Generally Recognized as Safe (GRAS) Notice for

Lactobacillus plantarum MCC1 DSM 23881

Prepared for:

NORDWISE BioTech OÜ
Riia 181a, 51014 Tartu
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1 SIGNED STATEMENT AND CERTIFICATIONS (21 CFR § 170.225)

1.1 GRAS Notice Submission

NORDWISE BioTech OÜ is hereby submitting this Generally Recognized as Safe (GRAS) notice to the United States (U.S.) Food and Drug Administration (FDA), in accordance with 21 CFR part 170, Subpart E

1.2 Name and Address of the Sponsor

NORDWISE BioTech OÜ
Riia 181a, 51014 Tartu
Estonia

1.3 Common or Usual Name

Lactobacillus plantarum MCC1, *Lactiplantibacillus plantarum* MCC1, *Lactobacillus plantarum* DSM 23881, *Lactiplantibacillus plantarum* DSM 23881

1.4 Intended Conditions of Use

The microbial ingredient *Lactiplantibacillus plantarum* MCC1 is intended to be used as a general ingredient in conventional foods at a minimum level of $5x10^8$ CFU/serving and up to $1x10^{10}$ CFU/serving. *Lactiplantibacillus plantarum* MCC1 is not intended for addition to infant foods, such as formula, or products regulated by the USDA.

1.5 Basis for GRAS Status

The conclusion of GRAS status for the intended uses of *Lactiplantibacillus plantarum* MCC1 DSM 23881 is made through scientific procedures, in accordance with 21 CFR §170.30 (a) and (b).

1.6 Premarket Exempt Status

The notified substance is not subject to premarket approval requirements under the Food, Drug, and Cosmetic Act based on the conclusion that the substance is GRAS under the conditions of intended use.

1.7 Availability of Information

The data and information that serve as the basis for the conclusion for the GRAS conclusion will be available to the FDA for review and copying upon request either in paper or electronic format.

1.8 Freedom of Information Act Statement (FOIA)

The data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that are privileged or confidential. Therefore, none of the data and information presented herein are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. Section 552.

1.9 FSIS Statement

Not applicable

1.10 Certification and Signature

To the best of our knowledge, this GRAS notice is a complete, representative, and balanced compilation that includes all relevant information, both favorable and unfavorable, that are pertinent to the evaluation of the safety and GRAS status of *Lactiplantibacillus plantarum* MCC1 DSM 23881 under its intended use.

Signature of Notifier:

November 24, 2023

Marie-Eve Boyte
President
NutraPharma Consulting Services Inc

Date

2 IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT (21 CFR § 170.230)

2.1 Names of the GRAS Organism

Lactiplantibacillus plantarum MCC1 DSM 23881

2.2 Sources of the GRAS Organism

Lactiplantibacillus plantarum MCC1 was isolated from a one-year-old healthy child in 1995. The microbial ingredient was deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) culture collection under accession number DSM 23881 on 05/08/2010 under the Budapest Treaty.

2.3 Description of the GRAS Organism

2.3.1 Taxonomy and Origin

Scientific Classification

Domain: Bacteria
Phylum: Bacillota
Class: Bacilli
Order: Lactobacillales
Family: Lactobacillaceae
Genus: *Lactiplantibacillus*
Species: *Lactiplantibacillus plantarum*
Strain*: Lactiplantibacillus plantarum* MCC1

2.3.2 Phenotypic Identification

Lactiplantibacillus plantarum MCC1 is a non-motile, non-spore forming, rod-shaped, in pairs or in short chains, anaerobic, Gram-positive bacteria.
Lactiplantibacillus plantarum MCC1 is catalase and oxidase negative, facultatively heterofermentative, no gas production from glucose and no arginine hydrolysis.

Colonies of *L. plantarum* MCC1 on MRS agar after 48h of growth at 37°C in microaerobic conditions ($CO_2/O_2/N_2$: 10/5/85) are round, 2-2.5 mm of diameter, smooth, entire, convex, shiny, and creamy-white.

Carbohydrate utilization profile according to API 50 CHL system (bioMérieux, France)

Positive reaction for ribose, galactose, D-glycose, D-fructose, D- mannose, maltose, mannitol, sorbitol, N acetyl-glucosamine, amygdalin, arbutine, esculine, salicin, cellobiose, maltose, lactose, saccharose, trehalose, inositol, adonitol, melezitose, β-gentiobiose, D-turanose, 2 keto-gluconate. Weak reaction for starch.

Negative reaction for melibiose, gluconate, α methyl-D-mannoside , L- arabinose, D-raffinose, α methyl-D-glucoside, glycerol, erythrol, D-arabinose, D-xylose, L-xylose, β methyl-xyloside, L-sorbose, rhamnose, dulcitol, inulin, glycogen, xylitol, D-xylose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, , 5 keto-gluconate.

Enzyme activity according to API ZYM (bioMérieux, France)

Positive reaction for leucine arylamidase, valine arylamidase, α-glucosidase, and β-glucosidase. Negative reaction for acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, cystine arylamidase, esterase (C4), esterase (C8) and, N-acetyl- β-glucosaminidase.

2.3.3 Genotypic Identification

Confirmation of the identity of the microbial ingredient MCC1 was at the species level by whole genome sequencing (WGS) data analysis according to the guideline of EFSA (2021). WGS of the microbial ingredient *Lactiplantibacillus plantarum* MCC1 was performed by BGI Genomics.

The sequencing was performed by high throughput Illumina paired-end sequencing on the Miniseq platform. The genome was assembled using SPAdes (Prijbelski *et al*., 2020) ~~v~~ersion 3.15.4. The resulting genome is 3,273,561 bp with a GC% of 44.34%.

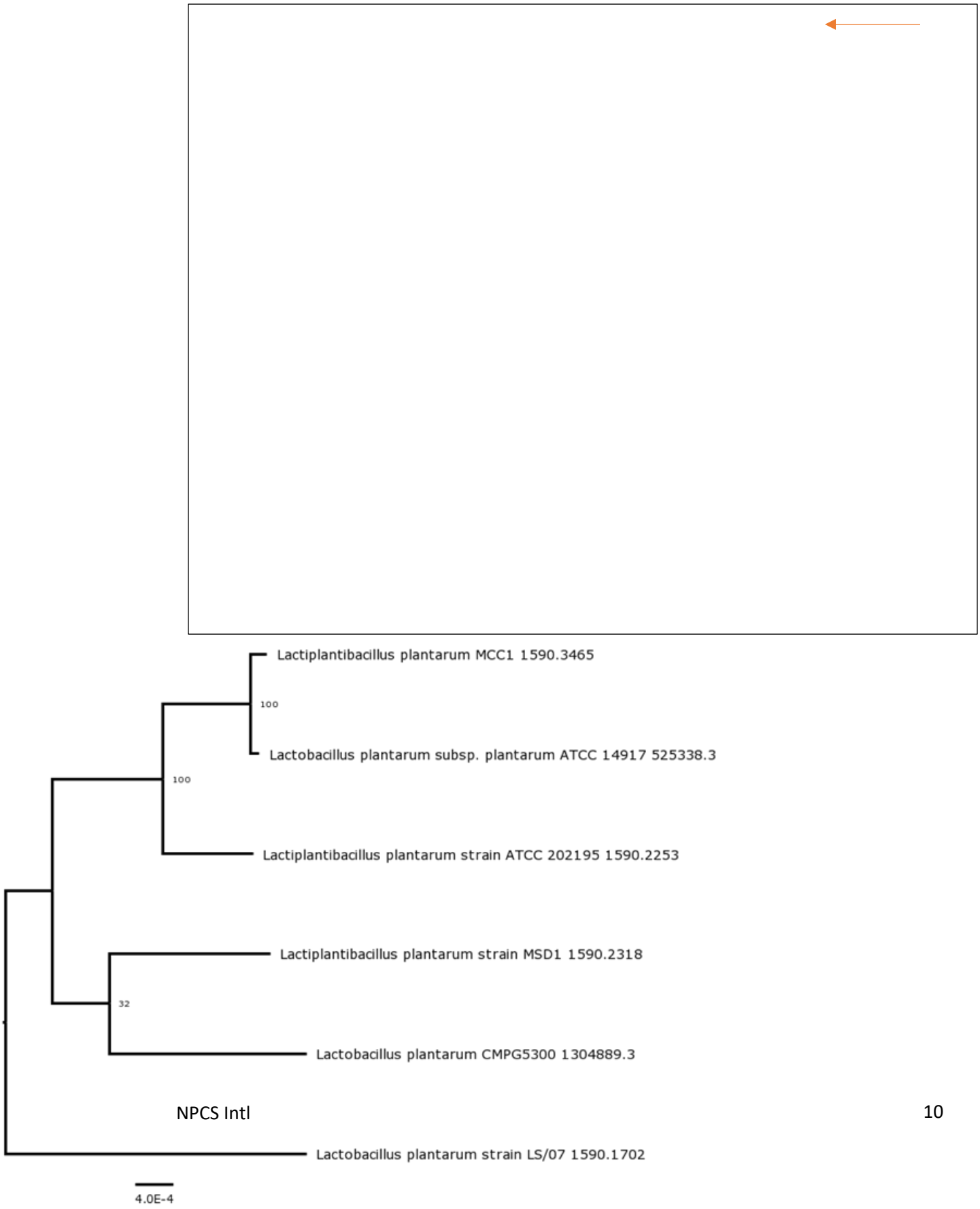
L. plantarum MCC1 taxonomic identity of *Lactiplantibacillus plantarum* was confirmed using Type (Strain) Genome server (TYGS) based on the digital DNA-DNA hybridization (dDDH) of the assembled genome to closely related strains (determined automatically by TYGS pipeline) according to Meier-Kolthoff and Göker (2019). The taxonomic identity of microbial ingredient MCC1 as *Lactiplantibacillus plantarum* is presented in Table 1.

