylococcus, Streptococcus* and *Listeria*. Most of the hits were associated with Clp and heat shock proteins, surface structures, and transport or secretion systems. None of the hits were assessed to be virulence factors and all hits could be regarded as 'niche factors' (Hill, 2012), since they are also found in commensal bacteria.

The screening of the *Bifidobacterium longum subsp. infantis* DSM 33361 genome revealed 23 unique hits. Of these hits 19 were to genes in VFDB which could be categorized either as 'niche factors' or housekeeping genes in bacteria (Clp genes, transporters, capsule genes, or genes involved in heat shock). The remaining three hits were present in five out of the six *B. longum* subsp. *infantis* genomes in the NCBI NR database. The genes were annotated as urease subunits or as urease accessory protein UreG. When assessing the genes and flanking sequences the

entire gene cluster for urea uptake and metabolism was found. This gene cluster has previously been described as conserved in *B. longum* subsp. *Infantis* but absent in *B. longum* subsp. *longum* (Locascio et al., 2010). Due to the presence of this gene cluster it is believed that *B. longum* subsp. *infantis* has a role in the early life of infants to use the urea made available in the gastro intestinal tract while generating ammonia to satisfy the host nitrogen needs (Locascio et al., 2010). So, the gene cluster is considered a beneficial niche factor and is not considered a safety concern in *Bifidobacterium longum subsp. infantis* DSM 33361.

The remaining hit was to a gene annotated as a sialidase and present in four out of the six *B. longum* subsp. *infantis* in the NCBI NR database. Sialidases can e.g. help the bacteria adhere to the host mucus layer and have been described also to enable *B. longum* subsp. *infantis* and other *Bifidobacterium* species to cleave sialyl-human milk oligosaccharides and mucin glycans to produce oligosaccharides that supports growth of the bacteria (Nishiyama et al., 2017; Sela et al., 2011; Audy et al., 2010). Genes encoding sialidases were in a previous study found to be present in the majority of *B. longum* subsp. *infantis*, while absent in the majority of *B. longum* subsp. *longum* (Locascio et al., 2010). The ability to adhere inside the host gut and to produce oligosaccharides that supports the growth are beneficial effects for probiotic bacteria and is considered niche factors. So, the gene is considered of no safety concern.

In addition to *in silico* genome screening, phenotypic tests for cytotoxicity and hemolysis were also performed (see section 2.2.6.4). As described below, the findings from these phenotypic tests showed that *Bifidobacterium longum subsp. infantis* DSM 33361 did not cause cytotoxic activity in a Vero cell assay and the strain is non-hemolytic.

2.2.5.3 Search of Gene annotations

The genome sequence of *Bifidobacterium longum* subsp. *infantis* DSM 33361 was subjected to annotation using published methods. The gene annotations were searched to identify terms that could be linked to antibiotic resistance. A total of 39 genes included one or more of these words in its annotation. Many proved to be housekeeping genes or transporters. Six hits were further assessed but were in all cases annotated with names of antibiotics to which the strain shows no resistance and homologs of the genes were observed in many *B. longum* strains. All were dismissed as safety concerns.

In a similar fashion the gene annotations were searched for terms that could be linked to virulence. This search identified 33 genes; most genes were determined to be housekeeping genes of no safety concern. Those containing the word “toxin” in the annotation were found to be bacterial toxin-antitoxin systems, part of the cellular regulatory machinery. One gene was to Fibrinectin/fibrinogen binding proteins and was found in all 27 *B. longum* genomes in the NR NCBI database and may be involved in adhesion of the cell. Adhesion is considered a niche factor and beneficial for a probiotic strain. Three genes were to hemolysins homologues and two of the genes were found in all *B. longum* genomes present in the NCBI NR database. One of

the hemolysin homologues (annotated as predicted membrane protein hemolysin III homologue) was found in 22 out of 27 *B. longum* genomes in the NR NCBI database. Although hemolysin homologue genes were present, the strain is not hemolytic (see section 2.2.6.4) so these genes are probably membrane proteins with other functions and of no safety concern.

