# JHeimbach LLC



April 9, 2020

Susan J. Carlson, Ph.D., Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Carlson:

Pursuant to 21 CFR Part 170, Subpart E, Kaneka Americas Holding, Inc., and AB-Biotics, through me as their agent, hereby provide notice of a claim that the addition of a blend of *Lactobacillus plantarum* strain KABP-011, *Lactobacillus plantarum* strain KABP-012, and *Lactobacillus plantarum* strain KABP-013 to conventional foods is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Kaneka and AB-Biotics have determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, one copy of the GRAS monograph and one signed copy of the conclusion from each member of the Expert Panel are provided. Additionally, I have enclosed a virus-free CD-ROM with the GRAS monograph and the signed statements of the Expert Panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5543 or jh@jheimbach.com.

Sincerely,

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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# Generally Recognized as Safe (GRAS) Determination for the Use of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013 in Conventional Foods

**Prepared by**
**Kaneka Americas Holding, Inc.**
**Pasadena, Texas**

**and**

**AB-Biotics**
**S.A. Barcelona, Spain**

**Edited by**
**JHeimbach LLC**
**Port Royal, Virginia**

**April, 2020**

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# Part 1. Signed Statements and Certifications

## 1.1. GRAS Notice Submission

Kaneka Americas Holding, Inc., of Pasadena, Texas ("Kaneka") submits this GRAS notification in accordance with subpart E of 21 CFR part 170.

## 1.2 Name and Address of Notifier

Kaneka Americas Holdings, Inc.
6161 Underwood Road
Pasadena, Texas 77507

Joshua Garey, Sr. R&D Scientist
Tel: 281-474-1826
Email: joshua.garey@kaneka.com

## 1.3. Names of Notified Microorganisms

*Lactobacillus plantarum* KABP-011 (CECT 7527), *Lactobacillus plantarum* KABP-012 (CECT 7528), and *Lactobacillus plantarum* KABP-013 (CECT 7529). The strains, when combined in a 1:1:1 ratio as lyophilized powder, are denoted "AB-Life" and "Floradapt Cardio" by the microorganism supplier.

## 1.4. Intended Conditions of Use

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, are intended to be used in conventional foods, such as yogurt and other dairy products, soy products, chewing gum, and confectionary snacks. The intended use does not include infant formula, other products intended for consumption by infants, or foods regulated by the U.S. Department of Agriculture. The purpose of the identified strains is to serve either individually or as a combination of microorganisms to be consumed by the general population, excluding infants.

When used individually, the intended level each strain of *L. plantarum* is intended to be at least $4x10^8$ cfu/serving over the shelf life of the product. The maximum level of addition is intended to be $4x10^9$ cfu/serving of the strain to allow for the loss of viability over the shelf life of the product.

When used as a combination, the intended combined level of the three *L. plantarum* strains is intended to be at least $1.2x10^9$ cfu/serving over the shelf life of the product. This is equivalent to a level of $4x10^8$ cfu/serving for each strain. The maximum level of addition of the combination is intended to be $1.2x10^{10}$ cfu/serving, equivalent to $4x10^9$ cfu/serving of each individual strain, to allow for the loss of microorganism viability over the shelf life of the product.

The identified strains of microorganisms will be present only in foods to which they are intentionally added.

## 1.5. Statutory Basis for GRAS Status

Kaneka Americas Holdings, Inc., has determined that the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is GRAS based on scientific procedures in accordance with 21 C.F.R. 170.30(a) and (b).

## 1.6. Premarket Exempt Status

Since Kaneka Americas Holdings, Inc., has determined that the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is GRAS, such use is not subject to premarket approval requirements under the Federal Food, Drug, and Cosmetic Act.

## 1.7. Data Availability

The data and information that serve as the basis for the conclusion that the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is GRAS will be made available to the FDA upon request. At FDA's option, a complete copy of the information will be sent to FDA in either paper or electronic format, or the information will be available for review at the home office of JHeimbach LLC, located at 923 Water Street, Port Royal, VA  22535, during normal business hours.

## 1.8. Freedom of Information Act Statement

No data or information submitted with this GRAS notification is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

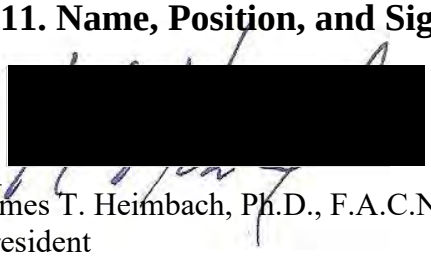
## 1.9. Certification

To the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination.

## 1.10. FSIS Statement

Not applicable.

## 1.11. Name, Position, and Signature of Notifier



James T. Heimbach, Ph.D., F.A.C.N.
President
JHeimbach LLC
Agent to Kaneka Americas Holding, Inc.

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

## 2.1. Names of the GRAS Organisms

The subjects of this GRAS notification are strains of bacteria designated *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013. The strains in combination (1:1:1 ratio) are named AB-Life and Floradapt Cardio by the supplier.

## 2.2. Sources of the GRAS Organisms

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 are from a bank of strains that were isolated from the feces of healthy South American infants. The strains were deposited in the Spanish Type Culture Collection (Colección Española de Cultivos Tipo/CECT) under accession numbers CECT 7527, CECT 7528, and CECT 7316, respectively.

## 2.3. Descriptions of the GRAS Organisms

"Lactic acid bacteria (LAB)" is a general term applied to species of bacteria belonging to any of 17 different genera, including *Lactobacillus* (Rovira and Molero, 2018). LAB are characterized by the phenotypic property of producing lactic acid as the major end product of sugar fermentation. LAB are Gram-positive, non-spore-forming, devoid of cytochromes, and catalase-negative (Holzapfel et al., 2001). LAB have a long history of use in food and food fermentation, and the human body is colonized by many species of LAB ( Douillard and Vos, 2014). The presence of *L. plantarum* has been confirmed in ziang sang (or ziansang), a traditional dish of Nagaland and Manipur in northeast India, composed of fermented mustard leaves (Tamang et al., 2005).

*L. plantarum* is Gram-positive; rod shaped; single, paired, or short-chained; non-motile; and facultatively heterofermentative, producing lactic acid during fermentation. *L. plantarum* has been isolated from dairy products, silage, sauerkraut, pickled vegetables, sourdough, and the feces, mouth, and intestinal tract of humans (Vos et al., 2009). Use of *L. plantarum* was documented in 1968 for the preparation of a Filipino fermented fish dish, burong dalag (Bourdichon et al., 2012; Orilla and Pederson, 1968).

2.3.1. Phenotypic Identification

*L. plantarum* KABP-011 (CECT 7527), *L. plantarum* KABP-012 (CECT 7528), and *L. plantarum* KABP-013 (CECT 7529) were evaluated for enzymatic activity using the API® ZYM test kit made by bioMérieux (Figure 1).

| | Enzyme of study | *L. plantarum* CECT 7527 | *L. plantarum* CECT 7528 | *L. plantarum* CECT 7529 |
|---|---|---|---|---|
| 1 | Control | - | - | - |
| 2 | Alkaline phosphatase | - | - | + |
| 3 | Esterase (C4) | - | - | + |
| 4 | Esterase Lipase (C8) | - | + | + |
| 5 | Lipase (C14) | - | - | - |
| 6 | Leucine arylamidase | + | + | + |
| 7 | Valine arylamidase | + | + | + |
| 8 | Cystine arylamidase | - | - | + |
| 9 | Trypsin | - | - | - |
| 10 | α-Chymotrypsin | - | - | - |
| 11 | Acid phosphatase | - | + | + |
| 12 | Naphthol AS-BI-phosphydrolase | + | + | + |
| 13 | α-galactosidase | - | - | - |
| 14 | β-galactosidase | - | - | + |
| 15 | β-glucoronidase | - | - | - |
| 16 | α-glucosidase | - | - | + |
| 17 | β-glucosidase | - | + | + |
| 18 | N-acetyl-β-glucosaminidase | - | + | + |
| 19 | α-mannosidase | - | - | - |
| 20 | α-fucosidase | - | - | - |

**Figure 1: API® ZYM Profile of Strains (AB-Biotics 2019, unpublished).**

The strains were analyzed by pulsed-field gel electrophoresis (PFGE) using two different restriction enzymes following the methodology described by Rodas et al. (2005), as shown in Figures 2 and 3.

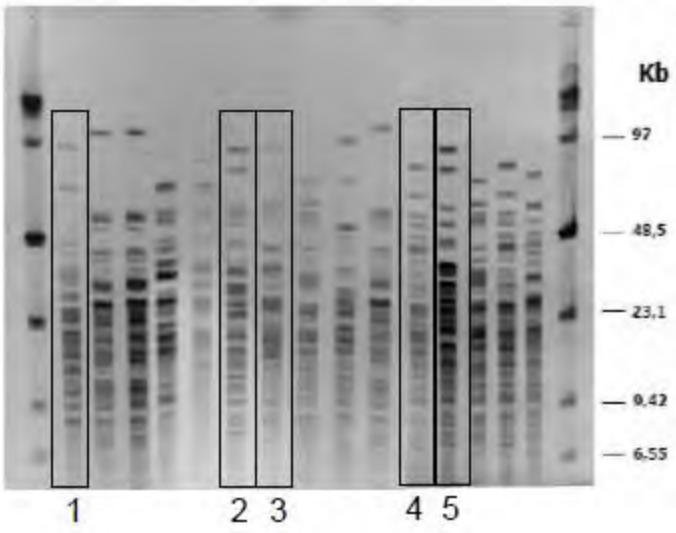



**Figure 2: PFGE, SmaI, (M) Markers and (1) *L. plantarum* 299v (2) KABP-011 (3) KABP-012 (4) *L. plantarum* VSL#3 and (5) KABP-013 (AB-Biotics 2017, unpublished).**

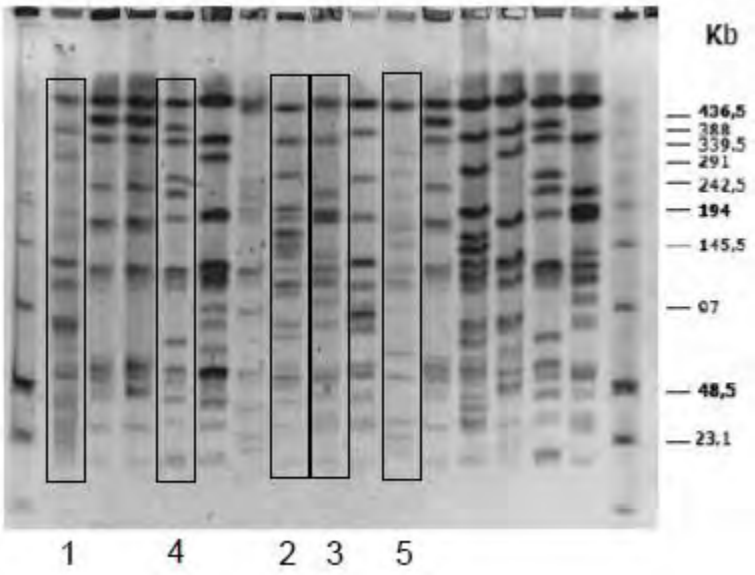



**Figure 3: PFGE, SfiI, (M) Markers and (1) *L. plantarum* 299v (2) KABP-011 (3) KABP-012 (4) *L. plantarum* VSL#3 and (5) KABP-013 (AB-Biotics 2017, unpublished).**

### 2.3.2. Genotypic Identification

The phylogenetic tree and species identification of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 were performed using the 16s ribosomal RNA gene near-full-length sequence. The lyophilized powder of each strain was suspended in PBS and grown on MRS agar plates. DNA was extracted from the grown colonies with commercial kit DNeasy Tissue and Blood (Qiagen) per manufacturer's protocol. The 16s rRNA

gene was amplified by PCR using universal primers (Eub27f and Eub1492R) following conventional methodology. The DNA obtained was washed using the commercial kit Quiaquick by Quiagene. The sequencing was performed on a Genetic Analyzer 3130 by Applied Biosystems using BigDye kit v. 3.1. Data collection and chromatograms were built using DNA Sequence Analysis v.5.2 software (Applied Biosystems) and checked by visual analysis with Chromas (Technelysium Pty Ltd.) and BioEdit (Ibis Biosciences).

| Step | Primer | Orientation | 5' → 3' sequence |
|---|---|---|---|
| Amplification | Eub27f | forward | GAGTTTGATCCTGGCTCAG |
| | Eub1492r | reverse | TACGGYTACCTTGTTACGACTT |
| Sequencing | 27f | forward | AGAGTTTGATCCTGGCTCAG |
| | 357f | forward | CGCCCGCCGCGCCCCGCGCCCGGCCCGCC |
| | | | GCCCCCGCCCCCCTACGGGAGGCAGCAG |
| | 907r | reverse | CCGTCAATTCCTTTGAGTTT |
| | 1492r | reverse | GGTTACCTTGTTACGACTT |

**Figure 4: Primers used for amplifying and sequencing of the 16s gene.**

The obtained sequences were compared by BLAST to the NCBI database (Blast) and the phylogenetically closest type strains were determined using the RDP database (Cole et al., 2014). The summary phylogenetic tree is shown in Figure 5. The sequences of the analyzed strains were aligned to reference strains and *S. salivarius* ATCC 19258 was selected as an outgroup of the *Lactobacillae* family for the construction of the phylogenetic tree. The comparison confirms that the sequenced strains have been correctly assigned at the species level.

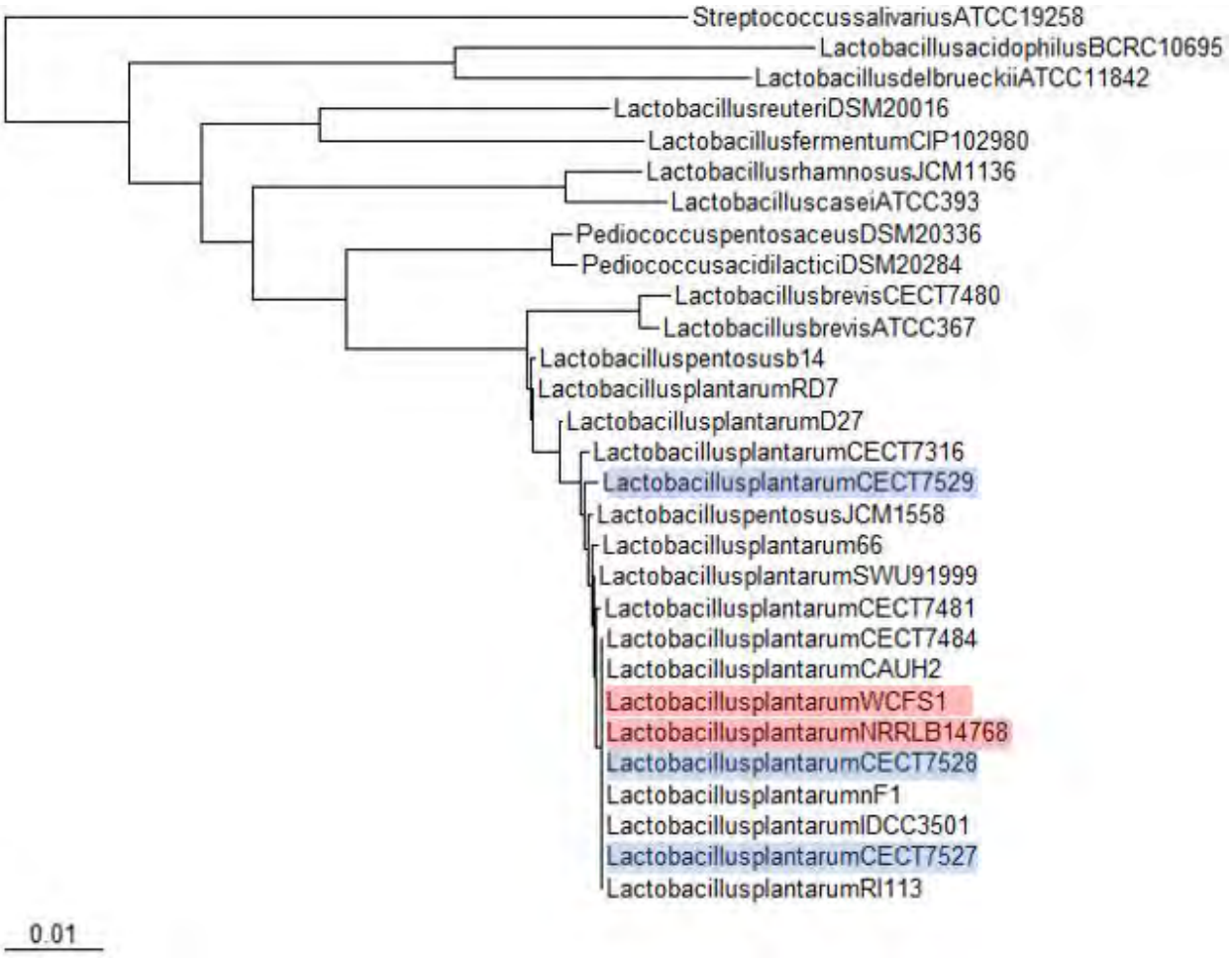



**Figure 5: Phylogenetic Tree (AB-Biotics 2019, unpublished).**

The strains are closely related to representative strains (highlighted in orange) for their species. *L. plantarum* KABP-011 (CECT 7527), KABP-012 (CECT 7528), and KABP-013 (CECT 7529) are closely related to other strains of *L. plantarum*.