Table 1. Taxonomic identity of MCC1 as *Lactiplantibacillus plantarum*

Query strain	**Subject strain**	**dDDH (d0, in %)**	**C.I. (d0, in %)**	**dDDH (d4, in %)**	**C.I. (d4, in %)**	**dDDH (d6, in %)**	**C.I. (d6, in %)**	**G+C content difference (in %)**
MCC1	*Lactiplantibacillus plantarum* DSM 20174	95.6	[93.5 – 97.1]	98.9	[98.3 – 99.3]	97.6	[96.3 – 98.4]	0.2
MCC1	*Lactiplantibacillus plantarum* ATCC 14917	95	[92.7-96.6]	98.9	[98.3 – 99.3]	97.2	[95.8 – 98.1]	0.18
MCC1	*Lactobacillus arizonensis* DSM 13273	84.2	[80.4 – 87.3]	94	[92.2 – 95.4]	88.6	[85.7 – 91.0]	0.06
MCC1	*Lactiplantibacillus argentoratensis* DSM 16365	76.7	[72.7 – 80.2]	62.9	[60.0 – 65.7]	76.6	[73.1 – 79.7]	0.61
MCC1	Lactiplantibacillus paraplantarum DSM 10667	52.3	[48.8 – 55.8]	31.2	[28.8 - 33.8]	48.4	[43.4 – 49.4]	0.61

Using the Pathosystems Resource Integration Center (PATRIC), the whole genome sequence from *L. plantarum* MCC1 was compared against whole genome sequences from different strains of *Lactiplantibacillus plantarum* and closely related species (Davis et *al* 2020). Figure 1 shows that MCC1 clusters with other strains of *Lactiplantibacillus plantarum*, including type strain ATCC 14917 confirming that MCC1 is a *Lactiplantibacillus plantarum*.

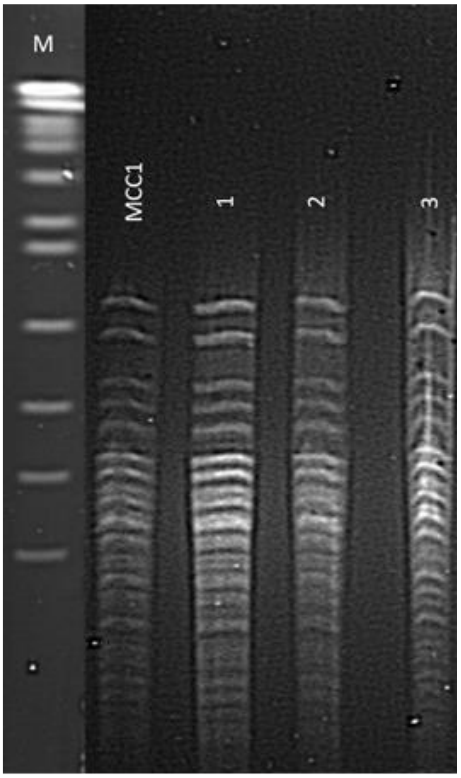
Figure 1: Phylogenic tree based on whole genome sequence.



2.3.3.1 Genomic stability

The genomic stability of *L. plantarum* MCC1 was demonstrated by comparing the DNA fingerprints of deposited microorganism (from August 5, 2010) of the BioCC OÜ culture collection with isolates from randomly selected freeze-dried batches of MCC1 produced by BioCC OÜ of batch 10-44 from 19.05.2016, batch 10-155 from 09.06.2016 and batch MM-22-003 from 17.01.2022. The DNA fingerprints were obtained with pulse field gel electrophoresis (PFGE) (Figure 2)

Figure 2: PFGE Profiles of *L. plantarum* MCC1



Legend :

PFGE profiles of isolates:
MCC1: original deposited microorganism - 2010
1: Freeze-dried lot 10-44 - 2016
2: Freeze-dried lot 10-155 - 2016
3: Freeze-dried lot MM-22-003 - 2022
M: marker (1kb)

Identical patterns with original *L. plantarum* MCC1 from the deposited microorganism and from the seedbank demonstrate the genome stability of the microbial ingredient.

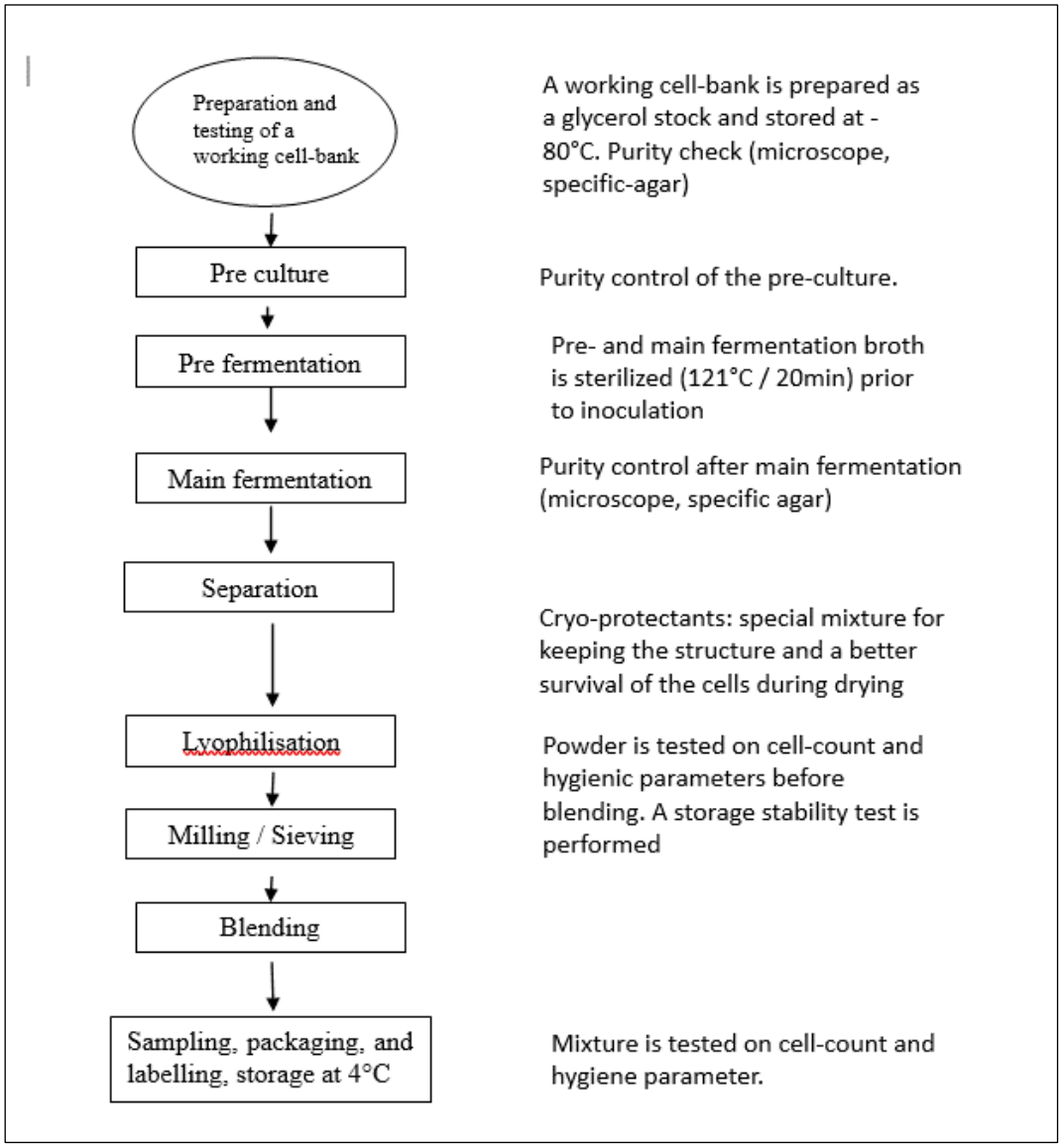
2.4 Production Process

The freeze-dried culture of the microbial ingredient *L. plantarum* MCC1 is manufactured in a production facility with fully implemented general principles of food hygiene (GMP) including HACCP plan, standard operating procedures incl. quality control to ensure the product quality. The production facility has implemented the food safety management system which complying the requirements of standard ISO 22000:2018 (Registration No.: 00590/0).

The company have applied General Principles of Food Hygiene (GMP) incl. HACCP hazard analysis and Critical Control Points in conformity with requirements in document since 1969, last reviewed in 2020 (valid until 26.09.2025).

The general outline of the manufacturing process for the microbial ingredient is presented in the flow chart in Figure 3.

Figure 3. Production flow chart for *Lactiplantibacillus plantarum* MCC1



Manufacturing Process brief description:

The microbial ingredient *L. plantarum* MCC1 culture is maintained in the production facility microbial culture collection MC as master seed which is operated according to written procedures.

The storage conditions employed have proven to ensure both genetic and physiological stability. Each master seed and working cell-bank vial is labelled with an internal microbial culture collection and a batch number. From the master seed, a working cell-bank is prepared as a glycerol stock and stored at -80°C. Identification of the microbial during the working cell-bank production is confirmed by purity control (microscope, specific-agar etc.).

Preparation and sterilisation of the fermentation broth ingredients: All ingredients used in fermentation broth are suitable for human consumption.

The microbial quality of the fermentation medium is checked before inoculation the pre-culture into fermenter. Pre-culture preparation by inoculating into sterile LAB media and incubating for 16 h in a microaerobic environment.

The pH is controlled during fermentation by the addition of food-grade ammonium hydroxide to maintain a fixed pH. The fermentation process is checked for purity after main fermentation (microscope, specific agar).

Fermentation is terminated by cooling the fermenter. After fermentation, the bacterial cells are collected and concentrated by nozzle centrifuge.

The concentrated bacterial cells are mixed with cryoprotectants. All ingredients used in cryoprotectants are suitable for human consumption.

The concentrated bacterial cell mass undergoes lyophilization process for approximately 48 hours in clean-room area. The lyophilized bacterial culture is ground and sieved into a fine powder, packaged into clean tightly closed containers, labelled, and stored at 4 °C. The milled powder is blended with excipients to achieve the necessary viable cell count. Each batch undergoes quality control including hygiene parameters and cell-count.