2.2.5.4 Conclusion of the Genomic Characteristics and Safety Assessment

The genome of *Bifidobacterium longum* subsp. *infantis* DSM 33361 was analyzed for the presence of antibiotic resistance genes and no antibiotic resistance genes were detected. No virulence genes were identified indicating a very low virulence potential of the strain. In conclusion, there are no indications that *Bifidobacterium longum subsp. infantis* DSM 33361 is a safety concern based on the genome safety assessment.

2.2.6 In Vitro Studies of Bifidobacterium longum subsp. infantis DSM 33361

2.2.6.1 Biogenic Amines

The *Bifidobacterium longum* subsp. *infantis* DSM 33361 strain was tested for the production of histamine, tyramine, cadaverine and putrescine using an in-house procedure based on published methods (Cid et al., 2008). The strain did not produce any of the four biogenic amine compounds tested when grown in presence of specific amino acid precursors known to induce production of the biogenic amines.

2.2.6.2 Antibiotic Resistance In Vitro

Minimum inhibitory concentrations (MICs) of 9 antibiotics were determined for *Bifidobacterium longum* subsp. *infantis* DSM 33361 according to the ISO 10932 I IDF 223 international standard. These MICs were compared with the cut-off values established for *Bifidobacterium* (Table 2) by the European Food Safety Authority (EFSA, 2018). *Bifidobacterium longum subsp. infantis* DSM 33361 is sensitive to all the antibiotics tested with MIC values that are less than or equal to the EFSA 2018 cut-off values for *Bifidobacterium*. According to those breakpoints, *Bifidobacterium longum subsp. infantis* DSM 33361 is not considered resistant to any of the tested antimicrobial agents.

Table 3: MIC values for Bifidobacterium longum subsp. infantis DSM 33361

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

[a]EFSA cut-off values for *Bifidobacterium* as listed in 'Guidance on microorganisms used as feed additives or as production organisms' (EFSA Journal 2018, 16(3):5206); NR= not relevant.

2.2.6.3 Production of D-Lactate

Bifidobacterium longum subsp. *infantis* DSM 33361 was analyzed for production of D- and L-lactate.This strain was found to produce 100% L-lactate; the D-lactate isomer is not produced. Hence the strain is characterized as an L-lactate producing strain in line with published literature (McCartney, 2003). D-Lactate has been implicated in the etiology of acidosis in children with short small bowel syndrome as well as patients with intestinal bypass (Bongaerts et al, 2000; Hove and Mortensen 1995). Various strains of the genus *Lactobacillus* have been reported produce D-lactate (Kaneko et al, 1997; Iino et al, 2003), while there are no reports in the literature of D-lactate production by genus *Bifidobacterium*.

2.2.6.4 Cytotoxic and Hemolytic Activity

Bifidobacterium longum subsp. *infantis* DSM 33361 was tested for cytotoxic activity in a Vero cell assay in accordance with the guideline provided by EFSA with a few modifications (EFSA, 2014). The strain was inoculated in MRS broth supplemented with CysHCl instead of BHI broth and incubated at incubated at 37°C anaerobically. The cells were harvested after 24 hours and 48 hours. Hemolytic activity was tested on 5% sheep blood agar incubated anaerobically at 37°C for 48 hours. The result showed that the strain was non-hemolytic and not causing cytotoxic activity in the Vero cell assay.

2.3 Genetic Modification Status

According to the European Union (EU) regulations; 1829/2003 and 1830/2003 the use of Chr. Hansen cultures, including *Bifidobacterium longum* subsp. *infantis* DSM 33361, do not trigger Genetic Modification (GM) labeling of the final food product.

According US regulation, Chr. Hansen culture and enzyme products are not subject to bioengineered (BE) labeling under NBFDS, codified in 7 CFR Part 66.