### 2.3.3. Genome Summaries

The genomes of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 were obtained using Whole Genome Sequencing (WGS) with the sequencing platform PacBio [DNA Sequencing Reagent 4.0 v2, SMRT Cell 8 Pac v3 and SMRT Cell Oil (Pacific Bioscience)] and assembled with SMRT Analysis version 2.3.0 software and RS_HGAP_Assembly.3 program. The DNA was isolated with NucleoBond AXG20, NucleoBond Buffer Set III. SMRTBell Template Prep Kit 1.0 (Pacific Bioscience) was used for library preparation and sequencing templates larger than 15 kb were prepared by BluePippen size-selection system (P/N 100-286-000 Version 10, Sage Science). The sequences from each strain were compared to the reference database GenomesDB offered by JspeciesWS (Richter et al., 2015), for similarity scores (z-scores). The program returned a list of 100 strains ranked by

similarity, from which a reference strain was identified. A pairwise comparison of the reference strain and the sequenced strain was performed and the Alignment Nucleotide Identity (ANI) value was calculated using JSpeciesWS. ANI values greater than 95% indicate that the genomes are considered to be from the same species (Figueras et al., 2014; Goris et al., 2007; Richter and Rossello-Mora, 2009). Additionally, the sequenced strains and the reference strains were aligned using the MAUVE[1] program (Darling et al., 2010). The genomes were aligned using the default parameters.

For genome mining of antibiotic resistance genes, virulence factors, and mobile genetic elements, Quality-filtered subreads longer than 500 bp were joined to their corresponding paired reads and assembled with SMRT Analysis version 2.3.0 software and RS_HGAP_Assembly.3 program. The genomes were annotated with the help of the Prodigal algorithm (Hyatt et al., 2010) and Glimmer (Delcher et al., 2007). The results of the search for antibiotic resistance genes, virulence factors, and mobile genetic elements are discussed in Section 6.2.

For *L. plantarum* KABP-011, 145,869 subreads (adaptor-removed reads) were obtained with an average length of 10 kb per read. The reference genome of *Lactobacillus plantarum* is 3.27 Mb, giving an estimated sequencing coverage of 448x. This coverage implies that the information extracted for the genome sequencing of this strain is reliable due to the ultra-deep coverage (> 100x). The KABP-011 assembled genome was 3.19 Mb, consisting of three contigs. Within these contigs, 3,425 coding sequences were obtained. The assembled genome was compared to the database GenomesDB. When compared to the reference strain, *L. plantarum* WCFS1, the z-score was 0.99923 and the ANI value was 98.86%. This confirms the taxonomy of the strain, indicating that the strains belong to the same species and are closely related. KABP-011 was aligned with WCFS1 using the MAUVE program (Figure 6).

[1] MAUVE is free-of-charge open-source software for constructing multiple genome alignments in the presence of large-scale evolutionary events such as rearrangement and inversion.


**Figure 6: *L. plantarum* KABP-011 MAUVE Alignment (AB-Biotics 2019, unpublished).**

The relative lack of white space in the color regions of the MAUVE diagram demonstrates the high degree of genomic conservation between KABP-011 and the reference strain. The strains show a high degree of homology, evidenced by the ANI value of 98.86%.

For *L. plantarum* KABP-012, 139,703 subreads (adaptor-removed reads) were obtained with an average length of 10.8 kb per read. The reference genome of *Lactobacillus plantarum* is 3.27 Mb, giving an estimated sequencing coverage of 460x. This coverage implies that the information extracted for the genome sequencing of this strain is reliable due to the ultra-deep coverage (> 100x). The KABP-012 assembled genome was 3.44 Mb, consisting of five contigs. Within these contigs, 3,761 coding sequences were obtained. The assembled genome was compared to the database GenomesDB. When compared to the reference strain, *L. plantarum* WCFS1, the z-score was 0.99877 and the ANI value was 98.58%. This confirms the taxonomy of the strain, indicating that the strains belong to the same species and are closely related. KABP-012 was aligned with WCFS1 using the MAUVE program (Figure 7).


**Figure 7: *L. plantarum* KABP-012 MAUVE Alignment (AB-Biotics 2019, unpublished).**

The relative lack of white space in the color regions of the MAUVE diagram demonstrates the high degree of genomic conservation between KABP-012 and the reference strain. The strains show a high degree of homology, evidenced by the ANI value of 98.58%.

For *L. plantarum* KABP-013, 132,259 subreads (adaptor-removed reads) were obtained with an average length of 10.1 kb per read. The reference genome of *Lactobacillus plantarum* is 3.27 Mb, giving an estimated sequencing coverage of 409x. This coverage implies that the information extracted for the genome sequencing of this strain is reliable due to the ultra-deep coverage (> 100x). The KABP-013 assembled genome was 3.38 Mb, consisting of five contigs. Within these contigs, 3,688 coding sequences were obtained. The assembled genome was compared to the database GenomesDB. When compared to the reference strain, *L. plantarum* WCFS1, the z-score was 0.99888 and the ANI value was 98.37%. This confirms the taxonomy of the strain, indicating that the strains belong to the same species and are closely related. KABP-013 was aligned with WCFS1 using the MAUVE program (Figure 8).


**Figure 8: *L. plantarum* KABP-013 MAUVE Alignment (AB-Biotics 2019, unpublished).**

The relative lack of white space in the color regions of the MAUVE diagram demonstrates the high degree of genomic conservation between KABP-013 and the reference strain. The strains show a high degree of homology, evidenced by the ANI value of 98.37%.

In summary, *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 all show high degrees of homology with their reference strain, as indicated by the ANI values. Strain alignment with the MAUVE program demonstrates that these strains are highly homologous with their reference strain, but still have unique regions that can be used to develop strain-specific primers for strain identification.

## 2.4. Production Method

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 are manufactured in FDA registered facilities according to applicable current Good Manufacturing Practice (cGMP) by Kaneka or manufacturing companies under contract with Kaneka. All ingredients, processing aids, and excipients used in the manufacturing process are food grade, non-allergenic, and approved for use by the FDA.

Strains are stored in the strain collection as master seed. From the master seed, a working cell-bank is prepared as a glycerol stock and stored at -80°C. The identity of the seed is confirmed by 16s RNA sequencing. All strains are fermented independently and later combined at the appropriate ratio when they are to be used in combination. The working seed is transferred from a storage vial to a sterilized shake flask containing sterilized nutrient broth and allowed to

grow for 16 hours with shaking at the strain-specific temperature. This pre-culture is then transferred aseptically to the main fermentation tank containing broth that has been sterilized at 121°C for 20 minutes. Fermentation usually lasts for 3-18 hours, depending on the strain. The pH is controlled during fermentation by the addition of food-grade ammonium hydroxide to maintain a fixed pH, typically between 5.0 and 6.0. Fermentation is terminated by cooling the fermenter to 10°C. The endpoint of fermentation is determined by monitoring the flow rate of base addition and the pH to determine when the growth has entered the stationary phase. Every step in the fermentation process is checked for purity by microscopic control.

The fermentation broth is then separated and concentrated by removing excess water by centrifuge. The concentrated broth is fed into a clean sanitized stainless-steel tank. Food-grade cryoprotectants are added to the concentrated broth to protect the bacteria from death and damage caused by subsequent freeze drying. The bacteria undergo lyophilization by storage under freezing temperatures and vacuum to allow water sublimation for approximately 72 hours.

The lyophilized powder is then milled and passed through a 1-mm sieve to create a uniform particle size. The sieve also functions to remove any foreign material. The milled powder is blended with excipients (food grade maltodextrin, microcrystalline cellulose, or corn starch, for example) to achieve the necessary product concentration. For products using the strains in combination, the three separate strains are blended to create a uniform bulk powder. The blended powder is packaged in aluminum laminated bags approved for food contact, labelled for identity, lot tracking, and manufacturing date, and stored at -20°C.

The production process is illustrated in Figure 9.

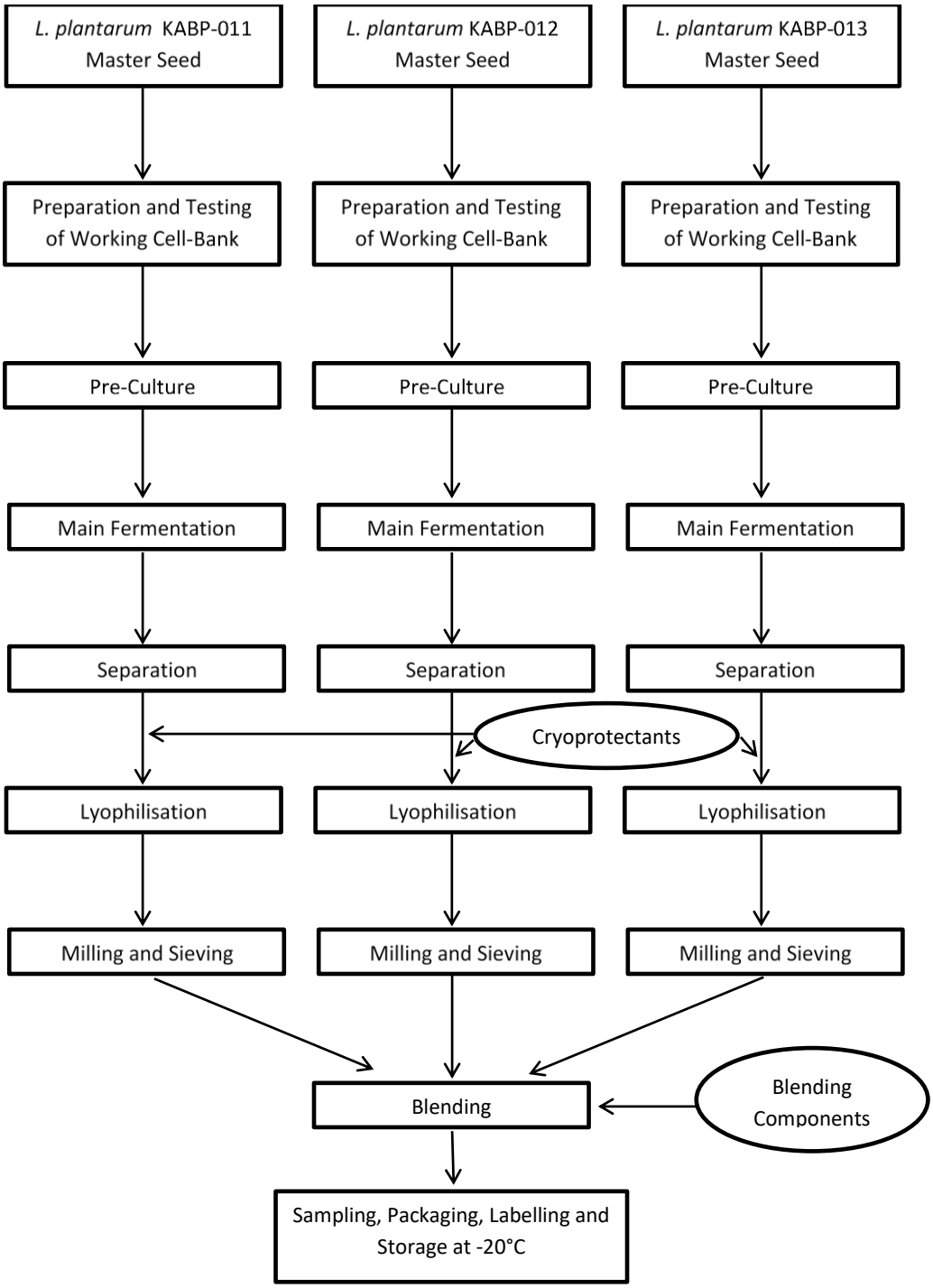



**Figure 9: Manufacturing Flow Chart (Kaneka 2019, unpublished).**

## 2.5. Specifications

Kaneka has established food-grade specifications for products containing the three strains *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, as shown in Table 1. All testing is done using up-to-date analytical methods that have validated for their specific spplications.

**Table 1. Specifications (Kaneka 2019, unpublished).**

| Parameter | Specification |
|---|---|
| Bacterial Count | >1.2x10$^{11}$ cfu/g |
| Yeasts and Molds | <10 cfu/g |
| Enterobacteria | <10 cfu/g |
| *E. coli* | <1 cfu/g |
| *Staphylococci* (coag +) | <10 cfu/g |
| *B. cereus* | <100 cfu/g |
| *Listeria monocytogenes* | n.d. in 25 g |
| *Salmonella* spp. | n.d. in 25 g |
| cfu = colony-forming unit | n.d. = not detected |

Table 2 shows the results of analyses of three non-consecutive lots of the notified strains combined as bulk lyophilized powder.

**Table 2. Analyses of Three Lots of Combination Product (Kaneka 2019, unpublished).**

| Parameter | Specification | Lot | | |
|---|---|---|---|---|
| | | 8820.01 | 8685.01 | 8685.07 |
| Bacterial Count | >1.2x10$^{11}$ cfu/g | 1.75x10$^{11}$ cfu/g | 1.48x10$^{11}$ cfu/g | 1.44x10$^{11}$ cfu/g |
| Yeasts and Molds | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| Enterobacteria | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| *E. coli* | <1 cfu/g | <1 cfu/g | <1 cfu/g | <1 cfu/g |
| *Staphylococci* (coag +) | <10 cfu/g | <10 cfu/g | <10 cfu/g | <10 cfu/g |
| *B. cereus* | <100 cfu/g | <100 cfu/g | <100 cfu/g | <100 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 |
| cfu = colony-forming unit | n.d. = not detected | | | |

Separate analyses were completed on the heavy metal content of 3 non-consecutive lots of the lyophilized powder blend, as shown in Table 3.

**Table 3. Analyses of Heavy Metals in Three Lots of Combination Products (Kaneka 2019, unpublished).**

| Heavy Metal | Unit | Lot | | |
|---|---|---|---|---|
| | | 8685.04a | 8685.06c | 8685.05b |
| Arsenic | mg/kg | <0.02 | 0.047 | 0.047 |
| Cadmium | mg/kg | <0.005 | 0.018 | 0.019 |
| Mercury | mg/kg | <0.005 | <0.005 | <0.005 |
| Lead | mg/kg | <0.005 | 0.006 | <0.005 |

## 2.6. Stability

The stability (viability of cells) of the three individual strains and the 1:1:1 strain combination mixed with a carrier, stored in the original packaging, and stored under frozen conditions (-20°C) was measured over 18 months (Figure 10). The intended use of the microorganisms is limited to applications that can sufficiently support microorganism viability throughout the shelf-life of the product.