2.5 Specifications

2.5.1 Specifications

Before release, each batch of the freeze-dried microbial ingredient undergoes the microbiological purity. Food grade specifications have been established and are summarized in Table 2.

Table 2: Specifications of *Lactiplantibacillus plantarum* MCC1 powder

Parameter	Specification	Method
Bacterial Count	≥ 4.0 x 10^{11} CFU/g	EVS-EN 15787:2021
Coagulase-positive staphylococci	< 1.0 x 10^{1} CFU/g	EVS-EN 6888-1:2021
Yeasts & Molds	< 1.0 x 10^{1} CFU/g	EVS-ISO 21527-2:2009
Enterococci	< 1.0 x 10^{1} CFU/g	ISO 7899-2:2002 – modified
Enterobacteriaceae	< 1.0 x 10^{1} CFU/g	EVS-EN 21528-2:2017
Coliforms	< 1.0 x 10^{1} CFU/g	EVS-EN ISO 4832:2010
Listeria monocytogenes	n.d. in 25 g	EVS-EN ISO 11290-1:2017
Salmonella spp	n.d. in 25 g	EVS-EN ISO 6579-1:2017
Heavy Metals		
Lead	< 0.1 ppm	ICP-MS EVS-EN ISO 17294-2:2016
Mercury	< 0.1 ppm	
Cadmium	< 0.1 ppm	
CFU = colony forming unit	n.d. = not detected	

2.5.2 Batch Analysis

Tables 3 and 4 show the microbiological and heavy metals analysis results for three non-consecutive lots of the microbial ingredient.

Table 3: Microbiological analysis of three lots of the notified microbial ingredient

Parameters	Specifications	10-156	10-174	10-231
Bacterial Count	≥ 4.0 x 10^{11} CFU/g	≥ 4.0 x 10^{11} CFU/g	≥ 4.0 x 10^{11} CFU/g	≥ 4.0 x 10^{11} CFU/g
Coagulase-positive staphylococci	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g
Yeasts & Molds	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g
Enterococci	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g
Enterobacteriaceae	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g
Coliforms	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g	< 1.0 x 10^{1} CFU/g
Listeria monocytogenes	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g
Salmonella spp	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g	n.d. in 25 g

Table 4: Heavy metal analysis of three lots of the notified microbial ingredient

Lead	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
Mercury	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
Cadmium	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm

2.6 Stability

A stability study was conducted on two random lots packaged in aluminium foil pouches to assess the viability of the cells during the storage at -18ºC over 24 months. The stability study is ongoing for storage at 4°C and 20°C.

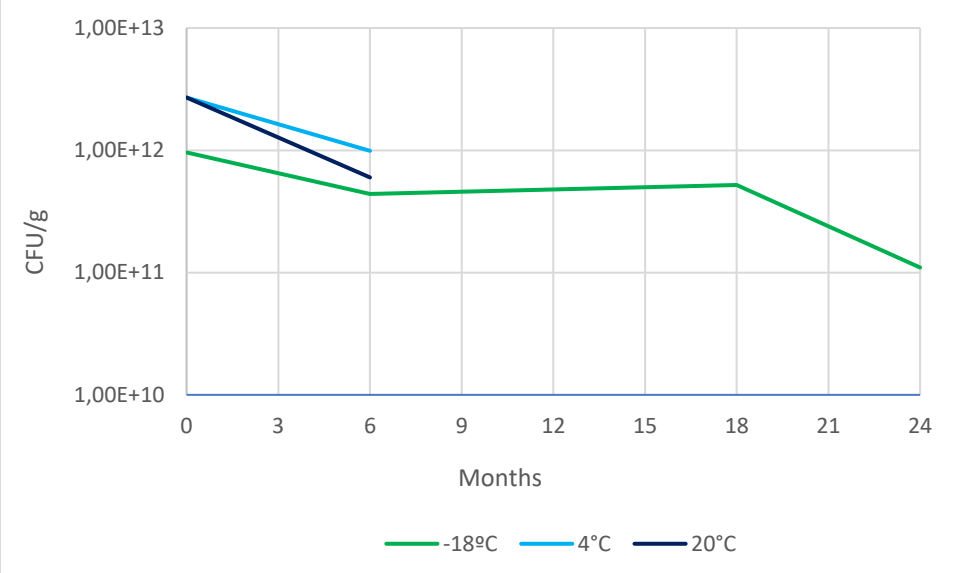


Figure 4: Microorganism viability at different temperatures (unpublished).

3 INTENDED USE AND DIETARY EXPOSURE (21 CFR § 170.235)

L. plantarum MCC1 is intended to be used as a microbial ingredient in conventional foods, such as, but not limited to, dairy products (cheese, yoghurt, sour cream, cottage cheese, processed cheese, soft ice cream etc), fruit and vegetable juices, water, confectionary, dairy alternatives (e.g., plant - based vegan products), shelf-stable products (bars) and functional and nutritional products when not precluded by a standard of identity, at levels consistent with current good manufacturing practice (cGMP). It is not intended to be added to infant formula, other products intended for infants or foods regulated by the U.S Department of Agriculture.

Under the intended conditions of use, the maximum level of the microbial ingredient incorporated in the conventional foods will be $5x10^8$ CFU per serving, taking into consideration the viability loss throughout the shelf life of the foods. A healthy individual consumes an average of 20 servings of food per day (Health Canada 2011, NHMRC, USDA 2020). If all the combined foods contain the microbial ingredient, the maximum daily exposure to microbial ingredient from all food sources is approximated to $1x10^{10}$ CFU. Such estimated dietary exposure under the intended use conditions is conservative as it assumes no viability loss of the microbial ingredient during storage and freight. Furthermore, the dietary exposure of $1x10^{10}$ CFU is lower than the highest concentration showing no adverse effects in human clinical studies for other *L. plantarum* (Ducrotte et al 2012, Songisepp et al 2012a,b, Sharafedtinov et al 2013, Hütt et al 2015, Huang et al 2018). When compared, the expected dietary intake of *L. plantarum* MCC1 under the specified conditions aligns with the consumption of other *L. plantarum* mentioned in GRAS notices (CFSAN 2023, CFSAN 2019, CFSAN 2018, CFSAN 2017) that received an FDA letter of "no questions."

4 SELF-LIMITING LEVELS OF USE (21 CFR § 170.240)S

This part does not apply

5 EXPERIENCE BASED ON COMMON USE IN FOOD (21 CFR § 170.240)

This part does not apply since the GRAS conclusion is based on scientific procedures and not on common use in foods prior 1958.

6 NARRATIVE (21 CFR § 170.250)

6.1 History of safe use in foods

Lactic acid bacteria (LAB) are commonly found in human commensal microflora and are omnipresent in fermented and non-fermented foods (Adams 1999). Food fermentation for food preservation has been around for many centuries (Adams 1999, Behera et al. 2018, Bourdichon et al. 2012). In the past, fermented foods were naturally fermented by indigenous strains without deliberate intervention. However, over the last century, the practice of using inoculation materials containing specific fermenting microorganisms has become well-established (Mogensen 2002). In today's world, fermentation plays many different roles in food processing in addition to food preservation such as improving food safety and nutritional value and organoleptic quality of food (Bourdichon et al. 2012).

Lactic acid bacteria (LAB) are ingested in substantial quantities, predominantly through the consumption of fermented foods. Lactobacilli constitute the dominant microbiota in many fermented foods (Rossi et al. 2022) In Europe, the average annual consumption of fermented milk products per capita is 22 kg. This equates to a total of approximately 8.5 billion kg of fermented milk consumed each year. Given the average microbial content of 10^8 bacteria per gram or milliliter in these fermented products, the overall intake amounts to a staggering total of $8.5x10^{12}$ lactic acid bacteria (Mogensen 2002).

L. plantarum is found in many different fermented foods including sourdoughs, fermented plant material, fermented sausages, stockfish, and fermented dairy. This species is responsible for the majority of fermented vegetables, as it possesses the capability to withstand the high levels of salinity and acidity present in these foods, particularly in cucumber, sauerkraut, and olives (Behera 2018). Furthermore, *L. plantarum* is recognized as a suitable starter cultures, contributing to the production of a diverse range of fermented vegetable products such as sweet potato, pickled vegetables, cereals such as bran, rice, and wheat (Behera et al. 2018, Bested et al. 2013, Wang et al. 2020). Additionally, *L. plantarum* frequently occurs spontaneously in plant based fermented foods such as brined olives, capers, sauerkraut, salted gherkins, and sourdough (Katcho 2019, Bernardeau 2006). Its wide dispersion indicates that individuals consuming such products also consume large amounts of *L plantarum* (Katcho 2019).

LAB are also involved in fermented meats such as sausage (Behera 2018, Corsetti et al 2011). This species has demonstrated its dominance in numerous traditionally fermented sausages worldwide, spanning regions such as the Mediterranean, Asia, South America, and Africa. The characterization of the prevalent *L. plantarum* strains from fermented sausages has identified many strains that are proposed as potential functional starter cultures, possessing desirable technological and safety attributes. Among the extensively studied properties of these indigenous *L. plantarum* strains is their ability to produce bacteriocins known as plantaricins—antimicrobial peptides that can be applied in various food matrices to reduce sensitive bacteria, including foodborne pathogens or spoilage bacteria. The use of starter cultures or protective cultures is a widely adopted strategy to enhance the safety and quality of fermented sausages (Behera 2018).

LAB play an important role throughout the fermentation process of traditional cheeses (Coelho et al 2022). *L. plantarum* is found in different cheeses such as Emmental, batzos, mozzarella, ricotta, Italian grana, fioro sardo and ripened cheese (Coelho et al 2022, Bernardeau 2006). *L. plantarum* can reach levels up to 10^7 CFU per gram during prolonged aging of the cheese (Behera et al. 2018, Corsetti et al. 2011, Kacho 2019).