2.4 Method of Manufacture

Viable *Bifidobacterium longum* subsp. *infantis* DSM 33361 is manufactured following Chr. Hansen's global protocol for production of cultures. Currently, the freeze-dried product is manufactured by site Roskilde (Chr. Hansen A/S, Sdr. Ringvej 22, 4000 Roskilde, Denmark). Subsequently it is transported to site Copenhagen (Chr. Hansen A/S, Jernholmen 1-27, 2650 Hvidovre, Denmark) where it is grinded into a powder and, in some cases, blended with other products. All manufacturing is done in accordance with current good manufacturing practices (cGMP) consistent with 21 CFR Parts 110 and 117. All Chr. Hansen plants comply 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 available from our website: www.chr-hansen.com. In addition, all plants have an appointed local Operational Pre-Requisite Program that includes PRP issues and Critical Control Points, which are documented and are classified as specifically critical for the safety of food ingredients produced in the plant. Both plants maintain the following certifications: FSSC incl ISO 22000, ISO 14001, and OHSAS 18001.

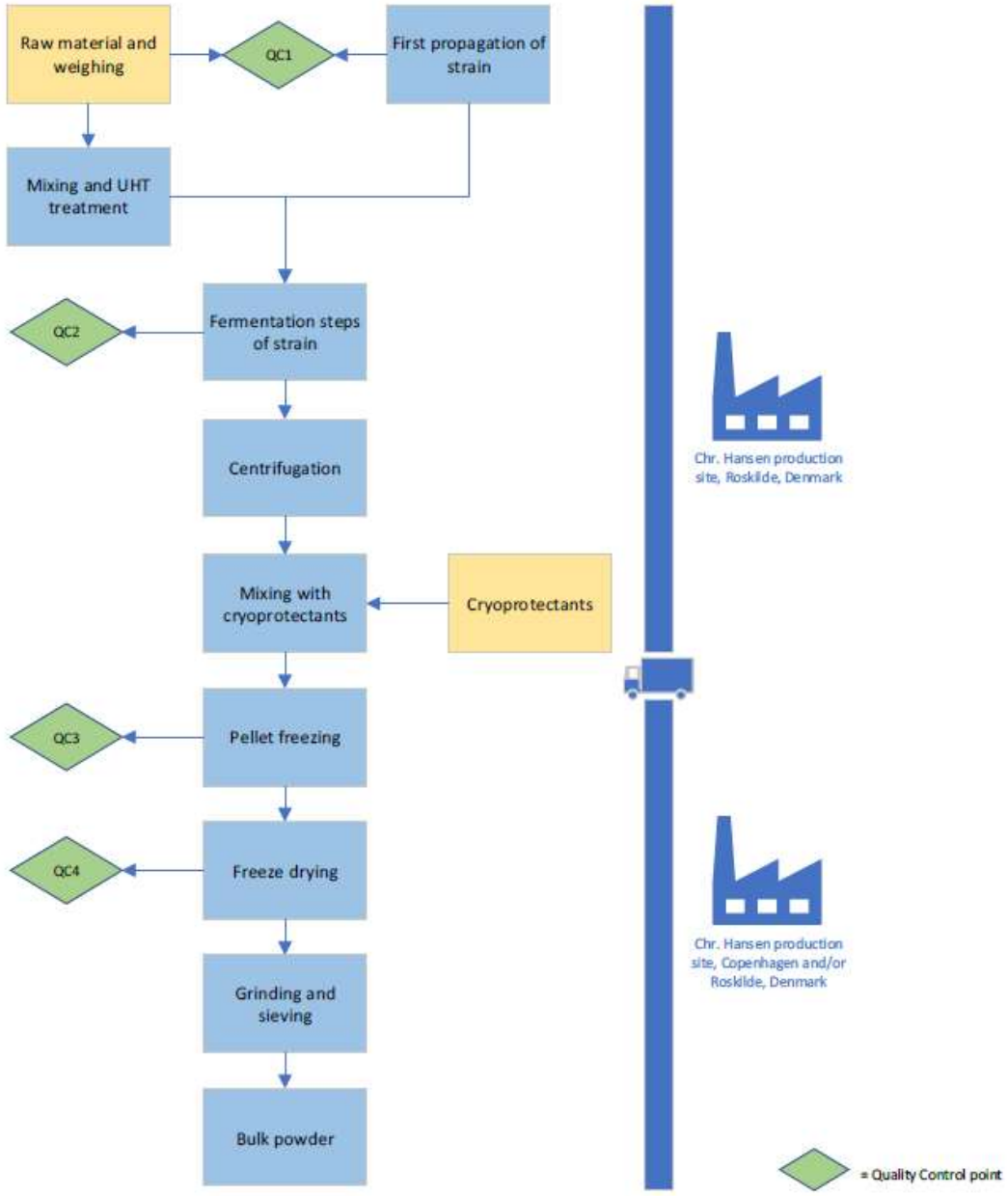

Figure 1: Manufacturing procedures for concentrates

The individual production steps are as follows:

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

***Inoculation and fermentation*.** The initial material is a pure culture from Chr. Hansen's Culture Collection. *Bifidobacterium longum subsp. infantis* DSM 33361 working cell bank (inoculation

culture) is propagated throughout different production steps. This includes the first propagation from a small vial followed by a number of fermentation processes using the above-mentioned media for fermentation. Upon completion of the fermentation processes the bacterial cells are harvested and proceed to the concentration step.

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

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

Freeze-drying. The frozen pellets are lyophilized resulting in very low water activity and ensuring stability of the culture. The freeze-dried granules may be grinded to a powder and blended with excipients to a standardized cell count and sold as an individual product. The powder may also be blended with other strains and excipients to be sold as blends.

2.4.1 Raw Materials and Processing Aids