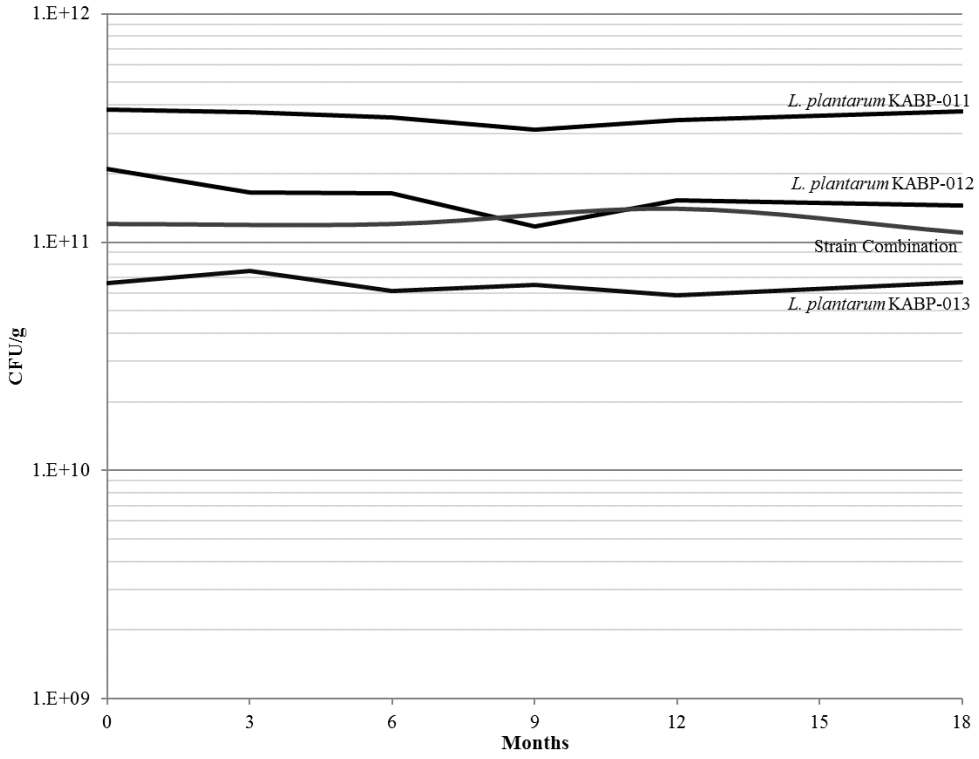



**Figure 10: Microorganism Viability at -20°C (Kaneka 2019, unpublished).**

# Part 3. Intended Use and Dietary Exposure

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, are intended to be used in conventional foods, such as yogurt and other dairy products, soy products, chewing gum, and confectionary snacks. The intended use does not include infant formula, other products intended for consumption by infants, or foods regulated by the U.S. Department of Agriculture. The purpose of the identified strains is to serve either individually or as a combination of microorganisms to be consumed by the general population, excluding infants.

When used individually, the intended level of each strain of *L. plantarum* is intended to be at least $4x10^8$ cfu/serving over the shelf life of the product. The maximum level of addition is intended to be $4x10^9$ cfu/serving of the strain to allow for the loss of viability over the shelf life of the product.

When used as a combination, the intended combined level of the three *L. plantarum* strains is intended to be at least $1.2x10^9$ cfu/serving over the shelf life of the product. This is equivalent to a level of $4x10^8$ cfu/serving for each strain. The maximum level of addition of the combination is intended to be $1.2x10^{10}$ cfu/serving, equivalent to $4x10^9$ cfu/serving of each individual strain, to allow for the loss of microorganism viability over the shelf life of the product.

The strains, individually or in combination, are expected to be present in a limited number of foods at between $10^9$ and $10^{10}$ cfu/serving. The likely maximum ingestion is less than $10^{10}$ cfu/day, well within levels that have been shown to be safe. The identified strains of microorganisms will be present only in foods to which they are intentionally added, and this amount thus represents the total daily intake from all sources.

## Part 4. Self-Limiting Levels of Use

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 have no self-limiting levels of use. However, uses are restricted to foods that can maintain the survival of the strains.

## Part 5. Experience Based on Common Use in Food

The GRAS conclusion is based on scientific procedures and not on common use in food before 1958.

# Part 6. Narrative

## 6.1. History of Safe Ingestion

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 in combination at a minimum concentration of $1.2x10^9$ cfu/serving has been marketed in Mexico (2012), Europe (2013), Asia (2015), and Canada (2016) under the brand name AB-Life.

## 6.2. Properties of the Strains

Bosch et al. ( 2012a) investigated the properties of *L. plantarum* strains *L. plantarum* KABP-011 (CECT 7527), KABP-012 (CECT 7528), and KABP-013 (CECT 7529) *in vitro.* Survival and growth in simulated oral and gastrointestinal conditions was assessed in 10-30 µg $H_2O_2$/ml, 100-300 µg lysozyme/ml, and 0.3-1.0% bile salts, and acidity of pH 2-4. The strains were compared to *L. plantarum* 299v and VSL#3. Under oral conditions, the strains had a relative growth between 93.7% and 99.7% versus growth ranging from 81.2% to 92.9% by the comparison strains.

The adhesion capabilities of the strains to mucus and intestinal pig epithelium were compared with those of commercial strains *L. rhamnosus* GG and *L. plantarum* 299V and were found to be approximately four times as high for mucus adhesion at $6.8x10^5$ cfu/cm$^2$ to $7.31x10^6$ cfu/cm$^2$. Higher adhesion values were also observed for the strains on the epithelial cells, ranging from $5.19x10^5$ cfu/cm$^2$ to $3.12x10^6$ cfu/cm$^2$ versus $8.53x10^4$ cfu/cm$^2$ and $2.5x10^5$ cfu/cm$^2$ for the comparison strains.

The capability of the strains to generate bile salt hydrolase (BSH) was determined. BSH can hydrolyze bile salts used for fat and cholesterol absorption. BSH activity of the three strains were all higher than the reference strains *L. rhamnosus* GG, *L. plantarum* 299v, and *L. plantarum* VSL#3. The combination of the three strains had higher BSH activity than any individual strain. The combination of strains also reduced cholesterol *in vitro* by 13% and 50.9% when a 1% concentration of bile salts was added to solution. The ability of the strains to adhere cholesterol was also measured. Of the three strains, 2.26%, 2.36%, and 1.05% of the cells, respectively, adhered cholesterol to their surface. The production of two short-chain fatty acids (propionic acid and butyric acid) by the strains was evaluated. Propionic acid production for the strains was (in mg/L) 15.5, 12.2, and 44.9, compared to 12.9 for the reference 299v strain. Butyric acid production for the strains was (in mg/L) 14.2, 12.3, and 21.6, compared to 9.2 for the reference 299v strain.

## 6.3. Safety-Related Issues

### 6.3.1. Antibiotic Resistance

Mukerji et al. (2016) and Bosch et al. (2014) analyzed the phenotypic antibiotic resistance of *L. plantarum* KABP-011, KABP-012, and KABP-013 per ISO 10932:2010 (IDF, 2010) as recommended by the European Food Safety Authority (EFSA, 2012). The antibiotics tested and the minimum inhibitory concentration (MIC) cutoff values were selected based on the EFSA guidance.

The results of the MIC testing are shown in Figure 11. *L. plantarum* KABP-011 and *L. plantarum* KABP-012 exceeded the thresholds for clindamycin and kanamycin.

| | Breakpoint | KABP-011 | | KABP-012 | | KABP-013 | |
|---|---|---|---|---|---|---|---|
| | | Mukerji | Bosch | Mukerji | Bosch | Mukerji | Bosch |
| Ampicillin | 2 | 0.25 | nr | 1 | 2 | 0.5 | nr |
| Gentamicin | 16 | 4 | nr | 8 | 16 | 2 | nr |
| Kanamycin | 64 | 128 | nr | 128 | 64 | 64 | nr |
| Erythromycin | 1 | 0.5 | nr | 0.5 | 1 | 0.25 | nr |
| Clindamycin | 2 | 8 | 4 | 8 | 1 | 0.5 | nr |
| Tetracycline | 32 | 16 | nr | 16 | 32 | 16 | nr |
| Chloramphenicol | 8 | 4 | 8 | 8 | 8 | 4 | 16 |

nr = not reported μg / mL

**Figure 11: Antibiograms (Kaneka 2019, unpublished).**

Mukerji et al. (2016) also investigated the antimicrobial resistance of these two strains using genomic analysis. A *cat* gene associated with resistance to chloramphenicol was observed in all three strains even though the breakpoint was not exceeded. The gene was not encoded on plasmids or near any insertion elements or prophages. In the two strains showing resistance to kanamycin and clindamycin, mutations as single nucleotide polymorphisms (SNPs) were observed in the 23S gene. These mutations were not present in the strain that did not show the resistance. The authors discussed the risk of horizontal gene transfer of the antibiotic resistance and concluded that these strains were not at risk for horizontal gene transfer.

### 6.3.2. Gene Mining for Antibiotic Resistance Genes, Virulence Factors, and Mobile Genetic Elements

As mentioned in the previous section, the genomes of *L. plantarum* KABP-011, KABP-012, and KABP-013 were analyzed by Mukerji (2016) for antibiotic resistance. The isolated DNA from the strains was analyzed using the MiSeq platform using 250 bp paired-end reads. Assembly was performed *de novo* with SeqMan NGen 2.0 and annotated with RAST. Comparative genomics was performed using progressive MAUVE 2.3.1. The genomes were searched using the BLAST contigs function in RAST. Specific gene sequences that had been associated with antibiotic resistance were downloaded from NCBI. A total of 92 individual genes were evaluated which had been reported to show resistance to the lincosamide and aminoglycoside families of antibiotics, of which clindamycin and kanamycin are members, respectively. Additionally, the three strain genomes were compared with BLAST against the Antibiotic Resistance Gene-ANNOTation (ARG-ANNOT) database (Gupta et al., 2014) last modified on June 2, 2015. The authors noted that modifications of specific nucleotides in the 23S rRNA gene have been observed to confer antibiotic resistance (Vester and Douthwaite, 2001). Also, point mutations in the gene sequences of L4 and L22, which are ribosomal proteins, have been shown to generate antibiotic resistance (Zaman et al., 2007). Base substitutions in the 5 loci of the 23S rRNA gene were evaluated for the strains and the L4 and L22 gene sequences were evaluated for SNPs.

The KABP-011 draft genome consisted of 14 contigs and 3,225,239 bp for a coverage of 186x. The KABP-012 draft genome consisted of 30 contigs and 3,172,685 bp for a coverage of 162x. The KABP-013 draft genome consisted of 18 contigs and 3,098,101 bp for a coverage of 114x. No significant gene matches to the 92 genes from the NCBI database were found. When

blasted against the ARG-ANNOT database, one match to the chloramphenicol acetyltransferase gene, *cat*, was found in all three strains. The resistance to clindamycin and kanamycin in KABP-011 and KABP-012 could not be attributed to any known genes. In these two strains, the ribosomal mutations were evaluated. SNPs were observed in the 23S rRNA loci of the two strains but not in KABP-013. The three strains had identical L4 and L22 gene sequences, which were identical to the type strain. The strains were also evaluated for mobile genetic elements. The identified *cat* gene was not encoded on any plasmids and was not near any insertion elements or prophages. The authors concluded that the transfer of antibiotic resistance genes by the strains was not a risk.

An unpublished genome sequencing and mining was performed using a different NGS platform and compared to different updated databases. The genomes of *L. plantarum* KABP-011, KABP-012, and KABP-013 were sequenced as described previously in Section 2.3.3. The genomes were evaluated for the presence of antibiotic resistance genes, virulence factors, and mobile genetic elements.

In order to identify the presence of possible antibiotic resistance genes, the 3 annotated contigs from *L. plantarum* KABP-011 were compared against the last version of the Comprehensive Antibiotic Resistance Database (CARD), a bioinformatic database of resistance genes, their products, and associated phenotypes (Jia et al., 2017) updated April 25, 2019. This database contains 2,570 reference sequences, 1,233 SNPs of 82 pathogens, and more than 76,000 WGS assemblies. Contigs were compared by similarity using the program Resistance Gene Identifier (Jia et al., 2017), identifying potential hits after filtering them by having minimum sequence identity of 80% of sequence length and coverage of the reference gene greater than 50%. No antibiotic resistance genes were found in the sequenced strain of *L. plantarum* KABP-011.

The 5 annotated contigs from *L. plantarum* KABP-012 were also compared using the same criteria. No antibiotic resistance genes were found in the sequenced strain of *L. plantarum* KABP-012.

The 5 annotated contigs from *L. plantarum* KABP-013 were also compared using the same criteria. No antibiotic resistance genes were found in the sequenced strain of *L. plantarum* KABP-013.

The most up-to-date version of the Virulence Factors Database (VFDB) contains DNA sequences from 1,079 virulence factors from 951 bacterial strains having 32,436 virulence factor-related non-redundant genes information (Chen et al., 2016). All the sequences from each microorganism strain were compared to this database for the presence of virulence factors. Similarity was compared using the VF analyzer (Liu et al., 2019). Once basic housekeeping genes were discarded, *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 showed no hits in the database. Additionally, the genomes were compared to another database, VirulenceFinder (Joensen et al., 2014), using the ARIBA software. No virulence factors were identified. This indicates that the strains lack known genes for surface antigens, toxins, secretion systems, adhesion molecules, virulence-related nutrition mechanisms, hemolysis, or any other virulence factors.

Intrinsic antibiotic resistance in microorganisms consumed in food is not considered to be harmful. However, the transfer of genetic resistance via mobile genetic elements like prophages,

phages, plasmids, and transposons to pathogenic bacteria sharing the gastrointestinal habitat is of concern (Zheng et al., 2017). The genome sequences of the notified strains were compared against the latest version of the A CLAssification of Mobile genetic Elements (ACLAME) database (Leplae et al., 2009). Similarity was compared using Blast+ to identify potential hits. The thresholds used were having a minimum sequence identity of 80% and coverage of the reference gene greater than 50%.

Results for *L. plantarum* KABP-011 against the assembled contigs are shown in Figure 12. Data indicate the origin of detected mobile elements, the bacterial species in the ACLAME database with maximum sequence similarity, mobile element ACLAME identifier code (Gene ID), and the contig (assembled part of the genome) where the gene is located. The right columns indicate the parameters of the BLAST-identified hits, including the starting and ending points of the gene inside the contig, the sequence alignment length (in bp), the DNA sequence identity percentage and a brief description of the pathway in which the identified gene participates.

| CD | MGE Name | MGE Type | Host | Length (bp) | Identity (%) | GbkAnnot |
|---|---|---|---|---|---|---|
| contig01-CD1739 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 87 | 85 | hypothetical protein |
| contig01-CD2337 | pGdh442 | plasmid | Lactococcus lactis | 106 | 94 | putative truncated membrane protein |
| contig01-CD2338 | pGdh442 | plasmid | Lactococcus lactis | 251 | 99 | transport protein |
| contig01-CD2339 | pGdh442 | plasmid | Lactococcus lactis | 216 | 97 | ABC transporter |

**Figure 12: *L. plantarum* KABP-011 ACLAME hits (Kaneka 2019, unpublished).**

Results show 4 hits in all 3 annotated contigs obtained from the sequencing procedure followed in this analysis. All of them were described in the *Lactobacillus* genus except for the gene prophinder:42846 described in *P. pentosaceus* and 3 consecutive CDs with homology to pGdh442 plasmid described in *Lactococcus lactis*. Gene exchange events between species belonging to the same genus occur often in nature. Identified genes involve transporters, replication and repair proteins, secretion systems, transcriptional regulators and inner defense system proteins, none of them implying any risk of pathogenicity themselves.

These same sequences were compared against another database called PlasmidFinder 2.0.1 (Carattoli et al., 2014) which identifies genes and single nucleotide polymorphisms directly from NGS short reads; no mobile genetic elements were found.

No virulence or antibiotic resistance genes were found in a contiguous region to the detected mobile genetic elements. Thus, it is concluded that the sequenced strain from *L. plantarum* KABP-011 has no potential for transfer of pathogenicity or antibiotic resistance genes due to the presence of mobile genetic elements in its genome.

Results for similar analysis of *L. plantarum* KABP-012 are given in Figure 13.

| CD | MGE Name | MGE Type | Host | Length (bp) | Identity (%) | GbkAnnot |
|---|---|---|---|---|---|---|
| contig01-CD0867 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 135 | 84 | hypothetical protein |
| contig01-CD0868 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 126 | 84 | hypothetical protein |
| contig01-CD0869 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 217 | 92 | hypothetical protein |
| contig01-CD0870 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 124 | 95 | hypothetical protein |
| contig01-CD0871 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 61 | 85 | hypothetical protein |
| contig01-CD0878 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 119 | 91 | hypothetical protein |
| contig01-CD1047 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 152 | 94 | Phage terminase, small subunit |
| contig01-CD1048 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 632 | 95 | Phage terminase-like protein, large subunit |
| contig01-CD1049 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 64 | 95 | hypothetical protein |
| contig01-CD1050 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 387 | 93 | hypothetical protein |
| contig01-CD1051 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 410 | 91 | phage phi-C31 gp36 major capsid-like protein |
| contig01-CD1052 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 110 | 82 | hypothetical protein |
| contig01-CD1067 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 87 | 85 | hypothetical protein |
| contig01-GL1209 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 169 | 94 | restriction endonuclease |
| contig01-GL1214 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 251 | 95 | protease subunit of ATP-dependent Clp protease |
| contig01-PR1192 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 169 | 92 | restriction endonuclease |
| contig01-PR1197 | prophinder:42846 | prophage | Pediococcus pentosaceus ATCC 25745 | 251 | 95 | protease subunit of ATP-dependent Clp protease |

**Figure 13: *L. plantarum* KABP-012 ACLAME hits (Kaneka 2019, unpublished).**

After discarding all the hits described in *Lactobacillus* genus, the 17 hits remaining presented in Figure 13 belong to prophages described in *P. pentosaceus* ATCC 25745. Identified genes involve transporters, replication and repair proteins, secretion systems, transcriptional regulators and inner defense system proteins, none of them implying any risk of pathogenicity themselves.