L. plantarum was listed in the initial "Inventory of Microorganisms with a Documented History of use in Foods", a list compiling microorganisms for safe use in foods, published by the International Dairy Federation (IDF) and done in collaboration with the European Food and Fed Cultures Association (EFFCA) in 2002 (Mogensen 2002) and is still present on the current list (Bourdichon et al 2022).

Lactiplantibacillus plantarum MCC1 is currently sold in international markets. It is added in a starter culture kit for home made sour yoghurt and fermented vegetable acidifed drink. For both products, one sachet contains $1x10^6$ CFU viable freeze-dried cells, which generates one liter of sour yoghurt or vegetable acidified drink (Nordwise® juuretiste tootesari). It is also sold internationally as a dietary supplement with a concentration of 2.5×10^9 CFU per capsule (Flexellent from Nordwise®) and 1×10^9 CFU per capsule (PlantaBif® from Nordwise®)

Other *Lactiplantibacillus plantarum* are available in the United States and the rest of the world. As mentioned in GRN 722, *L plantarum* Lp-115 has been commercially available for a minimum of 20 years (CSFAN 2018). And *L. plantarum* 299v has been sold worldwide since 1994 (CFSAN 2017).

6.2 Antibiotic resistance

6.2.1 Phenotypic analysis

The antibiotic resistance profile of *L. plantarum* MCC1 was tested in accordance with the ISO 10932 standard using microdilution plate panels (VetMIC™ panels Vet-Mic Lact-2 ja Lact-1, SVE, Sweden). The minimum inhibiting concentration was determined according to the epidemiological cut-off values recommended to antimicrobials of human and veterinary importance suggested by the European Food Safety Authority (EFSA, 2018b).
A microbial strain is considered susceptible when it is inhibited at an equal or lower concentration to the cut-off value of the specific antimicrobial compound (S ≤ × mg/L). A microbial strain is considered resistant when it is inhibited at a higher concentration than the cut-off value of the specific antimicrobial compound (R > × mg/L).

Table 5: Antibiotic resistance profile for *L. plantarum* MCC1

Antimicrobial agent	EFSA cut-off values (µg/ml)	*L. plantarum* MCC1 (µg/ml)
Ampicillin	2	0.12
Vancomycin	n.r.	n.r.
Gentamicin	16	0.5
Kanamycin	64	16
Streptomycin	n.r.	n.r.
Erythromycin	1	0.06
Clindamycin	4	0.25
Tetracycline	32	16
Chloramphenicol	8	4
Ciprofloxacin	n.r.	n.r.

n.r. Not required

L. plantarum MCC1 shows no phenotypic resistance against the tested antibiotics.

6.2.2 Genotypic analysis

The whole genome sequence of the microbial ingredient was screened for the presence of genes of potential concern for safety, including genes coding for or contributing to resistance to antimicrobials relevant to their use in humans and animals, using ResFinder, Comprehensive Antibiotic Resistance Databases.

ResFinder is a database that captures antimicrobial resistance genes from whole-genome sequence. The database uses BLAST to accomplish the screening and analysis. Whole genome sequence antimicrobial susceptibility testing using ResFinder 4.1 provides *in silico* antibiograms as reliable as those obtained by phenotypic antimicrobial susceptibility testing at least for the bacterial species/antimicrobial agents of major public health relevance considered (Bortolaia et *al* 2020, Zankari et *al* 2020, Clausen et *al* 2018, Camacho et *al* 2009).

The Comprehensive Antibiotic Resistance Database (CARD) is a bioinformatic database of resistance genes, their products, and associated phenotypes. It is a rigorously curated collection of characterized, peer-reviewed resistance determinants and associated antibiotics, organized by the Antibiotic Resistance Ontology (ARO) and AMR gene detection models. The CARD includes tools for analysis of molecular sequences, including BLAST and the

Resistance Gene Identifier (RGI) software for prediction of resistome based on homology and SNP models (Alcock et *al* 2023).

The whole genome screening of microbial ingredient using both databases did not reveal any candidates for clinically relevant antimicrobial genes.

Based on the whole genome sequence analysis, *L. plantarum* MCC1 does not harbour any acquired antimicrobial resistance genes.

6.3 Production of antimicrobial substances

The antimicrobial activity was tested *in vitro* on modified agar and the level of inhibition was determined by the growth inhibition zone of the pathogen.

Table 6: *Lactiplantibacillus plantarum* MCC1 antimicrobial activity against pathogens on modified MRS agar medium (pathogen growth inhibition zone, mm)

Pathogen	**Growth inhibition zone (mm)**
Listeria monocytogenes	12.8 ± 1.7
Yersinia enterocolitica	12.8 ± 1.0
Salmonella enteritidis	7.1 ± 2.8
S. typhimurium	17.8 ± 1.8
Shigella sonnei	20.3 ± 2.1
Escherichia coli	18.0 ± 1.0
Enterococcus faecalis	14.7 ± 2.7
Staphylococcus aureus	15.0 ± 1.0

L plantarum MCC1 expresses antagonistic activity against several enteric pathogens with the highest against *Shigella, E. coli*, *Salmonella typhimurium* and followed by *Staphylococcus aureus* and *Enterococcus faecalis.* This property enables to use the microbial ingredient for prolongation of shelf-life of food products which is in line with what is described in the literature.

6.4 Production of Biogenic amines

The whole genome was screened for the presence of encoding biogenic amines production genes. Since biogenic amines are mainly generated by decarboxylation of amino acids, the genome was screened for the presence of decarboxylases required to generate cadaverine, putrescine, histamine, and tyramine. The genome of the microbial ingredient *L plantarum* MCC1 does not contain decarboxylases for cadaverine, putrescine, histamine, and tyramine.

The amounts of biogenic amines produced by *Lactiplantibacillus plantarum* MCC1 do not pose a threat to human health.

6.5 Pathogenicity and toxicity

Whole genome sequence of the microbial ingredient was screened for the presence of genes of potential concern for safety, including genes coding for, for virulence and pathogenicity factors.

The whole genome was examined to detect virulence factors using the online tool PATRIC which includes PATRIC VF, VFDB and Victors curated databases (Davis et *al* 2020), and VirulenceFinder, a bioinformatic tool identifying acquired virulence genes by analysing the input genome (Camacho et *al*. 2009, Clausen et *al* 2018, Joensen et *al*. 2014, Malberg Tetzschner AM et *al* 2020). There were no hits for virulence factors in both platforms.

The bioinformatic tool PathogenFinder 1.1 is a validated tool for the prediction of bacterial pathogenicity by analysing the input proteome, genome, or raw reads. The method relies on groups of proteins, created without regard to their annotated function or known involvement in pathogenicity. The method has been built to work with all taxonomic groups of bacteria. The approach here proposed is not biased on sets of genes known to be associated with pathogenicity. This platform allows to predict the pathogenicity of a microorganism (Consentino et al. 2013). *L. plantarum* MCC1 was predicted as non human pathogen.

Hemolysin is also an important virulence factor in many *Escherichia coli* strains including the O157 strain. In hemolytic *E. coli*, the hemolysin determinant is composed of four genes: *hlyC, hlyA, hlyB* and *hlyD* (Koronakis 2002). While in hemolytic streptococci, beta-hemolysis (β-hemolysis) is caused by two hemotoxins: Streptolysin S and Streptolysin O (Nizet). β-hemolysis is defined as clear and complete lysis of red blood cells surrounding the colony on blood agar (buxton 2005 Patterson 1996).

The whole genome sequence of the microbial ingredient was screened for the presence of hemolysins *hlyC, hlyA, hlyB, hlyD, sagA* and *slo* using PATRIC (Davis et *al* 2020). *L. plantarum* MCC1 came up negative for the presence of hemolysins.

Lactiplantibacillus plantarum MCC1 was tested for its lack of hemolytic activity to further demonstrate its safety.

L. plantarum MCC1 was grown in MRS media for 48 h and streaked onto blood agar plates containing either human or sheep blood. Hemolysis was evaluated after 24 and 48 h of incubation in aerobic, microaerobic (10% CO2), and anaerobic (90% N2, 5% CO2, 5% H2) environments. *L. plantarum* MCC1 did not cause the lysis of erythrocytes of human and sheep blood in aerobic, microaerobic, or anaerobic environments.

Based on the whole genome sequence analysis, *L. plantarum* MCC1 does not harbour virulence genes or factors, including hemolysins, and is not predicted to be pathogenic to humans.

6.6 Preclinical Studies

6.6.1 *In vivo* animal trials

An *In vivo* study for *Lactiplantibacillus plantarum* MCC1 was conducted on mice to assess the safety of the microbial ingredient. The mice from the test group were fed the microbial ingredient *Lactiplantibacillus plantarum* MCC1 at a daily dose of 2 x 10^{8} CFU for 30 consecutive days. No translocation of the administered microbial ingredient or other microbes into the blood or organs was detected. The heart blood, liver, kidney, and lung samples obtained at autopsy were sterile in all mice. No pathological shifts and no micro abscesses, granulomas, or inflammation were found by morphological and histological evaluation of the spleen, liver, ileum, and colon of mice (data not published).

6.7 Clinical Data

6.7.1 Studies Conducted with *Lactiplantibacillus plantarum* MCC1

Different clinical trials were conducted with *Lactiplantibacillus plantarum* MCC1.

Kullisaar et al (2013) conducted a randomized double-blind control group study to investigate the effect of pasteurized whey fermented with *L. plantarum* MCC1 and *L. gasseri* MCC2 on lower urinary tract symptoms (LUTS) in men with benign prostatic hypertrophy. Fifty-eight male subjects of 55 ± 5 years of age were randomized into 2 groups: the control group (n=19), consumed 200 g of apple juice per day, and the intervention group (n=39) consumed 50 g/day of yellow pasteurized fermented whey product, for 2 weeks. There was a correlation between LUTS symptoms change and level of inflammation and oxidative stress-related indices. Statistically significant changes in mentioned parameters occurred only in study group. There were no adverse events reporting in the study.