Bifidobacterium longum subsp. infantis DSM 33361 is produced using standard fermentation techniques. This includes the use of fermentation and standardizing ingredients that are safe and suitable for use in human food. These ingredients have no technical function in the finished food product and are all permitted for the applications specified in this notice and meet the specifications of the Food Chemical Codex.

2.4.2 Quality Program

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

2.4.3 Allergen Control

Chr. Hansen controls all allergens listed in EU Labeling Regulation 1169/2011 and the US Food Allergen Labeling and Consumer Protection Act of 2004. Chr. Hansen also communicates the allergen status of our products in accordance with these two regulations. Allergen control is managed via our GMP and HACCP programs that are FSSC 22000 certified at all of our production sites. Allergen communication is managed via our Quality Management and HACCP programs that are ISO 22000 certified. Chr. Hansen produces *Bifidobacterium longum subsp. infantis DSM 33361* products for dietary supplements and conventional foods. Due to this, there may be different formulations for different products. Milk allergen may be present in finished product ingredients for some forms of *Bifidobacterium longum subsp. infantis* DSM 33361. We have dairy-free products as well which contain no allergens in either the

fermentation media or finished product ingredients. Please see the attached statement regarding our allergen management program (Allergen Management in Chr. Hansen).

2.5 Specification, Product Release, Product Stability

2.5.1 Specification and Microbiological

Bifidobacterium longum subsp. infantis DSM 33361 freeze-dried product is a light beige fine ground powder. Purity is controlled as described in Table 4.

Table 4: Microbiological Specification

Specification	Criteria	Frequency of analysis
Total aerobic microbial count (cfu/g)	≤ 2000	Every batch

Further microbiological testing occurs after the bulk product is further processed into different forms and/or blended with other strains. All products must pass microbiological criteria prior to their release in order to guarantee total cell count and purity.

2.5.2 Product Stability

Bifidobacterium longum subsp. infantis DSM 33361 freeze-dried products, in the form of granulate or grinded, have a shelf-life of 24 months from the date of manufacture when stored at 2-8 °C in original or tightly closed aluminum foil pouch.