These same sequences were compared against PlasmidFinder 2.0.1 (Carattoli et al., 2014) and no mobile genetic elements were found.

No virulence or antibiotic resistance genes were found in a contiguous region to the detected mobile genetic elements. Thus, it is concluded that the sequenced strain from *L. plantarum* KABP-012 has no potential for transfer of pathogenicity or antibiotic resistance genes due to the presence of mobile genetic elements in its genome.

Results for similar analysis of *L. plantarum* KABP-013 are given in Figure 14.

| CD | MGE Name | MGE Type | Host | Length (bp) | Identity (%) | GbkAnnot |
|---|---|---|---|---|---|---|
| contig02-CD0019 | pMRC01 | plasmid | Lactococcus lactis | 672 | 83 | protein TrsE |
| contig02-CD0044 | pMD136 | plasmid | Pediococcus pentosaceus | 186 | 80 | DNA invertase-like protein |
| contig02-CD0078 | pMRC01 | plasmid | Lactococcus lactis | 530 | 80 | protein TrsK |
| contig02-CD0085 | pMRC01 | plasmid | Lactococcus lactis | 672 | 81 | protein TrsE |
| contig02-CD0086 | pMRC01 | plasmid | Lactococcus lactis | 672 | 80 | protein TrsE |
| contig02-CD0087 | pMRC01 | plasmid | Lactococcus lactis | 672 | 92 | protein TrsE |
| contig02-CD0089 | pMRC01 | plasmid | Lactococcus lactis | 217 | 81 | protein TrsD |
| contig02-GL0026 | pMRC01 | plasmid | Lactococcus lactis | 217 | 81 | protein TrsD |
| contig02-GL0106 | pMRC01 | plasmid | Lactococcus lactis | 672 | 80 | protein TrsE |
| contig02-GL0108 | pMRC01 | plasmid | Lactococcus lactis | 672 | 92 | protein TrsE |
| contig02-GL0112 | pMRC01 | plasmid | Lactococcus lactis | 217 | 84 | protein TrsD |
| contig02-PR0027 | pMRC01 | plasmid | Lactococcus lactis | 217 | 81 | protein TrsD |
| contig02-PR0106 | pMRC01 | plasmid | Lactococcus lactis | 672 | 93 | protein TrsE |
| contig02-PR0110 | pMRC01 | plasmid | Lactococcus lactis | 217 | 84 | protein TrsD |
| contig04-CD0015 | pGdh442 | plasmid | Lactococcus lactis | 141 | 87 | truncated transposase of ISLpl1 |
| contig04-CD0016 | pGdh442 | plasmid | Lactococcus lactis | 141 | 80 | truncated transposase of ISLpl1 |
| contig04-CD0029 | pMD136 | plasmid | Pediococcus pentosaceus | 186 | 82 | DNA invertase-like protein |
| contig05-CD0029 | pMD136 | plasmid | Pediococcus pentosaceus | 186 | 83 | DNA invertase-like protein |
| contig05-GL0045 | pGdh442 | plasmid | Lactococcus lactis | 141 | 84 | truncated transposase of ISLpl1 |
| contig05-PR0045 | pGdh442 | plasmid | Lactococcus lactis | 141 | 84 | truncated transposase of ISLpl1 |

**Figure 14: *L. plantarum* KABP-013 ACLAME hits (Kaneka 2019, unpublished).**

After discarding all the hits described in *Lactobacillus* genus, 3 out of the 20 hits remaining presented in Table 1 belong to a pMRC01 plasmid of *Lactococcus lactis* and 17 hits of the pGdh442 plasmid described in *P. pentosaceus*. Identified genes involve transporters, replication and repair proteins, secretion systems, transcriptional regulators and inner defense system proteins, none of them implying any risk of pathogenicity themselves.

These same sequences were compared against PlasmidFinder 2.0.1 (Carattoli et al., 2014) and no mobile genetic elements were found.

No virulence or antibiotic resistance genes were found in a contiguous region to the detected mobile genetic elements. Thus, it is concluded that the sequenced strain from *L. plantarum* KABP-013 has no potential for transfer of pathogenicity or antibiotic resistance genes due to the presence of mobile genetic elements in its genome.

### 6.3.3. Biogenic Amines

*L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 were individually plated on petri dishes and inoculated in synthetic liquid medium by the Enolab at the University of Valencia. The microorganisms were allowed to grow for 3 days at 28°C. Histidine, tyrosine, ornithine, and lysine were added as precursors for the biogenic amines histamine, tyramine, putrescine, and cadaverine. The production of biogenic amines was evaluated by

HPLC as described in Bover-Cid and Holzapfel (1999). None of the three strains was able to produce biogenic amines under the assayed conditions.

### 6.3.4. Lactic Acid

Most lactic-acid bacteria produce some amount of D-lactate (from 1% of all lactate produced up to 97%, depending on the strain; with 40% being a typical amount). The notified strains were evaluated for lactic acid production by Enolab at the University of Valencia. A Boehringer Mannheim kit was used to quantify the L-lactic and D-lactic acid content directly. *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 were determined to produce L-lactic acid at (all g/L) 5.38, 5.90, and 6.36 concentrations and D-lactic acid at 4.16, 3.93, and 3.82 concentrations respectively.

There has in the past been concern expressed about the potential of D-lactic acid producing bacteria to contribute to D-lactic acidosis, a rare metabolic occurrence that has been reported only in individuals suffering from short bowel syndrome (Vitetta et al., 2017). The only medical indication that D-lactate producing strains should not be used is derived from older studies in which infants were fed formulas that were acidified with known amounts of D- and L-lactate (Stolley and Droese, 1971). Subsequent studies with acidified formulas have not supported these initial findings. The acidification was a direct result of the addition of chemical lactic acid and not naturally occurring acidification due to the fermentation of food matter.

Connolly et al. (2005) reviewed D-lactic acid and its metabolism and reported as follows:

1. There is no evidence to show that the normal gastrointestinal tract biota can induce D-lactic acidosis in the healthy human adult or infant.

2. D-lactic acid acidosis only occurs in subjects with a disturbed gastrointestinal function following bowel resection.

3. Well-controlled clinical trials (doses of $10^5$ to $10^9$ cfu/day for 28-30 days) in which the DL-lactic acid producing bacteria *Lactobacillus reuteri* was given to over 160 human newborn term and preterm infants clearly indicated that clinical signs of acidosis did not occur after *L. reuteri* administration at any dose tested.

4. Exposure of infants to the bacterium *L. reuteri* does not result in abnormal levels of D-lactic acid in the blood.

A more recent review of the literature (Lukasik et al., 2018) was performed to evaluate the ability of D-lactic acid producing bacteria, acidified infant formulas, and fermented infant formulas to cause pediatric D-lactic acidosis. The authors identified five randomized controlled trials from 2005 to 2017 which covered 544 healthy infants. No clinically relevant adverse events related to D-lactic acid were observed. Two case studies (5-year-old girl and 5-year-old boy) observed the development of acidosis from bacteria. Both children had short bowel syndrome secondary to intestinal resection. D-lactic acid producing bacteria do not pose a safety hazard to healthy children.

## 6.4. Research Studies of the Organisms

### 6.4.1. Studies of *L. plantarum* KABP-011, KABP-012, and KABP-013

#### 6.4.1.1. Animal Toxicity Study of *L. plantarum* KABP-011, KABP-012, and KABP-013

A seven day oral toxicity study in Wistar rats (Bosch et al., 2014) was performed for the *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 strains. Twelve 9-week-old Wistar rats (sex not reported) were equally divided into two groups (placebo and 1:1:1 strain combination) and dosed in the morning on two consecutive days at a level of $5x10^{10}$ cfu/kg bw/day with the bulk powder strain suspended in PBS solution or just PBS solution in the placebo group. Clinical observations were made for 5 additional days after dosing. Individual body weights were recorded on Day 0, 1, and then every two days. Water and feed consumption were monitored. At the end of the seven-day observation period, the animals were sacrificed with carbon dioxide. All animals were subjected to gross necropsy and mesenteric lymph node samples were taken to determine whether bacterial translocation had occurred. No signs of systemic toxicity were noted during the observation period and all animals showed expected gains in body weight; no differences in consumption of feed or water were observed. No abnormalities were noted at necropsy or histopathological examination. Bacterial translocation to the mesenteric lymph nodes and liver were similar between groups. The authors concluded that, "The results of the toxicity assay showed that *L. plantarum* CECT 7527, CECT 7528 and CECT 7529 were safe since they did not affect the animals' well-being and did not facilitate bacterial translocation even when administered at a high dose."

In an 8-week repeated-dose oral study in 5-week-old male Sprague Dawley rats, the effect of the combination of the strains on cholesterol metabolism was evaluated (Kim et al., 2014). Forty rats were divided into four equal groups. Hyperlipidemia in the rats was induced and maintained with a high-fat diet that was consumed throughout the study. Five rats were fed a control diet to confirm that the high fat diet induced the hyperlipidemia. The four groups were a control group, low-dose $0.6x10^9$ cfu/day, medium-dose $1.2x10^9$ cfu/day, and high-dose $2.4x10^9$ cfu/day administered daily by gavage in sterile saline solution. Dietary intake was measured every two days and body weight was measured weekly. Blood lipids, blood glucose, liver lipids, and organ weights were analyzed at the end of the experiment. The high-dose group had significantly less food intake and significantly less weight gain. All groups had significantly less gain in liver weight than the control group. The medium and high-dose groups had significantly lower blood serum levels of total cholesterol and LDL cholesterol. The high- dose group also had significantly lower liver levels of total lipids and total cholesterol and lower serum leptin. The authors concluded that, "The results of this study indicate that the *L. plantarum* mixture may exhibit anti-obesity and cholesterol-lowering effects, which suggest that the *L. plantarum* mixture has the potential to be [of use] in the management of obesity and hypercholesterolemia."

A 90-day repeated-dose oral toxicity study in 7-week-old male and female Crl:CD®(SD) rats according to OECD and FDA Redbook 2000 guidelines was reported by Mukerji et al., (2016). Three groups of rats with 20 rats per group (10 of each sex) were individually housed, provided feed and water *ad libitum*, and dosed by gavage daily for 90 days with PBS solution, $5.5x10^{10}$ cfu/kg bw/day, and $1.85x10^{11}$ cfu/kg bw/day of the strains in a 1:1:1 ratio. Enumeration of the bacteria count was performed at the beginning, middle, and end of the study, confirming the cfu count. Mortality and morbidity were assessed at least twice daily, and an additional

clinical examination was conducted daily 1-3 hours after dosing. Body weights and food consumption were measured weekly and detailed clinical observations were conducted weekly. Ophthalmologic examinations were conducted prior to initiation of dosing and near the end of the study. Fecal samples for bacterial and chemical analysis were collected from each animal approximately monthly, starting prior to initiation of dosing. At the end of the dosing period, rats were fasted in metabolism cages overnight for at least 15 hours for urine collection. On the day of sacrifice, blood samples for hematology, clinical chemistry, and coagulation parameters were collected. Urinalysis results were tabulated for volume, specific gravity, pH, total protein, and urobilinogen. The following blood parameters were assessed:

> Red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, absolute reticulocyte count, platelet count, white blood cell count and differential white blood cell count (absolute neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells, prothrombin time and activated partial thromboplastin time, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, creatinine, total cholesterol, triacylglycerol, glucose, total protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium, chloride, and total bile acids.

The external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera were evaluated. Absolute and relative (to body and brain weight) organ weights were determined for the liver, kidneys, adrenal glands, thymus, brain, spleen, heart, and reproductive organs. Tissues from the following organ systems were preserved in fixative, processed to slides, and stained with hematoxylin and eosin:

> Digestive system (liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands, and pancreas), urinary system (kidneys and urinary bladder), respiratory system (lungs, trachea, nose, larynx, and pharynx), cardiovascular system (heart and aorta), hemato-poietic system (spleen, thymus, mandibular lymph node, mesenteric lymph node, bone marrow, and Peyer's patches), endocrine system (pituitary, thyroid, parathyroid, and adrenal glands), nervous system (brain, spinal cord and sciatic nerve), musculoskeletal system (skeletal muscle, femur/knee joint, sternum), reproductive system of males (testes, epididymides, prostate, and seminal vesicles) and females (ovaries, uterus, mammary glands, and vagina), Harderian glands, skin, and eyes (including retina and optic nerve).

Tissues collected from animals in the control and 1000 mg/kg BW/day groups were evaluated microscopically.

Samples of whole blood, liver, and mesenteric lymph nodes were collected at necropsy and incubated under both aerobic and anaerobic conditions; bacterial colonies on anaerobic plates were evaluated for identification of the test strains. Each colony was assessed morpho-logically for shape, margin, elevation, size, texture, appearance, pigmentation and optical property.

Wet fecal samples were collected and analyzed for bacterial identification and enumeration and for primary and secondary bile acids, neutral sterols, short-chain fatty acids, branched-chain fatty acids, and lactic acid.

One animal died due to an intubation error, but all other animals survived to sacrifice. Body weight, weight gain, feed consumption, and feed efficiency showed no effects from the test article. The authors stated that there were no effects or abnormalities in the hematology, clinical chemistry, coagulation parameters, and urinalysis parameters attributable to the intervention. Macroscopic observation at necropsy showed no treatment-related abnormalities. The test strains

were not translocated to the blood of any animal. Translocation to the liver and mesenteric lymph nodes was observed at low to moderate numbers. The authors concluded that this was consistent with previous publications that showed low levels of lactobacilli translocation. This did not pose a safety concern at the low levels seen and the clinical, hematological, and microscopic findings were indicative of the safety. The no observed adverse effect level (NOAEL) in male and female rats was determined to be $1.85x10^{11}$ cfu/kg bw/day, the highest dose level evaluated (Mukerji et al., 2016).

These animal studies are summarized in Table 4.

#### 6.4.1.2. Human Clinical Studies of *L. plantarum* KABP-011, KABP-012, and KABP-013

The human clinical studies are summarized in Table 5.

**Table 4. Animal Studies of *L. plantarum* KABP-011, KABP-012, and KABP-013.**

| Study Design | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Acute oral toxicity study in the Wistar rat. | 12 9-week old Wistar rats | (a) 1.5 mL PBS solution<br><br>(b) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $5x10^{10}$ cfu/kg in 1.5 mL PBS | 2 consecutive daily doses and 7 total days | No signs of systemic toxicity were noted during the observation period. All animals showed expected gains in body weight over the observation period. No abnormalities were noted at necropsy. Bacterial translocation to the MLN and liver were similar between groups. | Bosch et al., 2014 |
| 8-week repeated-dose oral toxicity study in Sprague Dawley rats | 40 5-week old male Sprague Dawley rats induced into hyperlipidemia | (a) Sterile Saline solution<br><br>(b) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $0.6x10^{9}$ cfu/day<br><br>(c) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $1.2x10^{9}$ cfu/day<br><br>(d) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $2.4x10^{9}$ cfu/day | 60 days | The highest-dose group had significantly less weight gain and feed intake than the control group. All groups had significantly less gain in liver weight than the control group. The medium and high-dose groups had significantly lower blood serum levels of total cholesterol and LDL cholesterol. The high- dose group also had significantly lower liver levels of total lipids and total cholesterol and lower serum leptin. | Kim et al. 2014 |
| 90-day subchronic oral toxicity study in Crl:CD(SD) rats | 30 male and 30 female Crl:CD(SD) rats | (a) PBS solution<br><br>(b) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $5.5x10^{10}$ cfu/kg bw/day<br><br>(c) *L. plantarum* KABP-011, KABP-012, and KABP-013 (1:1:1) at $1.85x10^{11}$ cfu/kg bw/day | 90 days | A NOAEL was determined of $1.85x10^{11}$ cfu/kg bw/day for both male and female rats. Body weight, weight gain, food consumption, and food efficiency showed no effects from the test vehicle. The authors stated that there were no effects or abnormalities in the hematology, clinical chemistry, coagulation parameters, and urinalysis parameters attributable to the intervention. Macroscopic observation at necropsy showed no treatment related abnormalities. | Mukerji et al., 2016 |

**Table 5. Human Clinical Studies of *L. plantarum* KABP-011, KABP-012, and KABP-013.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on cholesterol and blood lipids in hypercholesterolemic subjects | Sixty subjects (34M and 26F, 51.8 ± 7.2 years) with 30 in the placebo and intervention arms. Spain | (a) *L. plantarum* KABP-011, KABP-012, and KABP-013 in a 1:1:1 ratio were consumed as a capsule at $3.01x10^9$ cfu/day at the start of the study which had been reduced to $1.28x10^9$ cfu/day at the end of the study<br><br>(b) Placebo capsule | Daily for 12 weeks<br><br>4-week follow-up | All subjects completed the study. Body weight and BMI significantly decreased from baseline for both groups but not between groups. Assayed blood glucose, creatinine, GOT, GPT, GGT, and liver enzymes stayed within normal physiological limits and were not significantly different than baseline values. There were no treatment related adverse events observed. | Fuentes et al., 2013, 2016 |
| An abstract for a presentation at the 10th Workshop of the Spanish Society of Probiotics and Prebiotics (SEPyP 2019) describes a prospective, observational study of subjects initiating consumption of KABP-011, KABP-012, and KABP-013. | 343 subjects (median age of 55 years, 63% female) | *L. plantarum* KABP-011, KABP-012, and KABP-013 in a 1:1:1 ratio were consumed as a capsule at $1.2x10^9$ cfu/day | Daily for 12 weeks | Seventeen percent of patients reported tolerability issues but none of them were considered severe. The tolerability issues correlated with antiplatelet use only. No adverse events were reported. | Espadaler et al., 2019 |

### 6.4.2. Studies of Other Strains of the Species

Selected human intervention studies of *L. plantarum* strains other than the notified strains are summarized in Table 6.