Ausmees et al (2018) a randomized double-blind placebo-controlled study to investigate the effect of pasteurized whey fermented with *L. plantarum* MCC1 and *L. gasseri* MCC2 on quality of life of males with moderate lower urinary tract symptoms. Forty-six male subjects were randomized into 2 groups: the control group (n=23), consumed 200 g of orange-peach drink per day, and the intervention group (n=23) consumed 200g of liquid pasteurized fermented whey product containing *L. plantarum* MCC1 and *L. gasseri* MCC2, for 4 weeks. Statistically significant changes in mentioned parameters occurred only in study group. The consumption of of fermented whey product for 4 weeks contributed to improve the quality of life in men with moderate lower urinary tract symptoms. There were no adverse events reporting in the study.

TABLE 7: Human clinical studies conducted with *Lactiplantibacillus plantarum* MCC1

Study Design and Objective	Subjects	Composition and Dosage	Duration	Safety end point results	Reference
Randomized double-blind placebo-controlled study	Fifty-eight male subjects of 55 ± 5 years of age with benign prostatic hypertrophy	Intervention: 50g of yellow pasteurized fermented whey product containing *L. plantarum* MCC1 and *L. gasseri* MCC2 Control: 200g of apple juice	2 weeks	There were no adverse events reporting in the study	Kullisaar et al (2013)
Randomized double-blind placebo-controlled study	Forty-six male subjects; 23 in the intervention arm and 23 in the control arm.	Intervention: 200g of liquid pasteurized fermented whey product containing *L. plantarum* MCC1 and *L. gasseri* MCC2 Control: 200g of orange-peach drink	4 weeks	There were no adverse events reporting in the study	Ausmees et al (2018)

6.7.2 Studies Conducted with Other *Lactiplantibacillus plantarum*

Table 8 lists human clinical studies with other *Lactiplantibacillus plantarum*.

Table 8: Human clinical studies conducted with other *Lactiplantibacillus plantarum*

Study Design and Objective	Subjects	Composition and Dosage	Duration	Safety end point results	Reference
Double-blind placebo-controlled cross-over study	Thirteen healthy adult subjects (5M and 8F, 29.1 ± 7.7 years),	Intervention: 50 g of test cheese containing 2.5 x 10^{10} CFU of *L. plantarum* TENSIA® Control: 50 g of cheese (without *L. plantarum* TENSIA®)	Consumption of the cheese containing the microbial ingredient daily for 3 weeks 2-week washout period Consumption of the control cheese daily for 3 weeks	12 subjects completed the study No adverse gastro-intestinal effects (negative shifts in general well-being, appetite, stool consistency and stool volume, no flatulence, stomachache, or bloating) were reported. No increased inflammatory indices (white blood cell count and U-CRP) were found after the 3-week treatment. No adverse side effects were reported.	Songisepp et al 2012 (a, b)
Double-blind placebo-controlled cross-over study health markers in healthy elderly participants	Twenty-one healthy elderly subjects (1M, 17F, 69.8 ± 5.5 years)	Intervention: 50 g of test cheese containing 1 x 10^{9} of *L. plantarum* TENSIA®	Consumption of the cheese containing the microbial ingredient daily for 3 weeks	18 subjects completed the study There were no abdominal discomfort	Songisepp et al 2012 (a, b)

		<u>Control:</u> 50 g of cheese (without *L. plantarum* TENSIA®)	2-week washout period Consumption of the control cheese daily for 3 weeks	(abdominal pain, flatulence, bloating), no increased inflammatory indices (white blood cell count and U-CRP and leucocytes), no allergenic sensibilization or cause harm to essential organs reported found after the 3-week treatment	
Open-label intervention study to investigate the safety and dose-response effects of semihard Edam-type cheese containing *L. plantarum* TENSIA® in healthy adults	Twenty-nine healthy adult subjects (8M, 18F, 37.2 ± 10.7 years)	<u>Intervention:</u> 100 g of test cheese containing 5 x 10^{10} of *L. plantarum* TENSIA®	3 weeks	26 subjects completed the study Single cases of abdominal pain, flatulence, bloating of their combination were observed but it was determined that these were caused by the excess intake of cheese and not the microbial ingredient	Songisepp et al 2012 (a)
Double-blind placebo-controlled cross-over study	Eighty-two healthy adult subjects (33M, 49F, 37.7 ± 11.1 years)	<u>Intervention:</u> 50 g of test cheese containing a dose of 1 x 10^{10} CFU of *L. plantarum* TENSIA®	Daily consumption of the cheese containing the microbial ingredient /or placebo for 3 weeks	There were no adverse events reporting in the study	Hütt et al 2015

		Control: 50 g of control cheese (placebo)	2-week washout period Daily consumption of the cheese containing the microbial ingredient /or placebo for 3 weeks		
Double-blind placebo-controlled cross-over study	Forty-three healthy adult subjects (11M, 32F, 34.2 ± 11.5 years)	Intervention: 150 g of yogurt containing a dose of 6×10^9 CFU of *L. plantarum* TENSIA® Control: 150 g of control yogurt (placebo)	Daily consumption of the yogurt containing the microbial ingredient /or placebo for 3 weeks 2-week washout period Daily consumption of the yogurt containing the microbial ingredient /or placebo for 3 weeks	There were no adverse events reporting in the study	Hütt et al 2015
Randomized double-blind placebo-controlled pilot study	Forty adults with metabolic syndrome between 30-69 years Intervention group: 9M, 16F, 52 ± 10.9 years, BMI 37.7 ± 4.3 Control group:	Intervention: 50 g of test cheese containing 1.5×10^{11} CFU/g of *L. plantarum* TENSIA® before renneting of the cheese	3 weeks	The consumption of Cheese with the microbial ingredient did not cause any adverse effects aside from some temporary constipation, which occurred at	Sharafedtinov et al 2013

	4M, 11F, 51.7 ± 12.1 years, BMI 36.3 ± 4.1	Control: 50 g of control cheese (placebo)		similar rates in the two groups	
Randomized, double-blind, placebo-controlled study	Sixty subjects (34M and 26F, 51.8 ± 7.2 years) with 30 in the placebo and intervention arms.	Oral capsule Intervention: Capsule containing *L. plantarum* KABP-011, KABP-012, and KABP-013 in a 1:1:1 ratio at a concentration of $3.01x10^9$ cfu at the start of the study then reduced to $1.28x10^9$ cfu at the end of the study Control: Placebo capsule	1 capsule daily for 12 weeks 4-week follow-up	All subjects completed the study. There were no treatment related adverse events observed.	Fuentes et al., 2013, 2016
Observational study	343 subjects (median age of 55 years, 63% female)	Oral capsule Intervention: Capsule containing *L. plantarum* KABP-011, KABP-012, and KABP-013 in a	1 capsule daily for 12 weeks	No adverse events were reported.	Espadaler et al., 2019

		1:1:1 ratio at a concentration of $1.2x10^9$ cfu			
Randomized, double-blind, placebo-controlled study	16 healthy males aged 20-40 years, eight were in each arm.	Oral capsule Intervention: Capsule containing *L. plantarum* TWK10 at a concentration of 1×10^{11} cfu Control: Placebo capsule	1 capsule daily for 6 weeks	There was no discussion of adverse events.	Huang et al., 2018
Randomized, prospective, placebo-controlled study	46 adults with 23 (5M, 18F; 52.3±10.7 years) in the intervention arm and 23 ((M,14F; 52.0 ±8.4) in the placebo arm.	Oral capsule Intervention: Capsule containing *L. plantarum* ECGC 13110402 at a concentration of $2x10^9$ cfu Control: Placebo capsule	2 capsules daily for 12 weeks	There was no discussion of adverse events	Costabile et al., 2017
Multi-center, randomized, double-blind, placebo-controlled study	214 subjects with 108 (70M, 38F; mean age 36.5± 12.1 years) in the intervention arm;	Oral capsule Intervention: Capsule containing *L. plantarum* 299v at	1 capsule daily for 4 weeks	No significant side effects were reported in the study. One participant reported a a transient vertigo	Ducrotte et al., 2012

	106 (81M, 25F, mean age 38.4±13.1 years) in the placebo arm.	a concentration of $1x10^{10}$ cfu Control: Placebo capsule (potato starch, magnesium stearate)		onset in the intervention group	
Randomized two-armed double-blind placebo-controlled study	One-hundred-thirty-two (132) adult subjects (48.2 ± 11.0 years)	Intervention: 150 g of yogurt containing 2x 10^9 CFU of *L. plantarum* INDUCIA® Control: 150 g of control yogurt (without *L. plantarum* INDUCIA®)	150g daily of test yogurt or control yogurt for 8 weeks	One-hundred and five (105) volunteers completed the study, and 10 participants were withdrawn from the analysis due to various reasons There were no adverse events reporting in the study	Stsepetova et al (2022)
Randomized double-blind controlled crossover intervention study	Sixty healthy adult subjects (36.9 ± 12.4 years)	Intervention: 150 g of yogurt containing 6 x 10^9 CFU of *L. plantarum* INDUCIA® Control: 150 g of control yogurt (without *L. plantarum* INDUCIA®)	150g daily of test/control yogurt for 3 weeks 2-week washout period 150g daily of test/control yogurt for 3 weeks	49 subjects completed the study No discomfort (flatulence, bloating), abdominal pain, or other negative symptoms were reported during the study.	Songisepp et al (2022)

Randomized double-blind placebo parallel two-armed study	One-hundred-fifty (150) healthy adult subjects (42.7 ± 11.9 years)	<u>Intervention:</u> 150 g of yogurt containing 5.9 x 10^9 CFU of *L. plantarum* INDUCIA® <u>Control:</u> 150 g of control yogurt (without *L. plantarum* INDUCIA®)	150g daily of test/control yogurt or control (placebo) yoghurt 8 weeks	One-hundred-thirty-six (136) completed the study No discomfort (flatulence, bloating), abdominal pain, or other negative symptoms were reported during the study.	Stsepetova et al (2022)

6.8 Adverse events reporting

Lactobacilli cause rare infections (Rossi et al 2022). The prevalence of lactobacilli bacteremia is generally low, comprising 0.1–0.2% of all isolates identified in blood cultures from hospitalized patients and 0.5% of blood isolates from immunocompromised patients (Kullar et al 2023).