The genetic stability of strain DSM 33361 has been demonstrated by DNA fingerprinting comparing the stock culture in the cell bank and various batches of inoculation material produced since 1992 (Figure 1). The genetic stability of *Bifidobacterium longum subsp. infantis* DSM 33361 shows that the strain safety analysis will hold true over time.

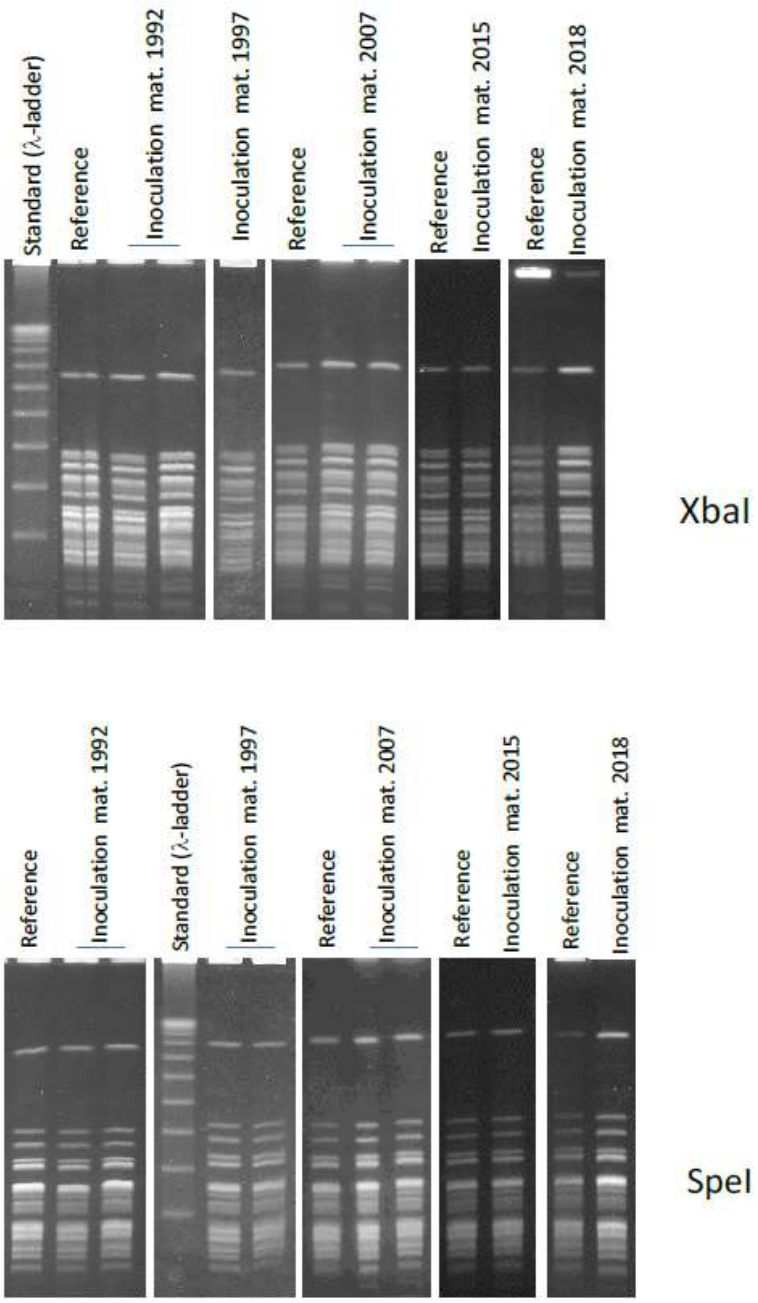

Figure 2: XbaI and SpeI fingerprints of Bifidobacterium longum subsp. infantis DSM 33361 reference and inoculation materials