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum.***

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|---|---|---|---|---|---|
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| - | | - | | | |
| - | - - | | | - | |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Case study of short-bowel patient with small-bowel bacterial overgrowth | 16-year-old boy<br>Received Lp299v | $10^{10}$ cfu *L. plantarum* 299v/day | Not reported | Replacement of the antibiotic with *L. plantarum* 299v produced good therapeutic response with no reported adverse effects. No indication of D-lactic acidosis was reported in this short-bowel patient receiving *L. plantarum*. | Vanderhoof et al., 1998 |
| Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of *L. plantarum* 299v on markers of CVD | 30 apparently healthy males with a mean age of 42.6 years<br>Lp299v: 15 | $1x10^{10}$ cfu *L. plantarum* 299v | 6 weeks | No adverse effects were reported from the ingestion of $1x10^{10}$ cfu/day of *L. plantarum* 299v. | Bukowska et al., 1998 |
| Prospective, randomized, double-blind, placebo-controlled study of the effects of *L. plantarum* 299v on metabolic endpoints and fecal bacteria | 48 apparently healthy adults (11 males and 37 females) with a mean age of 37 years.<br>Lp299v: 26 | $2x10^{10}$ cfu *L. plantarum* 299v | 21 days | No participants dropped out and there were no differences in adverse events reported by the 2 groups. 5 individuals in each group reported transient nausea or abdominal discomfort. Those receiving live bacteria had an increase in stool volume, a decrease in flatulence, and increases in fecal levels of carboxylic acids, but no change in fecal pH. *L. plantarum* 299v was found in large numbers in the feces of the test group at weeks 1 and 3, but in only 5 of the 26 individuals 8 days later. No other changes were seen in the fecal microbiota. | Johansson et al., 1998 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, single-blind placebo-controlled trial of the ability of *L. plantarum* 299v to reduce the incidence of infective diarrhea among children | 143 children aged 6 months to 3 years attending daycare in a region of Brazil with a high incidence of infectious diarrhea.<br>Lp299v: 71 | $10^{10}$ cfu *L. plantarum* 299v/day | 3 months | No adverse effects were reported from the ingestion of $10^{10}$ cfu/day of *L. plantarum* 299v by these young children. | Ribeiro and Vanderhoof, 1998 |
| Prospective, randomized, double-blind, placebo-controlled study of the effects of fermented and unfermented cereal gruel with *L. plantarum* 299v on the presence of fecal enteric bacteria | 151 apparently healthy children aged 6 months to 5 years.<br>Lp299v: 50 | The daily dose of *L. plantarum* 299v was not reported | 13 days | The proportion of children in the experimental and control groups harboring enteric bacteria (campylobacter, salmonella, shigella, *E. coli* O157, and enterotoxigenic *E. coli*) did not differ. There were no reported adverse effects due to consumption of fermented cereals. | Kingamkono et al., 1999 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on the symptoms of AIDS | 15 immunocom-promised children (5 males and 10 females) with HIV, aged 11.5 months to 14 years. All received Lp299v. | $2x10^{10}$ cfu *L. plantarum* 299v | About 1 month | No patient experienced bloating or other symptoms of intolerance, and none had to be withdrawn. No bacteria were detected in rectal swabs by the end of the first month after cessation of administration. The authors concluded, "The data suggest that *L. plantarum* 299v may be given safely to the immune-compromised host and may indeed have a positive effect on immune response." | Cunningham-Rundles et al., 2000 |
| Prospective, randomized, double-blind, placebo-controlled trial of the effect of attempted alteration of the gastrointestinal microecology of IBS patients with *L. plantarum* 299v | 52 adult patients with IBS.<br>Lp299v: 25 | $2x10^{10}$ cfu *L. plantarum* 299v/day | 4 weeks | *L. plantarum* 299v was found in the fecal samples from 84% of the test group, but there were no changes or differences between test and control groups in other bacterial counts. The authors noted that the products were well tolerated and no treatment-related adverse effects were reported from ingestion of $2x10^{10}$ cfu/day of *L. plantarum* 299v for 4 weeks. | Nobaek et al., 2000 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| 3 open label experiments on the ability of *L. plantarum* 299v to colonize tonsilar epithelia | 1st experiment: 6 adults aged 33-42 years (1 man and 5 women, mean age = 38 years);<br><br>2nd experiment: 2 women aged 41 and 42 years;<br><br>3rd experiment: same 2 women<br>All received Lp299v. | 1st experiment: 1 dose of $2x10^{11}$ cfu *L. plantarum* 299v;<br><br>2nd experiment: 1 dose of $10^{11}$ cfu *L. plantarum* 299v;<br><br>3rd experiment: 1 dose of $10^{10}$ cfu *L. plantarum* 299v | Single doses | The authors concluded that, since the bacteria could be isolated from tonsillar epithelia up to 8 hours after ingestion despite the constant flow of saliva and beverages over the tonsils, "the bacteria under investigation may possess the capacity to adhere to tonsillar cells." No adverse effects were reported. | Stjernquist-Desatnik et al., 2000 |
| Prospective, randomized, double-blind, placebo-controlled trial of the effect of *L. plantarum* 299v on IBS patients | 40 IBS patients (8 males and 32 females aged 27-63 years, mean = 45 years).<br>Lp299v: 20 | $2x10^{10}$ cfu *L. plantarum* 299v/day | 4 weeks | The authors noted that "No treatment related side-effects were observed." | Niedzielin et al., 2001 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, unblinded study to test if *L. plantarum* 299v administered before and after abdominal surgery reduces the incidence of sepsis | 129 patients (75 males and 54 females with median age = 68 years).<br>Lp299v: 64 | $2.5x10^{10}$ cfu *L. plantarum* 299v/day | median = 2 weeks | No differences were seen between the experimental and control groups in bacterial translocation to the lymph nodes or ileal serosa, gastric colonization, C-reactive protein levels, septic complications, or mortality. There were no reported adverse effects. | McNaught et al., 2002 |
| Prospective, randomized, double-blind, placebo-controlled trial of the ability of *L. plantarum* 299v to reduce symptoms of CVD risk factors in smokers | 36 apparently healthy 25-45-year-old smokers (18 of each sex).<br>Lp299v: 18 | $2x10^{10}$ cfu *L. plantarum* 299v/day | 6 weeks | No adverse events were reported; *L. plantarum* 299v was found in fecal samples from 13 of the 18 members of the test group. All of the biochemical changes were beneficial; no adverse changes were seen. | Naruszewicz et al., 2002 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, double-blind, placebo-controlled crossover trial of the effect of *L. plantarum* 299v on colonic fermentation of IBS patients | 12 (1 male and 11 female aged 23-61 years, mean age = 40.6 years) gastroenterologic IBS outpatients. All received Lp299v. | $6.3x10^9$ cfu *L. plantarum* 299v/day | 4 weeks | No difference was seen between the groups on any measure: exhalation of hydrogen and methane during calorimetry, breath hydrogen after lactulose ingestion, or daily symptom scores. The authors concluded that "*Lactobacillus plantarum* 299v in this study did not appear to alter colonic fermentation." There were no adverse effects. | Sen et al., 2002 |
| Prospective, randomized, double-blind, placebo-controlled study of the ability of *L. plantarum* 299v to reduce the likelihood of further recurrent episodes of *Clostridium difficile*-associated diarrhea | 21 patients (1 male and 20 females; mean age = 63.8 years) testing positive for *C. difficile* toxin and having a history of previous *C. difficile*-associated diarrhea. Lp299v: 12 | $5x10^{10}$ cfu of *L. plantarum* 299v/day | 38 days | There was a statistically insignificant reduction in the risk of recurrence among the patients receiving *L. plantarum* 299v, and the authors noted that "Treatment with the lactobacilli had no apparent side-effects." | Wullt et al., 2007; Wullt et al., 2003 |
| Prospective, randomized, double-blind, placebo-controlled study of the effect of a test beverage with *L. plantarum* 299v on plasma antioxidant capacity and fecal bacteria | 98 volunteers with a high working pace (39 men and 59 women aged 21-61 years [mean age = 35 years] Lp299v: 50 | $2.2x10^{10}$ cfu *L. plantarum* 299v/day | 4 weeks | There was no difference between the test and control groups in the incidence or nature of adverse effects and there were no adverse events that could reasonably be attributed to ingestion of *L. plantarum* 299v. | Onning et al., 2003 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled study to evaluate the effect of a fermented oat product containing *L. plantarum* 299v on children's intestinal function and microbiota | 69 apparently healthy children aged 6 months to 3 years<br>Lp299v: 33 | $1.4x10^{11}$ cfu *L. plantarum* 299v | 3 weeks | Product-related adverse events were reported for 5 children, 4 in the experimental group and 1 control: 3 children in the experimental group developed constipation and one child had regurgitations (which had begun before feeding commenced); one placebo-group child had softer than normal stools. No differences were seen between groups in stool frequency and consistency, flatulence, vomiting, or intestinal pain. The authors concluded that "the children tolerated the fermented oat product well." | Berggren et al., 2003 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on gut ecology and microbiota | 20 apparently healthy adults (9 males and 11 females); mean age = 32.9 years). Lp299v: 10 | $2x10^{11}$ cfu *L. plantarum* 299v | 4 weeks | No side effects were reported attributable to individuals consuming *L. plantarum* 299v; all had the strain in their feces, but it could be recovered from only one person a week after the end of ingestion. No adverse effects were reported. | Goossens et al., 2003 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, unblinded study of the ability of *L. plantarum* 299v to adhere to the gut mucosa of critically ill patients | 15 critically ill patients admitted to the ICU, 8 males and 9 females aged 33 to 84 years (mean = 64.6 years).<br>Lp299v: 8 | $2x10^{11}$ cfu *L. plantarum* 299v/day | Duration of stay in the ICU—4-37 days; median = 11 days | All patients tolerated total or partial enteral feeding; there were no differences in diarrhea, bloating, illness severity, length of ICU stay, 6-month mortality, levels of C-reactive protein, or leukocyte count. | Klarin et al., 2005 |
| Prospective, randomized, double-blind, placebo-controlled study of the survival of *L. plantarum* 299v in the GI tract and its effects on fecal microbiota, with and without gastric acid inhibition. | 29 apparently healthy volunteers (9 males and 20 females, mean age = 28.5 years). All received Lp299v. | $2x10^{11}$ cfu *L. plantarum* 299v | 2 weeks | No side effects were reported and there were no differences between groups in defecation frequency, stool consistency, fecal pH, or concentrations of SCFA. The administered strain was detected in the feces of all participants at the end of administration, but only in one 4 weeks later.. | Goossens et al., 2005 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, unblinded study of the effect of *L. plantarum* 299v on gut barrier function and systemic inflammatory response in critically ill patients | 103 patients (58 males and 45 females aged 28-90 years; median age = 71 years) within 24 hours of admission to the ICU.<br>Lp299v: 52 | $10^{10}$ cfu *L. plantarum* 299v/day | Until discharge from the hospital—3-17 days; median = 9 days | The mortality rate was 35% in both groups. 68 septic complications occurred; there were no differences in incidence, causes, or severity between test patients and controls. The authors concluded that "the results of this prospective randomised trial suggest that *Lactobacillus plantarum* 299v may attenuate the systemic inflammatory response in critically ill patients. This was not accompanied, however, by any significant changes in gastrointestinal microflora, endotoxin exposure, intestinal permeability, septic morbidity or mortality." | McNaught et al., 2005 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind crossover-design study to assess the effect of *L. plantarum* 299v on absorption of non-heme iron from a phytate-rich meal | 24 apparently healthy women with a mean age of 25 years. All received Lp299v. | $1.1x10^{11}$ cfu *L. plantarum* 299v | 2 days | There were no reported adverse events. | Bering et al., 2006 |
| Prospective, randomized, double-blind, placebo-controlled study to assess the effect of ingestion of *L. plantarum* 299v on fecal bacterial ecology and mucosal adhesion of bacteria | 29 apparently healthy patients (16 males and 13 females with a mean age of 56.9 years) undergoing colonoscopic examination for polyps.<br>Lp299v: 15 | $2x10^{11}$ cfu *L. plantarum* 299v | 2 weeks | No side effects were reported, and no differences were seen between groups in defecation frequency or stool consistency. | Goossens et al., 2006 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled crossover study to test the effect of lyophilized *L. plantarum* 299v on absorption of non-heme iron | 18 apparently healthy women with a mean age of 22 years. All received Lp299v. | $10^{11}$ cfu viable lyophilized *L. plantarum* 299v | 2 days | Iron absorption was no higher with *L. plantarum* 299v than without. The authors suggested that the lack of effect of the intervention could be explained by the bacteria not being in an active state, but there were no adverse effects. | Bering et al., 2007 |
| Prospective, randomized, double-blind, placebo-controlled study of the capacity of *L. plantarum* 299v to reduce *Clostridium difficile*-associated disease in critically ill patients | 44 ICU patients (26 males and 18 females aged 18-89 years; mean age = 64.7 years) receiving antibiotic therapy.<br>Lp299v: 22 | $1.6x10^{11}$ cfu (later $8x10^{10}$ cfu) *L. plantarum* 299v/day | Duration of stay in the ICU—2.5-22 days; mean = 5.5 days | Two patients from each group died in the ICU; 1 patient from the experimental group died in the hospital, and 4 patients from the control group died within 6 months. There were no differences between groups in sequential organ failure, length of ICU stays, or days on ventilators. There were no differences in C-reactive protein, TNF-α, IL-1β, or IL-6; IL-10 and white blood cell counts were higher in the control group than in patients receiving *L. plantarum* 299v. Gut permeability was higher in the control group than in the test group. The study product was well tolerated and the authors stated that "We found no adverse impact of the given preparation." | Klarin et al., 2008 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled study of the effect of *L. plantarum* 299v on GI symptoms during antibiotic therapy | 239 patients (93 males and 146 females; median age = 45 years) receiving antibiotic therapy for infectious disease.<br>Lp299v: 80 | $1x10^{10}$ cfu *L. plantarum* 299v/day | Until 7 days after the end of antibiotics | 76 patients withdrew or were excluded from the study, 38 each from the test and placebo groups; reasons for withdrawal did not differ between the groups. Diarrhea was infrequent (only 5 and 6 patients in the placebo and test groups, respectively). The authors reported that "No side effects of the treatment were recorded." | Lönnermark et al., 2010 |
| Prospective, randomized, double-blind, placebo-controlled, multi-center study of the capacity of *L. plantarum* 299v to reduce symptoms of IBS | 200 IBS patients (141 males and 59 females; mean age = 37.8 years).<br>Lp299v: 98 | $10^{10}$ cfu *L. plantarum* 299v/day | 4 weeks | No changes were observed in pulse or respiratory rates, blood pressure, or body temperature, and no side effects were reported. | Sawant et al., 2010 |
| Randomized, double-blind, placebo-controlled trial to evaluate microorganism effect on patients with IBS meeting the Rome III criteria. | 214 subjects with IBS with 108 (70M, 38F; mean age 36.5± 12.1 years) in the intervention arm; 106 (81M, 25F, mean age 38.4±13.1 years) in the placebo arm. | *L. plantarum* 299v at $1x10^{10}$ cfu/day as a capsule | Daily for 4 weeks | No significant side effects were reported in the study. Transient vertigo was noted by one patient in the intervention arm and no change in blood parameters was detected throughout the study. | Ducrotte et al., 2012 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective open-label study of treatment of ulcerative colitis with *L. plantarum* 299v | 39 ulcerative colitis patients (15 males and 24 females aged 19-50 years [median age – 35 years])<br>Lp299v: 39 | $2.5x10^{10}$ cfu for 2 days, then $5x10^{10}$ cfu thereafter | Up to 176 days | The authors reported: "No major AEs were reported and there were no dropouts due to AEs. An increased number of bowel movements were reported by 11 patients (28%), bloating by four (10%) and an increased number of bowel movements and bloating by three (8%). All AEs were self-limiting or managed by dose adjustments. For example, if a patient experienced a presumable AE during the introduction of Profermin®, the period with the low Profermin® dose was prolonged for up to 2 wk. None of the 8 dropouts or 4 excluded patients left the trial due to deterioration in UC symptoms." | Krag et al., 2012 |
| Prospective, randomized, double-blind, placebo-controlled trial of the effect of prophylactic *L. plantarum* 299v on pathogenic bacteria, translocation, and cell proliferation in colon surgery | 64 patients (36 males and 28 females aged 64 to 80 years; median age = 72 years) referred for colonic resection<br>Lp299v: 32 | $10^{11}$ cfu *L. plantarum* 299v | 14 days | There were no differences between groups in the incidence of enteric pathogenic bacteria, bacterial translocation, or postoperative complications. The authors noted that, "No adverse effects were recorded after the administration of high doses of *L. plantarum* 299v." | Mangell et al., 2012 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled pilot study of effects of *L. plantarum* 299v on obstructive jaundice during biliary drainage | 17 biliary-drainage patients (12 men, 5 women aged 48 to 75 years [median = 52 years])<br>Lp299v: 5 | The daily dose of *L. plantarum* 299v was not reported | 7 days | The findings of the hematological and biochemical analyses were not reported, suggesting that the findings were not remarkable. There were no reports of adverse effects from the treatment. | Jones et al., 2013 |
| Prospective randomized single-blind two-arm study comparing Profermin® and Fresubin as treatments for ulcerative colitis | 73 ulcerative-colitis patients (33 males and 40 females aged 20-78 years; mean age 41 years)<br>Lp299v: 32 | Median = $4.89 \times 10^{10}$ cfu of *L. plantarum* 299v | | The authors reported that, "No major adverse events (AEs) were reported, but 3 patients experienced AEs. In the Fresubin group, one experienced an 'obvious weight gain' and one felt it induced vomiting. In the Profermin group, one suffered from rumbling and bloating." They concluded that, "Supplementation with Profermin is safe, well tolerated, palatable." | Krag et al., 2013 |
| Prospective, randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on systemic immunity, blood chemistry, bowel movements, and influenza specific antibodies in the elderly | 50 institutionalized subjects with 47 completing the study (26M and 21F >65 years), 19 in the high-dose arm, 13 in the low-dose arm, and 15 in the placebo arm. Spain | (a) *Lactobacillus plantarum* KABP-031 and KABP-032 at $5 \times 10^8$ cfu/day in 20g of powdered skim milk diluted into 200 mL water<br>(b) *Lactobacillus plantarum* KABP-031 and KABP-032 at $5 \times 10^9$ cfu/day in 20g of powdered skim milk diluted into 200 mL water<br>(c) 20g of powdered skim milk diluted into 200 mL water | Daily for 12 weeks<br><br>12 weeks follow-up | There were no significant changes in the BMI, Barthel Index, and routine laboratory tests between the groups during the treatment or follow-up periods. The laboratory tests measured albumin, glucose, total cholesterol, triglycerides, creatinine, AST, ALT, ALP, GGT, total bilirubin, hemoglobin, leukocytes, and platelets with no reported adverse effects. | Mane et al., 2011<br><br>Bosch et al., 2011<br><br>Bosch et al., 2012b |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Randomized, double-blind, placebo-controlled trial to evaluate microorganism effects on patients with IBS symptoms meeting the Rome II criteria. | 81 adults (mean age 48 years) with 54 (2M, 52F; mean age 48.15±13.48 years) in the intervention arm; 27 (27F; mean age 47.27±12.15 years) in the placebo arm. | *L. plantarum* 299v at $0.5x10^9$ cfu/capsule ($1x10^{10}$ cfu/day) | 2x daily for 8 weeks | The treatment did not provide symptomatic relief versus the placebo. One patient suffered a severe rash as an adverse event, but the rash was attributed to an unrelated gynecologic condition. | Stevenson et al., 2014 |
| Retrospective open-label study of the use of *L. plantarum* 299v to reduce the incidence of *Clostridium difficile* infection | 356 organ trans-plant patients, 174 before *L. plantarum* 299v and 182 after<br>Lp299v: 182 | $10^9$ cfu *L. plantarum* 299v | Not reported | Of these patients, 21 in the first year and 2 in the second year were diagnosed with *C. difficile* infection, infection rates of 12.1 and 1.1%, respectively. No adverse effects were reported due to the treatment. | Kujawa-Szewieczek et al., 2015 |
| Prospective open-label multi-center pilot study of safety and efficacy of prophylactic use of *L. plantarum* 299v in children and adolescents undergoing hematopoietic cell transplantation | 30 children and adolescents (16 males and 14 females aged 7.7±4.7 years; the age range was 2.2 to 17.3 years)<br>Lp299v: 30 | $10^8$ cfu *L. plantarum* 299v/kg bw/day | 21 days | No episodes of *L. plantarum* bacteremia were observed. The authors reported that, "We did not observe any serious adverse events or unexpected severe adverse events attributed to [*L. plantarum*] in any patient enrolled to the study." The authors concluded that "Our study provides preliminary evidence that administration of [*L. plantarum*] is safe and feasible in children and adolescents undergoing [hematopoietic cell transplantation]." | Ladas et al., 2016 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Prospective, randomized, double-blind, placebo-controlled, multi-center study of the ability of *L. plantarum* 299v to treat *Salmonella* infection | 149 patients with *Salmonella* infections (40 males and 109 females aged 5 to 68 years; median age = 36 years)<br>Lp299v: 77 | $5x10^{10}$ cfu *L. plantarum* 299v | Median of 26 days | The authors reported a non-statistically significant tendency for a greater number of gastrointestinal symptoms to be reported by patients consuming *L. plantarum* 299v. The conclusion of the authors was that, "Our results give little support for positive effects of *L. plantarum* 299v treatment in nontyphoid salmonellosis." | Lönnermark et al., 2015 |
| 2 prospective, randomized, single-blind, placebo-controlled, cross-over trials to assess the ability of *L. plantarum* 299v to improve iron absorption | 22 apparently healthy Swedish women of reproductive age, 11 in each trial<br>Lp299v: 11 | $10^9$ or $10^{10}$ cfu *L. plantarum* 299v in trials 1 and 2 | 4 days | No adverse effects were reported. | Hoppe et al., 2015 |
| Prospective, randomized, double-blind, placebo-controlled trial of the effects of *L. plantarum* 299v and *Bifidobacterium infantis* Cure 21 on patients with poor ileal pouch function | 32 patients with impaired pouch function, 24 men and 8 women, aged 27-70 years (median age = 50 years)<br>Lp299v: 16 | $10^{10}$ cfu each of *L. plantarum* 299v and *B. infantis* Cure 21 | 21 days | There were no differences on any measures between the experimental and placebo groups and the authors concluded that, "The current study failed to confirm the hypothesis that [the interventions] improve function in patients with poor pouch function." There was no discussion of adverse effects of the treatment. | Bengtsson et al., 2016 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Randomized, double-blind, placebo-controlled trial to evaluate microorganism effect on side effects of outpatient antibiotic treatment of children. | 438 children (1-11 years) with 218 (113M, 105F; mean age 5.1±2.6 years) in the intervention arm and 220 (122M, 98F; 5.2±2.9 years) in the placebo arm. | *L. plantarum* 299v at $1x10^{10}$ cfu/day as a capsule | Daily for 15-28 days | No beneficial effect was observed related to the incidence of loose/watery stools, mean number of loose/watery stools, or the incidence of abdominal symptoms. 155 adverse events were reported in 99 children by the parents. Children with adverse events were significantly more common in the placebo arm (27.3% vs. 17.9%, *P*=0.02). The most frequent events were pyrexia, headache, rash, anorexia, cough viral infection, and ear pain. There were no serious adverse events reported. | Olek et al., 2017 |
| Randomized, prospective, placebo-controlled trial to evaluate the microorganisms' effect on blood lipid profiles. | 46 adults with mild hypercholesterolemia 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. | *L. plantarum* ECGC 13110402 at $2x10^9$ cfu/capsule ($4x10^9$ cfu/day) | 2x daily for 12 weeks | No findings of clinical significance were identified in pro-inflammatory biomarkers and no significant differences in bowel parameters were observed. | Costabile et al., 2017 |
| Double-blind, randomized, placebo-controlled, parallel-group trial to evaluate microorganism's effects on gingival inflammation (GI), plaque index (PII), angulated bleeding core (AngBS), and microbial composition. | 59 apparently healthy adults (31.7± 12.8 years) with 30 in the intervention arm. | *L. brevis* CECT 7480, *L. plantarum* CECT 7481, and *P. acidilactici* CECT 8633 (1:1:1 ratio) $2.0x10^9$ cfu/day ($1.0x10^9$ cfu/dose) in chewable tablets. | 2x daily for 6 weeks | One subject from the test group and 6 from the control group withdrew, none due to intervention-related adverse events. There was no significant difference in compliance between groups, nor in gingival inflammation. 4 patients in the intervention arm and one patient in the placebo arm reported adverse events; no serious adverse events were reported. | Montero et al., 2017 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' and FOS effects on pregnant women with diabetes. | 90 pregnant women with gestational diabetes with 45 receiving intervention (mean age 30.3±5.6 years). | *L. acidophilus* ($5\times10^{10}$ cfu/g), *L. plantarum* ($1.5\times10^{10}$ cfu/g), *L. fermentum* ($7\times10^{9}$ cfu/g), *L. Gasseri* ($2\times10^{10}$ cfu/g) and 38.5 mg of FOS as a capsule | Daily for 6 weeks | The supplementation did not show any significant effects on glycemia and insulin resistance/sensitivity indices. The authors note that "none of the participants have reported specific side effects of synbiotic supplements." | Nabhani et al., 2018 |
| Randomized, double-blind, placebo-controlled trial to evaluate the microorganism's ergogenic effect on endurance performance. | 16 healthy males aged 20-40 years with no professional athletic training. Eight were in each arm. | $1 \times 10^{11}$ cfu *L. plantarum* TWK10 as a capsule | Daily for 6 weeks | There was no discussion of adverse events. | Huang et al., 2018 |
| Randomized, double-blind, placebo-controlled trial to evaluate the synbiotics effects on constipated adults. | 85 constipated adults per Rome-III standards. 36F and 7M, aged 29.5±8.34 years in the n=43 intervention arm. | $1x10^{10}$ cfu/day of *L. plantarum* LP01, *B. lactis* BB12, and inulin-oligofructose as a 2.5g sachet | Daily for 12 weeks | There was no statistically significant improvement in the intervention arm versus the placebo for all measures of functional constipation. There was no discussion of adverse events. | Lim et al., 2018 |