Literature reviews were done on the potential pathogenicity of lactobacilli bacteremia. One survey reviewed the literature form 1980 to 2023 with search terms including Lactobacillus, bacteremia, probiotics, risk factors, treatment, antibiotic susceptibilities, safety, epidemiology, sepsis, endocarditis, and infection. Inclusion criteria included case reports, case series, observational studies, and reviews, while exclusion criteria included studies on non-*Lactobacilli* bacteremias, fungemias, or other types of diseases. Reviews and published papers were also inquired (Kullar et al 2023). It turns out that *Lactiplantibacillus plantarum* is one of the species less frequently reported as an infection agent (Kullar et al 2023).

Another account from this review detailed a group of six pediatric hematopoietic cell transplant recipients (ranging from 10 months to 16 years old) admitted to an intensive care unit who experienced lactobacilli bacteremia and had been prescribed probiotics. A potent probiotic, containing seven types of bacteria (*Strept. thermophilus*, *Bifido. breve*, *Bifido. lactis*, *L. acidophilus*, *Lpb. plantarum*, *Lcb. paracasei*, and *L. helveticus*) at a high dosage (1×10^{11} bacteria/day), was administered to 34 patients during their hospitalization to prevent nosocomial infections. Between January 2017 and December 2019, 17.6% of children receiving the probiotic developed lactobacilli bacteremia (4.7/1000 patient-days). After employing core-genome sequence typing to identify blood isolates, three patients had isolates matching at least one bacterial species present in the oral probiotic blend (Lcb. paracasei in one case and Lpb. plantarum in two cases). Unfortunately, the specific strains and the proprietary blend's identity were not disclosed (Gillam et al 2022, Kullar et 2023).

Rossi et al. also conducted a survey to reevaluate the pathogenic potential of lactobacilli based on the infection case reports published over a three-year period, 2019 to 2021 inclusively (Rossi et al 2022). These were retrieved from scientific literature databases by using the search terms “Lactobacillus”, or any of the names of the genera newly classified in 2020 and followed by the terms “infection” or “bacteremia”, or “endocarditis”, or “abscess”. A total of 48 cases were reported for those three years, but only three pertained to *L. plantarum* (Rossi et al 2022). The reported cases involved individuals experiencing opportunistic infections, who were either immunocompromised or had predisposed conditions such as cancer. (Biesiada et al 2019, Matsuura et al 2021, Rossi et al 2022).

A search of the FDA Center for Food Safety and Applied Nutrition Adverse Event Reporting System (CAERS) data files for the period of January 2004 – June 2023 returned one adverse event report using the search terms “*Lactobacillus plantarum* DSM 23881”, “*Lactobacillus plantarum* MCC1” and “*Lactobacillus plantarum*”. The suspected product caused increased blood creatinine, acute renal failure and venoocclusive liver disease requiring hospitalization for a 14.6-year-old female.

Moreover, as stated by the FDA, the adverse event reports related to a product and the overall count of adverse event reports in CAERS merely convey information as reported, without

implying any FDA determination regarding whether the product indeed caused the adverse events. In each report, the attribution of a suspected product as the cause of a reaction lacks certainty.

No results were found in the MedWatch, the FDA Safety information and adverse event reporting, and the FDA Recalls, market withdrawals & safety alerts search engines when querying for *Lactobacillus plantarum*. The information was accessed on November 23, 2023.

6.9 Recognition of safety by Authoritative Group of Qualified Experts

Lactiplantibacillus plantarum has been recognized as safe by different authoritative bodies because of its long history of safe use in food.

In 2002, the International Dairy Federation (IDF) and the European Food and Feed Cultures Association (EFFCA) published an inventory of food microorganism species with a history of safe use in foods. The inventory contains species that have a record for use in food production and their history of use in foods is documented by scientific literature references. The species *L. plantarum* was included in the initial list (Mogensen et al. 2002).

In 2007, the European Food Safety Authority (EFSA), introduced the Qualified Presumption of Safety (QPS) approach as a formal assessment of safety for microorganisms used in food/feed production. The safety assessment of a defined taxonomic group (*e.g.* genus or group of related species) is based on four pillars (establishing identity, body of knowledge, possible pathogenicity and end use). If the taxonomic group does not raise safety concerns or, if safety concerns exist, but could be defined and excluded (the qualification) the grouping could is granted QPS status. Thereafter, any strain of microorganism the identity of which could be unambiguously established and assigned to a QPS group would is freed from the need for further safety assessment other than satisfying any qualifications specified. Because of its long history of safe use, QPS status was attributed for *L. plantarum* by EFSA in 2007 and maintained ever since (EFSA 2007, EFSA 2011, EFSA 2012, EFSA 2013, EFSA 2014, EFSA 2015, EFSA 2016, EFSA 2017, EFSA 2018a, EFSA 2019, EFSA 2020, EFSA 2022).

In 2009, Health Canada issued a guidance document underlining the use of microbial ingredients in foods. The microbial ingredient is acceptable only if it has a history of safe use, such as *L. plantarum.* Otherwise, the microbial ingredient falls under the definition of a novel food. Furthermore, *L. plantarum* is an acceptable species to be used in food at a level of $1.0x10^9$ CFU/serving (Health Canada 2009, CFIA 2019).

Additionally, the Food and Drug Administration (FDA) completed its evaluation and issued a 'no question' response to multiple GRAS submission for *L. plantarum* such as GRN No. 1113 (CFSAN 2023), GRN No. 953 (CFSAN 2021), GRN No. 946 (CFSAN 2021a), GRN No. 847 (CFSAN 2019), GRN No. 722 (CFSAN 2018) and GRN No. 685 (CFSAN 2017) where some of them had an estimated daily intake up to 10^{11} CFU/serving (CFSAN 2023, CFSAN 2018, CFSAN 2017).

6.10 Pariza Decision Tree

Pariza and colleagues proposed a decision tree consisting of 13 questions to evaluate the safety of microbial cultures intended for human (and animals) consumption (Pariza et *al* 2015). With this decision tree approach, *L. plantarum* MCC1 can be concluded safe for human consumption as a food ingredient.

Table 9: Pariza Decision Tree for determining the safety of *L. plantarum* MCC1

#	Decision Tree Question	Response
1	Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding)	YES. The microbial ingredient has been properly characterized using whole genome sequence analysis approach. *L. plantarum* MCC1 clusters with other strains of *L. plantarum* including type strain ATCC 14917
2	Has the strain genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.)	YES. The genome of *L. plantarum* MCC1 has been fully sequenced
3	Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? (If YES, go to 4. If NO, go to 15.)	YES. *L. plantarum* MCC1 genome does not harbour any virulence genes, virulence factors or toxin and is not predicted to be pathogenic to humans.
4	Is the strain genome free of functional and transferable antibiotic resistance gene DNA? (If YES, go to 5. If NO, go to 15.)	YES. *L. plantarum* MCC1 genome does not harbour any acquired, functional, or transferable antibiotic resistance genes
5	Does the strain produce antimicrobial substances? (If NO, go to 6. If YES, go to 15.	NO. *L. plantarum* MCC1 does not produce antimicrobial substances detrimental to human health
6	Has the strain been genetically modified using rDNA techniques? (If YES, go to 7. If NO, go to 8.)	NO
7	Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in food? (If YES, go to 8. If NO, the expressed product(s) must be shown to be safe before proceeding to 8.)	NOT APPLICABLE
8a	Was the strain isolated from a food that has a history of safe consumption for which the species,	NO. *L. plantarum* MCC1 was isolated from the feces of a healthy child.

	to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? (If YES, go to 9. If NO, go to 13.)	
9	Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food use by an authoritative group of qualified scientific experts? (If YES, go to 10. If NO, go to 13.)	
10	Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9 continue to support the conclusion that the species, to which the strain belongs, is safe for use in food? (If YES, go to 11. If NO, go to 13.)	
11	Will the intended use of the strain expand exposure to the species beyond the group(s) that typically consume the species in “traditional” food(s) in which it is typically found (for example, will a strain that was isolated from a fermented food typically consumed by healthy adults be used in food intended for an 'at risk' group)? (If NO, go to 12. If YES, go to 13.)	
12	Will the intended use of the strain expand intake of the species (for example, increasing the number of foods beyond the traditional foods in which the species typically found, or using the strain as a probiotic rather than as a fermented food starter culture, which may significantly increase the single dose and/or chronic exposure)? (If NO, go to 14. If YES, go to 13.)	
13a	Does the strain induce undesirable physiological effects in appropriately	NO. *L. plantarum* MCC1 has been safely consumed by humans without

	designed safety evaluation studies? (If yes, go to 15. If no, go to 14a.) NO	adverse events in multiple double-blind placebo controlled clinical trials and products already in the market internationally
14a	The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.	

6.11 Summary

The data presented herein demonstrates that *L. plantarum* MCC1 is safe for its intended use as a general food ingredient. The safety of the microbial ingredient is supported by the following:

Lactiplantibacillus plantarum is a widely distributed and versatile LAB. It is a natural inhabitant of the human gastrointestinal tract. It also has a long history of safe consumption as it is responsible for the majority of fermented vegetables. It is found in fermented foods such as sourdoughs, fermented plant material, fermented sausages, fermented meats, stockfish, and fermented dairy.

This species remains listed in the latest "Inventory of food microorganism species with a history of safe use in foods" published by IDF/EFFCA (Bourdichon et al 2022). EFSA maintained its QPS status since 2007 (EFSA 2007, EFSA 2011, EFSA 2012, EFSA 2013, EFSA 2014, EFSA 2015, EFSA 2016, EFSA 2017, EFSA 2018a, EFSA 2019, EFSA 2020, EFSA 2022). Health Canada recognizes *L plantarum* as an acceptable species to be used in foods (Health Canada 2009, CFIA 2019). And, finally, six GRAS Notices (GRNs) on *L. plantarum* have received "no questions" letters from the FDA (GRN No. 1113, GRN No. 953, GRN No. 946, GRN No. 847, GRN No. 722, and GRN No. 685).