**Table 6. Human Clinical Studies of Other Strains of *L. plantarum*.**

| Study Design and Objective | Subjects | Strain and Dosage | Duration | Results | Reference |
|---|---|---|---|---|---|
| Double-blind, randomized, placebo-controlled pilot trial to evaluate microorganism's effects on swelling, pain, and eating difficulty after third molar extraction. | 37 apparently healthy teenagers and young adults with 20 in the intervention arm. | *L. brevis* CECT 7480 and *L. plantarum* CECT 7481 (1:1 ratio) $1.0x10^9$ cfu/day ($0.5x10^9$ cfu/dose) in chewable tablets. | 2x daily for 1 week | There was no difference between groups in swelling at the extraction site. There were two infections at the site of tooth removal in each arm, but no other adverse events were reported. | Pons et al., 2018 |
| Prospective, randomized, double-blind, placebo-controlled study of bacteria in *Helicobacter pylori* therapy | 209 adult patients with *H. pylori* infection; 103 in the intervention arm | $1x10^9$ cfu/day each of *P. acidilactici* and *L. plantarum* | Daily for 10 days | There were no differences in compliance or in eradication rates. Side effects at the end of the treatment were the primary outcome, but no differences between the intervention and placebo groups were reported. | McNicholl et al., 2018 |

## 6.5. Evaluations by Authoritative Bodies

Noting that a wide variety of microbial species are used in food, some with a long history of apparent safe use, and facing the need to set priorities for risk assessment, the European Food Safety Authority (EFSA) proposed a system referred to as "Qualified Presumption of Safety" (QPS; EFSA 2007a, 2007b). This system proposed basing the safety assessment of a defined taxonomic group (e.g., a genus or a species) on 4 pillars: established identity, body of knowledge, possible pathogenicity, and end use. If the taxonomic group did not raise safety concerns or, if safety concerns existed, but could be defined and excluded, the grouping could be granted QPS status. Thereafter, "any strain of microorganism the identity of which could be unambiguously established and assigned to a QPS group would be freed from the need for further safety assessment other than satisfying any qualifications specified" (EFSA 2007a, p1).

EFSA's Scientific Committee was asked to recommend organisms regarded as suitable for QPS status. The list of such organisms proposed by the Committee included *Lactobacillus plantarum*. In listing these species as suitable for QPS status, the Committee stated, "Where QPS status is proposed, the Scientific Committee is satisfied that the body of knowledge available is sufficient to provide adequate assurance that any potential to produce adverse effects in humans, livestock or the wider environment is understood and capable of exclusion" (EFSA 2007a, p8) and that the recommendations are "based on a thorough review of the available scientific literature and the knowledge and experience of the scientists involved" (EFSA 2007a, p8). EFSA (2007b) offered the following observations regarding lactobacilli:

- Many of the species are significant constituents of the normal gut flora of humans and livestock although their occurrence and numbers are host dependent. Several species of the genus are intentionally introduced in the food chains, being involved in a range of food and feed fermentations and applied as [microorganisms] for humans and animals.
- The characteristics and habitat of most of *Lactobacillus* species are well known. Some of the species of this genus have a long history of apparent safe use in industrial and agricultural applications. Lactobacilli are used as starter cultures in a variety of food fermentation, such as dairy products, fermented and cured meats, fermented vegetables, sourdough and silage. Moreover, they are among the dominant populations in microbial communities of traditional fermented foods.
- Members of the *Lactobacillus* genus are daily consumed in large quantities in a variety of fermented foods by people of all ages, ethnic groups and health status with apparently no ill effects. Apart from their possible involvement in the development of dental caries, lactobacilli have generally been considered to be non-pathogenic. However, there has been an increasing number of reports that these organisms might occasionally be involved in human disease. Clinical conditions from which these species were derived were chiefly subacute endocarditis and bacteremia or systemic septicemia, but also included abscesses, chorioamnionitis, and urosepsis. Many of the patients with apparent *Lactobacillus* infection were immunocompromised or had other severe underlying illnesses. In conclusion, most of the *Lactobacillus* species described to date can rightly be considered to be non-pathogenic to humans.
- There are apparently no specific safety concerns regarding a number of *Lactobacillus* species which have a long history of apparent safe use in the food chain. Due to the long history of safe use the following species are proposed for QPS status: [list of 33 species, including *L. plantarum*].

Updates to the QPS list have been issued at least annually since its inception. The review identifies any publicly available studies reporting safety concerns for humans, animals, or the environment caused by the listed QPS organisms since the previous review. As of the most recent review, *Lactobacillus plantarum* is still included on the QPS list as no safety concerns have been identified in the years since their inclusion on the list (EFSA, 2018).

The FDA has evaluated three NDI notifications for live *L. plantarum* strains. NDIN 171 for strain ATCC 202195 in combination with fructooligosaccharides from Kups International was reviewed in 2003 (CFSAN, 2003). NDIN 764 for strain L-137 from House Wellness Foods Corporation at a use level of $1.2x10^{10}$ cfu/day was reviewed in 2012 (CFSAN, 2012). NDIN 900 for strain CJLP133 from CJ Cheil Jedang at a use level of $1x10^{10}$ cfu/day was reviewed in 2016 (CFSAN, 2016). All three notices were accepted by the FDA.

In 2017, the FDA evaluated GRAS Notice No. GRN 000685 for *Lactobacillus plantarum* 299v from Probi. This strain was isolated from the healthy intestinal mucosa of a human. The intended use was as an ingredient added to conventional foods at a concentration of at least to $1x10^{10}$ cfu/serving and up to $1x10^{11}$ cfu/serving. The conventional foods included, but were not limited to, wet chilled and ambient products such as fruit drinks, yogurts, milk, and plant based products; dry chilled products; dry and shelf-stable products such as cereals, candy, bars, cookies, gums, and confectionary. On October 31, 2017, the FDA responded that it had no questions regarding the conclusion that the strain was GRAS for its intended use (CFSAN, 2017a).

In 2017, the FDA evaluated GRAS Notice No. GRN 000722 for *Lactobacillus plantarum* Lp-115 from DuPont Nutrition and Health. This strain was isolated from plant silage. The intended use was as an ingredient added to conventional foods at a concentration of at least to $1x10^{10}$ cfu/serving and up to $5x10^{11}$ cfu/serving. The conventional foods included yogurt and other dairy products, soy products, beverages, chewing gum, and confectionary snacks. On February 16, 2018, the FDA responded that it had no questions regarding the conclusion that the strain was GRAS for its intended use (CFSAN, 2017b).

## 6.6. Safety Assessment and GRAS Determination

### 6.6.1. Introduction

This section presents an assessment that demonstrates that the intended use of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013, individually or in combination, is safe, and is GRAS.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is demonstrated. Safety is established by demonstrating that the likely intake of the strains under their intended conditions of use is within allowable levels of intake. In the second step, the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is determined to be GRAS by demonstrating that the safety of these strains is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

The regulatory framework for establishing whether a substance or microorganism is GRAS, in accordance with Section 201(s) of the Federal Food Drug and Cosmetic Act, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components:

1) data and information relied upon to establish the scientific element of safety must be generally available; and

2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, is safe, and is GRAS. Once the intended use of these strains is determined to be GRAS, they are permitted to be used as intended, because they are by definition not food additives and therefore do not require promulgation of food additive regulations under 21 CFR prior to being marketed and sold in the United States.

A scientific procedures GRAS determination requires that information about the substance establish that the intended uses of the substance are safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

1) the probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);

2) the cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet; and

3) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

### 6.6.2. Estimated Daily Intake

The three strains, individually or in combination, are intended to be added to foods that can support their viability through the shelf life at a maximum addition level of $1.2x10^{10}$ cfu/serving in order to assure the presence of at least $1.2x10^{9}$ viable cfu through the shelf life.

The estimated daily intake of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 is less than $1.2x10^{10}$ cfu.

### 6.6.3. Safety of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, Individually or in Combination

*L. plantarum* was assigned QPS status at the origin of the program in 2007 and has maintained that status through the 2018 annual re-evaluation. The species has been safely used in food preparation for many years and is accepted by FDA. It has been approved for use in dietary supplements in the U.S. through submission and acceptance of New Dietary Ingredient notices, and *L. plantarum* has been the subject of GRAS notices that have been accepted by FDA with no questions. The specific strains *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 have been investigated in animal and human trials with no evidence of pathogenicity or toxigenicity.

The decision tree published by Pariza et al. (2015) indicates that the notified strains, *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, "are deemed to be safe for use in the manufacture of food . . . for human consumption" (Pariza et al., 2015).

The responses to each of the questions asked in the decision tree are as follows for the two strains:

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

2. Has the strain genome been sequenced? **YES**

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

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

5. Does the strain produce antimicrobial substances? **NO**

6. Has the strain been genetically modified using rDNA techniques? **NO**

7. Was the strain isolated from a food that has a history of safe consumption for which the species to which the strain belongs is a substantial and characterizing component (not simply an 'incidental isolate')? **NO**, *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 were isolated from the fresh feces of healthy South American children under 5 years old.

8. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? **NO**

## 6.7. Statement Regarding Information Inconsistent with GRAS

I have reviewed the available data and information and am not aware of any data or information that are, or may appear to be, inconsistent with our conclusion of GRAS status of the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination.

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.

## 6.8. Conclusion of the Expert Panel

The intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the bacterial product, estimating potential human exposure to the strains from their intended use, and demonstrating that this level of exposure is without significant risk of harm. Because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use is considered GRAS.

Determination of the safety and GRAS status of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 under their intended conditions of use has been made through the deliberations of an Expert GRAS Panel consisting of Robert J. Nicolosi, Ph.D., Michael W. Pariza, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They have critically reviewed and evaluated the publicly available information summarized in this document, including the potential human intake resulting from the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, and have individually and collectively concluded:

> Ingestion of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013 from their intended use results in levels of intake that are within safe limits established by the history of consumption of these strains and by published animal studies and human clinical trials. Therefore, the use of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013, individually or in combination, produced consistent with cGMP and complying with the specifications and use described in the GRAS monograph, at a maximum addition level of $1.2x10^{10}$ cfu/serving, is safe and GRAS based on scientific procedures.

It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion.

Robert J. Nicolosi, Ph.D. _____ Date: _____
Professor Emeritus
University of Massachusetts—Lowell
Lowell, Massachusetts

Michael W. Pariza, Ph.D. _____ Date: _____
Professor Emeritus
University of Wisconsin—Madison
Madison, Wisconsin

John A. Thomas, Ph.D. _____ Date: _____
Adjunct Professor
Indiana University School of Medicine
Indianapolis, Indiana

## 6.8. Conclusion of the Expert Panel

The intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the product, estimating potential human exposure to the strains from their intended use, and demonstrating that this level of exposure is without significant risk of harm. Because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use is considered GRAS.

Determination of the safety and GRAS status of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 under their intended conditions of use has been made through the deliberations of an Expert GRAS Panel consisting of Robert J. Nicolosi, Ph.D., Michael W. Pariza, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They have critically reviewed and evaluated the publicly available information summarized in this document, including the potential human intake resulting from the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, and have individually and collectively concluded:

> Ingestion of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013 from their intended use results in levels of intake that are within safe limits established by the history of consumption and by published animal studies and human clinical trials. Therefore, the use of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013, individually or in combination, produced consistent with cGMP and complying with the specifications and use described in the GRAS monograph, at a maximum addition level of $1.2 \times 10^{10}$ CFU/serving, is safe and GRAS based on scientific procedures.

It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion.

Robert J. Nicolosi, Ph.D. [redacted] Date: 05/16/2019
Professor Emeritus
University of Massachusetts—Lowell
Lowell, Massachusetts

Michael W. Pariza, Ph.D. _____ Date: _____
Professor Emeritus
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## 6.8. Conclusion of the Expert Panel

The intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the product, estimating potential human exposure to the strains from their intended use, and demonstrating that this level of exposure is without significant risk of harm. Because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use is considered GRAS.

Determination of the safety and GRAS status of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 under their intended conditions of use has been made through the deliberations of an Expert GRAS Panel consisting of Robert J. Nicolosi, Ph.D., Michael W. Pariza, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They have critically reviewed and evaluated the publicly available information summarized in this document, including the potential human intake resulting from the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, and have individually and collectively concluded:

> Ingestion of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013 from their intended use results in levels of intake that are within safe limits established by the history of consumption and by published animal studies and human clinical trials. Therefore, the use of *Lactobacillus plantarum* KABP-011, *Lactobacillus plantarum* KABP-012, and *Lactobacillus plantarum* KABP-013, individually or in combination, produced consistent with cGMP and complying with the specifications and use described in the GRAS monograph, at a maximum addition level of $1.2 \times 10^{10}$ CFU/serving, is safe and GRAS based on scientific procedures.

It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion.

Robert J. Nicolosi, Ph.D. _____ Date: _____
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## 6.8. Conclusion of the Expert Panel

The intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, individually or in combination, has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first establishing the identity and purity of the product, estimating potential human exposure to the strains from their intended use, and demonstrating that this level of exposure is without significant risk of harm. Because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use is considered GRAS.

Determination of the safety and GRAS status of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013 under their intended conditions of use has been made through the deliberations of an Expert GRAS Panel consisting of Robert J. Nicolosi, Ph.D., Michael W. Pariza, Ph.D., and John A. Thomas, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They have critically reviewed and evaluated the publicly available information summarized in this document, including the potential human intake resulting from the intended use of *L. plantarum* KABP-011, *L. plantarum* KABP-012, and *L. plantarum* KABP-013, and have individually and collectively concluded:

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It is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion.

Robert J. Nicolosi, Ph.D. _____ Date: _____
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## Part 7. List of Supporting Data and Information

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**To:** Hice, Stephanie; jh@jheimbach.com
**Cc:** "Garey, Joshua"
**Subject:** RE: GRN 000953 - Questions for Notifier
**Date:** Wednesday, December 16, 2020 7:41:05 PM
**Attachments:** image001.png
Hice Stephanie 20201216.pdf

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Dear Stephanie—

Here are our responses to your reviewers' questions. Best wishes to you and your colleagues for a Merry (and healthy!) Christmas.

Regards,
Jim

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**From:** Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
**Sent:** Thursday, December 3, 2020 4:20 PM
**To:** 'jh@jheimbach.com' <jh@jheimbach.com>
**Cc:** 'jheimbach@va.metrocast.net' <jheimbach@va.metrocast.net>
**Subject:** GRN 000953 - Questions for Notifier

Dear Dr. Heimbach,

During our review of GRAS Notice No. 000953, we noted further questions that need to be addressed and are attached to this email.

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

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

**Stephanie Hice, PhD**
*Staff Fellow (Biologist)*
*Division of Food Ingredients*
**Center for Food Safety and Applied Nutrition**
**Office of Food Additive Safety**
**U.S. Food and Drug Administration**
stephanie.hice@fda.hhs.gov


December 3, 2020

**Questions/Comments Regarding GRN 000953:**

**Comment:**

As the *Lactobacillus* genus recently underwent reorganization[1], we note that we will use the new nomenclature for the three notified strains (i.e., *Lactiplantibacillus plantarum* strains CECT 7527, CECT 7528 and CECT 7529) in our correspondence, but will provide the strains' previous names in a footnote, linking the old nomenclature with the new nomenclature. In a future GRAS notice, we recommend that the notifier use the new nomenclature for strains affected by the taxonomic reorganization, but should also provide the strains' previous names, clearly linking the old nomenclature with the new nomenclature, and include a reference to the publication.

**Questions:**

1. For the administrative record, please describe whether *L. plantarum* strains CECT 7527, CECT 7528 and CECT 7529 produce antibiotics.
2. Please state whether *L. plantarum* strains CECT 7527, CECT 7528 and CECT 7529 are genetically engineered.
3. In Part 1.3 (page 5), the notifier identifies the subjects of the notice as "*Lactobacillus plantarum* KABP-011 (CECT 7527), *Lactobacillus plantarum* KABP-012 (CECT 7528), and *Lactobacillus plantarum* KABP-013 (CECT 7529)". However, on page 8, the notifier states that the "... strains were deposited in the Spanish Type Culture Collection (Colección Española de Cultivos Tipo/CECT) under accession numbers CECT 7527, CECT 7528, and CECT 7316, respectively". For the administrative record, please clarify this discrepancy.
4. Please provide a physical description of the final product(s) (e.g., a white powder).
5. Please provide complete and appropriate citations for the analytical methods used to analyze for the microbiological specifications and heavy metals
6. In Table 3, the notifier provides batch analyses for heavy metals (page 20) but does not provide a specification limit for these heavy metals. Please provide a specification limit for the heavy metals in order to demonstrate that the final product can be manufactured in conformance with the stated specifications.

[1] Zheng, J., Wittouch, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'Toole, P. W. ... Leeber, S. (2020). A taxonomic note of the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology, 70*(4), 1-77. doi: 10.1099/ijsem.0.004107

7. Please clarify if “Enterobacteria” in Table 1 and 2 (page 19) refers to the *Enterobacteriaceae*.

8. On page 59 the notifier lists the intended use level for GRN 000685 as “... a concentration of at least to 1 x $10^{10}$ CFU/serving and up to 1 x $10^{11}$ CFU/serving” and for GRN 000722 as “... at a concentration of at least to 1 x $10^{10}$ CFU/serving and up to 5 x $10^{11}$ CFU/serving”. We note that the intended use level listed in our response letters for GRNs 000685 and 000722 are “... up to 1 x $10^{10}$ CFU/serving” and “... at 1 x $10^{10}$ colony forming units (CFU)/serving”, respectively. For the administrative record, please make a statement that corrects this reference.

9. Please state whether the fermentation process is conducted in a contained, sterile environment.

10. The notifier provides stability data under frozen conditions (-20 °C) over 18 months and indicates that the intended use is limited to applications that can sufficiently support microorganism viability throughout the shelf-life of the product. Please confirm if the shelf-life for the individual strains and the 1:1:1 combination of the strains is 18 months.

11. The notifier states that the intended use is in conventional foods, such as yogurt and other dairy products, soy products, chewing gum, and confectionary snacks. Please clarify if the intended use is in all conventional foods or just the specific categories indicated in the notice.

12. The notifier indicates that the likely maximum ingestion is less than $10^{10}$ CFU/d but does not provide an explanation as to how that value was derived. Please provide an exposure estimate that is based on the intended uses and describe how this value is derived.

13. Please include an updated literature search, with the specified source, search terms and date covered, for any new or additional relevant safety data and state if any such data contradicts the notifier’s GRAS conclusion.

JHeimbach LLC

December 16, 2020

Stephanie Hice, Ph.D.
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
U.S. Food & Drug Administration

Dear Dr. Hice:

In your e-mail to me of December 3, you reported that the FDA review team had a number of questions concerning GRN 953 and asked us to respond to them. Following are our responses to the questions.

1. For the administrative record, please describe whether *L. plantarum* strains CECT 7527, CECT 7528 and CECT 7529 produce antibiotics.

   *L. plantarum* strains CECT 7527, CECT 7528, and CECT 7529 do not produce antibiotics.

2. Please state whether *L. plantarum* strains CECT 7527, CECT 7528 and CECT 7529 are genetically engineered.

   *L. plantarum* strains CECT 7527, CECT 7528, and CECT 7529 are not genetically engineered.

3. In Part 1.3 (page 5), the notifier identifies the subjects of the notice as "*Lactobacillus plantarum* KABP-011 (CECT 7527), *Lactobacillus plantarum* KABP-012 (CECT 7528), and *Lactobacillus plantarum* KABP-013 (CECT 7529)". However, on page 8, the notifier states that the "... strains were deposited in the Spanish Type Culture Collection (Colección Española de Cultivos Tipo/CECT) under accession numbers CECT 7527, CECT 7528, and CECT 7316, respectively". For the administrative record, please clarify this discrepancy.

   Page 8 should read: "... strains were deposited in the Spanish Type Culture Collection (Colección Española de Cultivos Tipo/CECT) under accession numbers CECT 7527, CECT 7528, and CECT 7529, respectively." This was a typographical error.

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4. Please provide a physical description of the final product(s) (e.g., a white powder).

   *L. plantarum* strains CECT 7527, CECT 7528 and CECT 7529 appear as an ivory-white powder.

5. Please provide complete and appropriate citations for the analytical methods used to analyze for the microbiological specifications and heavy metals.

| Parameter | Specification | Method |
|---|---|---|
| Bacterial Count[I, II] | >1.2x10$^{11}$ cfu/g | ISO 15787:2009-09<br>ISO 29981:2010-02 |
| Yeasts and Molds[III] | <10 cfu/g | ISO 6611:2004 |
| Enterobacteriaceae[IV] | <10 cfu/g | ISO 21528-2:2017 |
| *E. coli*[V] | <1 cfu/g | Pharm EU 2.6.13 |
| *Staphylococci* (coag +)[VI] | <10 cfu/g | ISO 6888-1:1999+A1:2003;<br>Agar per Pharm EU 2.6.13 |
| *B. cereus*[VII] | <100 cfu/g | DIN 10198:2010-07 |
| *Listeria monocytogenes*[VIII] | n.d. in 25 g | ISO 11290-1:2005-01 |
| *Salmonella* spp.[IX] | n.d. in 25 g | ISO 6579:2002 |
| cfu = colony-forming unit n.d. = not detected | | |

[I] Standards Centre. 2009. British Standards Institute, Animal Feeding Stuffs - Isolation and Enumerations of *Lactobacillus* spp. EN 15787. London, British Standards Institute. 2009.