The microbial ingredient is manufactured in a production facility with fully implemented general principles of food hygiene (GMP) including HACCP plan, standard operating procedures including quality control to ensure the product quality. Moreover, the production facility has implemented the food safety management system which complying the requirements of standard ISO 22000:2018

The microbial ingredient has been properly characterized. The whole genome analysis confirmed proper identification. *L. plantarum* MCC1 clusters with other strains of *L. plantarum* including type strain ATCC 14917 confirming its identification.

Bioinformatic analysis of the genome demonstrates the absence virulence genes/factors, toxins and is not predicted to be pathogenic to humans. The microbial ingredient does not show any β-hemolysis activity and the genome does not contain any hemolysis genes.

L. plantarum MCC1 shows no phenotypic resistance against clinically relevant antibiotics. Furthermore, the bioinformatic analysis did not reveal any candidates for clinically relevant antimicrobial genes and does not carry any transferable gene conferring antibiotic resistance.

In vivo study in mice revealed that the daily administration of 2 x 10^8 CFU for 30 consecutive days of *L. plantarum* MCC1 did not show any translocation of the administered microbial ingredient or other microbes into the blood or organs was detected. The heart blood, liver, kidney, and lung samples obtained at autopsy were sterile in all mice. No pathological shifts and no micro abscesses, granulomas, or inflammation were found by morphological and histological evaluation of the spleen, liver, ileum, and colon of mice Furthermore, *L. plantarum* MCC1 was safe and well tolerated in human clinical trials with daily consumption.

Based on the Pariza decision tree to evaluate the safety of microbial cultures intended for human consumption, *L. plantarum* MCC1 is deemed to be safe for use in the manufacture of foods for human consumption.

L. plantarum MCC1 is intended for addition to comparable food categories and inclusion levels as other *L. plantarum* commercialized microbial ingredients with GRAS “no question” status (*L. plantarum* Lp115, Lp299v, *L. plantarum* ECGC 13110402 and *L. plantarum* NCIMB 30562).

6.12 Conclusions

We utilized the Pariza et al. (2015) decision tree framework and incorporated elements from the EFSA QPS approach to illustrate the safety of *L. plantarum* MCC1 for use as a microbial ingredient in conventional foods (excluding infant formula and meat and poultry products regulated by FSIS of the USDA). The data and information narrated in this GRAS document fully support the conclusion that *L. plantarum* MCC1 is GRAS under the intended uses as described. Based on the provided documentation, there is no reason to suspect harm to healthy individuals consuming foods supplemented with *L. plantarum* MCC1.

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Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

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

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001175	Nov 28, 2023
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

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

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

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

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

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

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

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Tonu Vetik	Business Development Manager

Organization *(if applicable)*: BioCC OÜ (LLC)

Mailing Address *(number and street)*: Kreutzwaldi 1

City	State or Province	Zip Code/Postal Code	Country
Tartu	N/A	51014	Estonia

Telephone Number	Fax Number	E-Mail Address
3725045662		tonu@nordwise.eu

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

Name of Contact Person	Position or Title
Marie-Eve Boyte	President

Organization *(if applicable)*: NutraPharma Consulting Services Inc

Mailing Address *(number and street)*: 32 Therese

City	State or Province	Zip Code/Postal Code	Country
Sainte-Anne-des-Plaines	Quebec (QC)	J5N 4B3	Canada

Telephone Number	Fax Number	E-Mail Address
5148983093		meboyte@npcs-intl.com

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

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

Lactobacillus plantarum MCC1

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

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

_____

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

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

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

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

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

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

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

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

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

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

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

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

☐ No

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

☐ Yes, a redacted copy of the complete submission

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

☐ No

SECTION D – INTENDED USE

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

L. plantarum MCC1 is intended to be used as a microbial ingredient in conventional foods, such as, but not limited to, dairy products (cheese, yoghurt, sour cream, cottage cheese, processed cheese, soft ice cream etc), fruit and vegetable juices, water, confectionary, dairy alternatives (e.g., plant - based vegan products), shelf-stable products (bars) and functional and nutritional products. Under the intended conditions of use, the maximum level of the microbial ingredient incorporated in the conventional foods will be 5x10E8 CFU per serving.). If all the combined foods contain the microbial ingredient, the maximum daily exposure to microbial ingredient from all food sources is approximated to 1x10E10 CFU. Such estimated dietary exposure under the intended use

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

(Check one)

☐ Yes ☒ No

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

(Check one)

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

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

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

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

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

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

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

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

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

Other Information

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

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Marie-Eve Boyte

(name of notifier)

has concluded that the intended use*(s)* of Lactobacillus plantarum MCC1

(name of notified substance)

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

2. NutraPharma Consulting Services Inc agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

(name of notifier)

32 Therese, Ste-Anne-des-Plaines, QC, Canada, J5N 4B3

(address of notifier or other location)

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

3. Signature of Responsible Official, Agent, or Attorney	**Printed Name and Title**	**Date (mm/dd/yyyy)**
MarieEve Boyte Signature numérique de MarieEve Boyte Date : 2023.11.28 10:00:23 -05'00'	Marie-Eve Boyte, President	11/28/2023

SECTION G – LIST OF ATTACHMENTS

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

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	COSM_3667_17340_NutraPharmaConsultin.pdf	Administrative

Follow-Up Questions for GRN 1175

Question 1:
The result for the analyses of batch M-22-003 was listed as <0.001 mg/kg. However, the limit of detection for this method was stated to be 0.01 mg/kg. Please clarify the discrepancy in the result for this batch.

Heavy metal	Detection limit	10-174	10-231	M-22-003
Lead	0.01 mg/kg	0.08±0.02	0.018±0.004	<0.01
Mercury	0.004 mg/kg	<0.004	<0.004	<0.004
Cadmium	0.007 mg/kg	0.040±0.008	<0.007	0.017±0.03

Answer:
The detection limit for lead is 0.01 mg/kg. Unfortunately, there was a typo when entering the results for M-22-003 lot. The accurate result for lead content for lot M-22-003 is <0.0.1

Question 2:
While you stated that the typical use level for the ingredient will be between $5x10^8$ and $5x10^9$ colony forming units (CFU) per serving, the maximum use level is stated to be $1x10^{10}$ CFU/serving. Therefore, please either revise the maximum use level to that used in your dietary exposure estimate ($5x10^8$ CFU/serving) or provide a dietary exposure estimate based on the maximum use level of $1x10^{10}$ CFU/serving.

Answer:
Under the intended conditions of use, the maximum level of the microbial ingredient incorporated in the conventional foods will be $1x10^{10}$ CFU per serving, taking into consideration the viability loss throughout the shelf life of the foods. A healthy individual consumes an average of 20 servings of food per day (Health Canada 2011, NHMRC, USDA 2020). If all the combined foods contain the microbial ingredient, the maximum daily exposure to microbial ingredient from all food sources is approximated to $2x10^{11}$ CFU. Such estimated dietary exposure under the intended use conditions is conservative as it assumes no viability loss of the microbial ingredient during storage and freight. Furthermore, the dietary exposure of $2x10^{11}$ CFU is within the same concentration range showing no adverse effects in human clinical studies for other *L. plantarum* (Sharafedtinov et al 2013, Huang et al 2018). When compared, the expected dietary intake of *L. plantarum* MCC1 under the specified conditions aligns with the consumption of other *L. plantarum* mentioned in GRAS notices (CFSAN 2023, CFSAN 2018, CFSAN 2017) that received an FDA letter of "no questions."

Question 3:
We note that you indicated in the amendment that all ingredients used in the manufacturing process are food grade. Please also confirm that all raw materials and processing aids used in the production of *Lactiplantibacillus plantarum* MCC1 are used in accordance with an appropriate U.S. regulation, are GRAS for their intended use, or are the subject of an effective food contact notification.

Answer:
The notifier confirms that all raw materials and processing aids used in the production of *Lactiplantibacillus plantarum* MCC1 are used in accordance with an appropriate U.S. regulation, are GRAS for their intended use, or are the subject of an effective food contact notification.

Questions from FDA on GRN 1175

Question 1:
In Figure 2, the notifier provides pulse field gel electrophoresis data. The bands in lane 3 (lot MM-22-003-2022) show noticeable deviation from the other profiles. Please explain the probable cause of the deviation. Please clarify if the marker (M) as shown in the figure was run on the same gel as the sample batches.

Answer:
The reason for deviation of third sample (MM-22-003 – 2022) may be in plug slice. It was probably too soft or cut too thin which explains the deviation seen in the image. However, all samples have the same profiles. The marker was run separately from these sample batches, but in the same PFGE machine with identical PFGE electrophoresis conditions.

Question 2:
In Section 2.4, the acronym "GMP" is given. For the administrative record, please clarify that this stands for "Good Manufacturing Practices" and indicate why the term general principles of food hygiene precedes the use of the acronym. Please confirm that *Lactiplantibacillus plantarum* MCC1 is produced in accordance with good manufacturing practices.

Answer:
The ingredient is produced in a plant certified under FAO/WHO Codex Alimentarius CXC 1-1969, Rev5 (2020) entitled General Principles of Food Hygiene (GMP). The acronym GMP stands for "Good Manufacturing Practices". Therefore, the term "general principles of food hygiene" that precedes the GMP acronym is in fact the name of the certification. Refer to Annex 1 for the accreditation certificate

Question 3:
In Table 4, the results of the batch analyses for heavy metals are reported as less than 0.1 mg/kg. It is not clear if the results are listed as below the limit of detection (LOD) for the method or if the results are listed as below the specification for the individual heavy metals. Please provide the LOD for the method for each heavy metal and provide the actual measured value for each heavy metal for each batch.

Answer:
Please see the LOD and the actual results in the table below:

Heavy metal	Detection limit	10-174	10-231	M-22-003
Lead	0.01 mg/kg	0.08±0.02	0.018±0.004	<0.001
Mercury	0.004 mg/kg	<0.004	<0.004	<0.004
Cadmium	0.007 mg/kg	0.040±0.008	<0.007	0.017±0.03

All the methods are accredited by a national accreditation center therefore validation for their intended us is mandatory. Furthermore, the laboratory is accredited ISO 17025:2017

Question 4:
Please indicate that all analytical methods are validated for their intended use
Answer:
All the methods are accredited by a national accreditation center therefore validation for their intended us is mandatory. Furthermore, the laboratory is accredited ISO 17025:2017. Refer to Annex 2 for the accreditation certificate.

Question 5:
There are references included in the text in Section 3 (e.g., Health Canada 2011, NHMRC, USDA 2020) that are missing from the reference list. Please provide an updated reference list that includes all the cited references.
Answer:
Refer to Annex 3 for the updated reference list.

Question 6:
The dietary exposure provided in Section 3 was based on a use level of $5x10^8$ colony forming units (CFU)/serving and the assumption that an individual consumes 20 servings of food per day containing the ingredient. We note that the maximum use level provided in Section 1.4 was stated to be $1x10^{10}$ CFU/serving. Please revise the dietary exposure estimate using the maximum use level for the ingredient.
Answer
The maximum use level in conventional foods is revised to $1x10^{10}$ CFU/serving. Yet, the typical levels of addition will range between $5x10^8$ and $5x10^9$ CFU per serving, therefore under the maximum use level of $1x10^{10}$ CFU/serving. When compared, the expected dietary intake of *L. plantarum* MCC1 under the specified conditions aligns with the consumption of other *L. plantarum* mentioned in GRAS notices GRN 1113 (CFSAN 2023), GRN 847 (CFSAN 2019), GRN 722 (CFSAN 2018) and GRN 685 (CFSAN 2017) that received an FDA letter of "no questions."

Question 7:
Please state that all raw materials, processing aids, filtration aids, and pH adjusters used in the production of *Lactiplantibacillus plantarum* MCC1 are food grade and are used in accordance with an appropriate U.S. regulation, are for their intended use, or are the subject of an effective food contact notification.
Answer:
The notifier confirms that all the ingredients used in the production process of the microbial ingredient *L. plantarum* MCC1 are food grade and are used in accordance with their intended use.

Question 8:
Please confirm that *Lactiplantibacillus plantarum* MCC1 is nonpathogenic and nontoxigenic.
Answer:
L. plantarum MCC1 is non pathogenic and nontoxigenic based on the screening of the whole genome sequence for the presence of genes of potential concern for safety since it does not harbour virulence genes or factors (refer to section 6.5) , including hemolysins (refer to section 6.5), decarboxylases genes required to generate cadaverine, putrescine, histamine, and tyramine (refer to section 6.4), any candidates for clinically relevant antimicrobial genes (refer to section 6.2). Additionally, PathogenFinder 1.1, a validated tool for the prediction of bacterial pathogenicity by analysing the input genome, predicted the microbial ingredient *L. plantarum* MCC1 as non pathogenic to humans (refer to section 6.5). *L. plantarum* MCC1 is as non pathogenic and nontoxigenic as other microbial ingredients from the same species with a "no question" response from the FDA such GRN No. 1113, GRN No. 953, GRN No. 946, GRN No. 847, GRN No. 722 and GRN No. 685.

Question 9:
Please confirm and state that there are no allergens in either the fermentation media and/or the final formulation of the article of commerce.
Answer:
There are no priority allergens as per FALCPA and FASTER in the fermentation media and/or the microbial ingredient.

ANNEX 1: GMP accreditation certificate

qualityaustria
Succeed with Quality

CERTIFICATE

LACTOSAN

Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH awards this **quality**austria certificate to the following organisation:

This **quality**austria certificate confirms the application and further development of an effective

Lactosan GmbH & Co.KG
AT-8605 Kapfenberg, Industriestrasse West 5

FOOD SAFETY MANAGEMENT SYSTEM
complying with the requirements of standard
ISO 22000:2018

Production of microorganisms and enzymes for use in food, food and feed additives, food supplements, dietary foods and in food processing packed in composite films and plastic containers.

Food Chain Category: K
(according ISO/TS 22003:2013)

The validity of the **quality**austria certificate will be maintained by annual surveillance audits and one renewal audit after three years.

Registration No.: 00590/0
Date of initial issue: 25 August 2022
Valid until: 26 September 2025

qualityaustria
MEMBER OF
IQNET

Vienna, 25 August 2022

Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Signatures removed for security reasons

Mag. Christoph Mondl
CEO

Mag. Dr. Werner Paar
CEO

DI Wolfgang Leger-Hillebrand
Specialist representative

Dok. Nr. FO_24_039

a9769d45-9f95-4284-baf2-63a1b12b841d

The current validity of the certificate is documented exclusively on the Internet under http://www.qualityaustria.com/en/cert EAC: 12

ANNEX 2 – Accreditation certificate

EESTI AKREDITEERIMISKESKUS
ESTONIAN ACCREDITATION CENTRE

Lisa kehtib perioodil 26.06.2024 kuni 19.04.2028
This annex is valid from 26.06.2024 to 19.04.2028

LISA BioCC OÜ akrediteerimistunnistusele nr **L250**
***ANNEX** to the accreditation certificate No **L250** of BioCC LLC*

1. Akrediteerimisulatus on:

Accreditation scope is:

Mikrobioloogilised analüüsid
Microbiological tests

Jrk nr. *No*	Määratav näitaja *Analysed parameter*	Uuritav materjal *Tested material*	Meetod *Method*
	Kvantitatiivne meetod *Quantitative method*		
1.	*Coli*-laadsed bakterid *Coliforms*	Toit, loomasööt *Food, feed*	EVS-ISO 4832:2010
2.	Pärm- ja hallitusseened *Yeasts and moulds*	Toit, loomasööt, mille veeaktiivsus on suurem kui 0,95 *Food, feed with water activity greater than 0.95*	EVS-ISO 21527-1:2009
3.	Pärm- ja hallitusseened *Yeasts and moulds*	Toit, loomasööt, mille veeaktiivsus on väiksem kui 0,95 *Food, feed with water activity is less than 0.95*	EVS-ISO 21527-2:2009
4.	*Enterobacteriaceae* *Enterobacteriaceae*	Toit, loomasööt *Food, feed*	EVS-ISO 21528-2:2017
5.	β-glükuronidaaspositiivne *Escherichia coli* *β-glucuronidase positive Escherichia coli*	Toit, loomasööt *Food, feed*	EVS-ISO 16649-2:2011
6.	Mesofiilsed piimhappebakterid *Mesophilic lactic acid bacteria*	Piimatooted, lüofiliseeritud bakterikultuur *Milk products, lyophilized bacterial cultures*	ISO 15214:1998
7.	*Lactobacillus spp.* *Lactobacillus spp.*	Loomasööt (va kõrge vasesisaldusega söödad), lüofiliseeritud bakterikultuur *Feed (excluding feeding stuffs containing high amounts of copper), lyophilized bacterial cultures*	EVS-EN 15787:2021
8.	Mikroorganismid *Microorganisms*	Toit, loomasööt *Food, feed*	EVS-EN ISO 4833-2:2013+A1:2022
9.	Koagulaaspositiivsed stafülokokid (*Staphylococcus aureus* ja teised liigid) *Coagulase-positive staphylococci (Staphylococcus aureus and other species)*	Toit, loomasööt *Food, feed*	EVS-EN ISO 6888-1:2021
10.	Eeldatav *Bacillus cereus*	Piimatooted, lihatooted, puu- ja köögiviljatooted	EVS-EN ISO 7932:2005

Jrk nr. *No*	Määratav näitaja *Analysed parameter*	Uuritav materjal *Tested material*	Meetod *Method*
	Presumptive Bacillus cereus	*Milk products, meat products, fruits and vegetables*	
11	Mesofiilsete ja termofiilsete bakterite spoorid *Mesophilic Aerobic Bacterial Spores and Thermophilic Bacterial Spores*	Piimatooted *Milk products*	NY/T 1331-2007
Kvalitatiivne meetod *Qualitative method*			
12.	Listeria monocytogenes *Listeria monocytogenes*	Toit, sööt, keskkonnaproovid toidu ja sööda käitlemisest *Food, feed, environmental samples from food and feed production*	EVS-EN ISO 11290-1:2017
13.	*Cronobacter spp* *Cronobacter spp*	Piimatooted, keskkonnaproovid toidu ja sööda käitlemisest *Milk products, environmental samples from food and feed production*	EVS-EN ISO 22964:2017
14.	*Salmonella spp* *Salmonella spp*	Toit, sööt, keskkonnaproovid toidu ja sööda käitlemisest *Food, feed, environmental samples from food and feed production*	EVS-EN ISO 6579-1:2017+A1:2020

2. Katsetamist teostav struktuuriüksus: BioCC OÜ labor
Part of legal entity that provides testing: laboratory of BioCC LLC

3. **Tegevuskohtade aadressid:** Riia mnt 181 a Tartu
Addresses of locations:

4. Labor on akrediteeritud standardi EVS-EN ISO/IEC 17025:2017 nõuete kohaselt
Laboratory is accredited against the requirements of standard EVS-EN ISO/IEC 17025:2017

Märkus: käesolev lisa asendab 30.03.2023 välja antud lisa seoses akrediteerimisulatuse laiendamisega ja EAK uue logo kasutuselevõtuga
Note: *this annex replaces the annex issued 30.03.2023 due to the extension of accreditation scope and due to introduction of EAK new logo*

Eire Endrekson
Eesti Akrediteerimiskeskuse juhataja / *Head of the Estonian Accreditation Centre*

Tallinn, 26.06.2024

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