[II] International Standard ISO 29981 (2010): Milk products – Enumeration of presumptive bifidobacteria – Colony count technique at 37°C. FIL-IDF, Brussels.

[III] ISO (2004) Milk and milk products—enumeration of colony-forming units of yeasts and/or moulds—colony-count technique at 25 degrees C (ISO 6611:2004 (IDF 94:2004)). International Organization for Standardization, Geneva.

[IV] International Organization for Standardization.（2017） ISO 21528-2. Microbiology of the food chain. Horizontal method for the detection and enumeration of Enterobacteriaceae. Part 2: Colony-count technique, ISO, Geneva.

[V] EP-European Pharmacopoeia, (2020), Microbiological examination of non-sterile products test for specified micro-organisms, 2.6.13.

[VI] International Organization for Standardization (2003) ISO 6888-3:2003. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species): part 3: detection and MPN technique for low numbers. Geneva.

[VII] German Institute for Standardization (2010) DIN 10198:2010-07. Microbiological Analysis Of Milk - Determination Of Presumptive Bacillus Cereus - Colony Count Technique At 37 Degree C. Berlin.

[VIII] German Institute for Standardization (2010) DIN EN ISO 11290-1:2005. Microbiology of the Food Chain – Horizontal Method for the Detection and Enumeration of Listeria monocytogenes and of Listeria spp. – Part 1: Detection Method. Berlin.

[IX] International Organization for Standardization (2002) ISO 6579:2002. Microbiology of food and animal feeding stuffs – horizontal method for the detection of Salmonella spp. Geneva.

6. In Table 3, the notifier provides batch analyses for heavy metals (page 20) but does not provide a specification limit for these heavy metals. Please provide a specification limit for the heavy metals in order to demonstrate that the final product can be manufactured in conformance with the stated specifications.

| Heavy Metal | Unit | Specification | Method | Lot | | |
|---|---|---|---|---|---|---|
| | | | | 8685.04a | 8685.06c | 8685.05b |
| Arsenic | mg/kg | ≤0.5 | PNTA0193[I] | <0.02 | 0.047 | 0.047 |
| Cadmium | mg/kg | ≤0.2 | PNTA0193[I] | <0.005 | 0.018 | 0.019 |
| Mercury | mg/kg | ≤0.1 | PNTA0193[I] | <0.005 | <0.005 | <0.005 |
| Lead | mg/kg | ≤0.2 | PNTA0193[I] | <0.005 | 0.006 | <0.005 |

[I] Heavy metals testing by ICP-MS by Mérieux NutriSciences lab with ISO 17025:2005 accreditation. International Organization for Standardization (2005) ISO 17025:2005. General Requirements for the Competence of Testing and Calibration Laboratories. Geneva.

7. Please clarify if "Enterobacteria" in Tables 1 and 2 (page 19) refers to the *Enterobacteriaceae*.

"Enterobacteria" in Table 1 and 2 refers to *Enterobacteriaceae.*

8. On page 59 the notifier lists the intended use level for GRN 000685 as "… a concentration of at least to 1 x $10^{10}$ CFU/serving and up to 1 x $10^{11}$ CFU/serving" and for GRN 000722 as "… at a concentration of at least to 1 x $10^{10}$ CFU/serving and up to 5 x $10^{11}$ CFU/serving". We note that the intended use level listed in our response letters for GRNs 000685 and 000722 are "… up to 1 x $10^{10}$ CFU/serving" and "… at 1 x $10^{10}$ colony forming units (CFU)/serving", respectively. For the administrative record, please make a

statement that corrects this reference.

The intended use level listed in FDA response letters for GRNs 000685 and 000722 are "… up to 1 x $10^{10}$ CFU/serving" and "… at 1 x $10^{10}$ colony forming units (CFU)/serving", respectively.

9. Please state whether the fermentation process is conducted in a contained, sterile environment.

The fermentation process for *L. plantarum* CECT 7527, CECT 7528, and CECT 7529 is conducted in a contained, sterile environment.

10. The notifier provides stability data under frozen conditions (-20 °C) over 18 months and indicates that the intended use is limited to applications that can sufficiently support microorganism viability throughout the shelf-life of the product. Please confirm if the shelf-life for the individual strains and the 1:1:1 combination of the strains is 18 months.

The stability data under frozen conditions is included for the individual strains and the 1:1:1 combination of strains. Stability of the live microorganisms in conventional foods is dependent on the storage temperature, water activity, and desired shelf life of the conventional foods within which the live microorganisms are included. Applications are limited to conventional foods that include up to 1 x $10^{10}$ colony forming units (CFU)/serving at time of manufacture and 1 x $10^{9}$ colony forming units (CFU)/serving at the end of shelf life.

11. The notifier states that the intended use is in conventional foods, such as yogurt and other dairy products, soy products, chewing gum, and confectionary snacks. Please clarify if the intended use is in all conventional foods or just the specific categories indicated in the notice.

The intended use is in all conventional foods that can support viability. The specified categories are indicative of the types of conventional foods that are currently conducive to the inclusion of live microorganisms.

12. The notifier indicates that the likely maximum ingestion is less than $10^{10}$ CFU/d but does not provide an explanation as to how that value was derived. Please provide an exposure estimate that is based on the intended uses and describe how this value is derived.

As described in the notice, "When used individually, the intended level of each strain of *L. plantarum* is intended to be at least $4x10^8$ cfu/serving over the shelf life of the product. The maximum level of addition is intended to be $4x10^9$ cfu/serving of the strain to allow for the loss of viability over the shelf life of the product." When used as a blend, the total intended content of the 3 strains combined is $1.2x10^9$ cfu/serving, i.e., $4x10^8$ cfu of each strain. Target foods are limited to those that support viability. Since mean food consumption is about 20 food servings/day*, and this estimate allows for ten or more servings of foods or drinks containing the bacteria, it is felt that this is an extremely conservative estimate.

(*Millen AE, D Midthune, FE Thompson, V Kipnis, AF Subar. 2006. The National Cancer Institute diet history questionnaire: validation of pyramid food servings. *Am J Epidemiol* 163:279-288.)

13. Please include an updated literature search, with the specified source, search terms and date covered, for any new or additional relevant safety data and state if any such data contradicts the notifier's GRAS conclusion.

A PubMed search was performed for "plantarum" with the results limited to clinical trials. Publications not included in the original GRAS submission are summarized below. Espadaler et al. (2019) was not from the PubMed search but is a new publication of research using the notified strains. None of the data conflicts with the notifier's GRAS conclusion.

| Citation | Description | Subjects | Intervention | Duration | Safety-Related Findings |
|---|---|---|---|---|---|
| Espadaler et al., 2019 | Prospective observational study of subjects initiating consumption of KABP-011, KABP-012, and KABP-013. [Abstract of a presentation at the 10th Workshop of the Spanish Society of Probiotics and Prebiotics (SEPyP 2019)]. | 343 subjects (median age = 55 years, 63% female) | *L. plantarum* KABP-011, KABP-012, and KABP-013 in a 1:1:1 ratio were consumed in a capsule at $1.2x10^9$ cfu/day | Daily for 12 weeks | 17% of patients reported tolerability issues but none of them were considered severe. The tolerability issues correlated with antiplatelet use only. No AEs were reported. |
| Madempudi et al., 2019 | Randomized, double-blind, placebo-controlled trial to evaluate the micro-organisms' effects in patients with Type 2 diabetes. | 79 subjects with Type 2 diabetes with 40 receiving intervention (62M, 17F, mean age = 52.4 years). | a) *L. salivarius* UBLS22, *L. casei* UBLC42, *L. Plantarum* UBLP40, *L. acidophilus* UBLA34, *B. breve* UBBr01, and *B. coagulans* Unique IS2, 30 billion cfu and fructo-oligosaccharide, 100 mg) b) placebo capsules of maltodextrin | 12 weeks | 2 participants experienced mild flatulence or moderate constipation assessed as likely unrelated to the intervention. No other SAEs or deaths occurred during the study. |

| Citation | Description | Subjects | Intervention | Duration | Safety-Related Findings |
|---|---|---|---|---|---|
| Håkansson et al., 2019 | Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' ability to suppress ongoing celiac disease in at-risk children. | 78 children with celiac disease with 40 receiving intervention (3-7 years of age). | a) 1g sachet of maltodextrin and *L. plantarum* HEAL9 / *L. paracasei* 8700:2 at $10^{10}$ cfu/sachet<br>b) 1g sachet of maltodextrin | 6 months | 3 children in the intervention group and 4 children in the placebo group reported AEs of pain, flatulence, or diarrhea. 1 in each group reported GI symptoms. |
| Huang et al., 2019 | Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on exercise physiological adaptation, performance, and body composition in healthy humans. | 54 healthy subjects (20-30 years, non-professional athletes, 27M and 27F) | a) Placebo<br>b) 3 x $10^{10}$ cfu/day *L. plantarum* TWK10<br>c) 9 x $10^{10}$ cfu/day *L. plantarum* TWK10 | 6 weeks | No significant differences were observed in AST, ALT, BUN, CREA, UA, FFA, and glycerol among groups. |
| Park et al., 2020 | Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects postprandial lipid levels and intestinal environment. | 70 subjects with 62 completing the study (24M/46F, mean age of 48 years) | a) *L. plantarum* Q180 4 x $10^{9}$ cfu twice a day<br>b) Placebo | 12 weeks | Four intervention and four placebo subjects dropped out of the trial. No safety data is presented. |
| Toshimitsu et al., 2020 | Randomized, double-blind, placebo-controlled trial to evaluate the microorganisms' effects on glucose metabolism and chronic inflammation in prediabetic adults. | 130 subjects aged 20-64 years with 65 in the intervention arm and 65 in the placebo arm. | a) Heat treated *L. plantarum* OLL2712 at 5 x $10^{9}$ cfu in yogurt<br>b) Placebo (yogurt) | 12 weeks | No SAEs occurred in the study, although a total of 21 nonserious AEs were accounted for through participants' diaries, medical interviews, and blood tests. There were no significant differences in the incidence of AEs between the groups, and none of these were judged by the physician in charge to be related to the consumption of the test yogurt. |

| Citation | Description | Subjects | Intervention | Duration | Safety-Related Findings |
|---|---|---|---|---|---|
| Ford et al., 2020 | Randomized, double-blind, placebo-controlled, crossover study to evaluate the effects of a high protein diet (HPD) with and without live microorganisms on gut microbiota and wellness in older women. | 26 healthy, older women aged 73.7 ±5.6 years. | a) HPD<br>b) HPD + multistrain probiotic formulation ($1.54\times10^9$ *Bifidobacterium bifidum* HA-132, $4.62\times10^9$ *Bifidobacterium breve* HA-129, $4.62\times10^9$ *Bifidobacterium longum* HA-135, $4.62\times10^9$ *Lactobacillus acidophilus* HA-122, and $4.62\times10^9$ *Lactobacillus plantarum* HA-119)<br>c) HPD + prebiotic (5.6g inulin)<br>d) HPD + multistrain probiotic blend + 5.6g inulin | 2 weeks per arm in the crossover study with 2 weeks washout between arms | No study-related AEs were reported during the study. |
| Nam et al., 2020 | Open label, single arm study evaluating the effects of the live microorganism on skin hydration. | 13 female volunteers aged 44.3 ±16.5 years. | a) $1 \times 10^{10}$ cfu of *L. plantarum* HY7714 as a capsule | 8 weeks | 2 of 15 recruited subjects failed to complete the study due to personal non-medical reasons. |
| Shin et al., 2020 | Evaluation of the effectiveness of the microorganism in children with rotaviral enteritis. | 23 children with acute gastroenteritis with 15 in the intervention arm and 8 in the control group. 27 children were evaluated retrospectively. | a) unknown cfu of *L. plantarum* LRCC5310<br>b) no intervention<br>c) *Saccharomyces* of unknown amount | 7 days | No information on safety was published. |

Espadaler, J., Audivert, S., Buj, D. (2019). Abstracts of the 10th workshop on probiotics and prebiotics. *Ann Nutr Metab* 74(suppl 1), 1–31. https://doi.org/10.1159/000496759

Ford, A. L., Nagulesapillai, V., Piano, A., Auger, J., Girard, S. A., Christman, M., Tompkins, T. A., Dahl, W. J. (2020). Microbiota stability and gastrointestinal tolerance in response to a high-protein diet with and without a prebiotic, probiotic, and synbiotic: A randomized, double-blind, placebo-controlled trial in older women. *J Acad Nutr Dietet* 120(4), 500-516.e10. https://doi.org/10.1016/j.jand.2019.12.009

Håkansson, Å., Aronsson, C. A., Brundin, C., Oscarsson, E., Molin, G., Agardh, D. (2019). Effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* on the peripheral immune response in children with celiac disease autoimmunity: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients* 11(8). https://doi.org/10.3390/nu11081925

Huang, W. C., Lee, M. C., Lee, C. C., Ng, K. S., Hsu, Y. J., Tsai, T. Y., Young, S. L., Lin, J. S., Huang, C. C. (2019). Effect of *Lactobacillus plantarum* TWK10 on exercise physiological adaptation, performance, and body composition in healthy humans. *Nutrients* 11(11). https://doi.org/10.3390/nu11112836

Madempudi, R. S., Ahire, J. J., Neelamraju, J., Tripathi, A., Nanal, S. (2019). Efficacy of UB0316, a multi-strain probiotic formulation in patients with type 2 diabetes mellitus: A double blind, randomized, placebo controlled study. *PloS One* 14(11), e0225168. https://doi.org/10.1371/journal.pone.0225168

Nam, B., Kim, S. A., Park, S. D., Kim, H. J., Kim, J. S., Bae, C. H., Kim, J. Y., Nam, W., Lee, J. L., Sim, J. H. (2020). Regulatory effects of *Lactobacillus plantarum* HY7714 on skin health by improving intestinal condition. *PLoS ONE* 15(4), e0231268. https://doi.org/10.1371/journal.pone.0231268

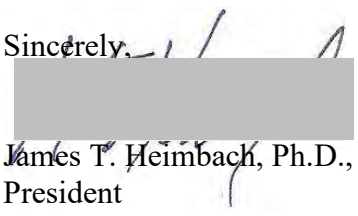
Park, Y. E., Kim, M. S., Shim, K. W., Kim, Y. Il, Chu, J., Kim, B. K., Choi, I. S., Kim, J. Y. (2020). Effects of *Lactobacillus plantarum* Q180 on postprandial lipid levels and intestinal environment: A double-blind, randomized, placebo-controlled, parallel trial. *Nutrients 12*(1). https://doi.org/10.3390/nu12010255

Shin, D. Y., Yi, D. Y., Jo, S., Lee, Y. M., Kim, J. H., Kim, W., Park, M. R., Yoon, S. M., Kim, Y., Yang, S., Lim, I. S. (2020). Effect of a new *Lactobacillus plantarum* product, LRCC5310, on clinical symptoms and virus reduction in children with rotaviral enteritis. *Medicine* 99(38), e22192. https://doi.org/10.1097/MD.0000000000022192

Toshimitsu, T., Gotou, A., Sashihara, T., Hachimura, S., Shioya, N., Suzuki, S., Asami, Y. (2020). Effects of 12-week ingestion of yogurt containing *Lactobacillus plantarum* OLL2712 on glucose metabolism and chronic inflammation in prediabetic adults: A randomized placebo-controlled trial. *Nutrients* 12(2). https://doi.org/10.3390/nu12020374

I hope these responses provide satisfactory answers to your questions. Please let me know if you have further questions.

Sincerely,



James T. Heimbach, Ph.D., F.A.C.N.
President

| | |
|---|---|
| **From:** | jheimbach@va.metrocast.net |
| **To:** | Hice, Stephanie; jh@jheimbach.com |
| **Cc:** | "Garey, Joshua" |
| **Subject:** | RE: GRN 000953 - Questions for Notifier |
| **Date:** | Wednesday, December 23, 2020 10:58:30 AM |
| **Attachments:** | image001.png<br>Hice Stephanie 20201223.pdf<br>alcance_iso17025_enac.pdf<br>Contfirmation_testing methods_Lactosan.pdf |

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Dear Stephanie—

As a Christmas present ☺, I am sending you our response to your email of December 18. The response includes a letter and two pdf documents.

Regards,
Jim

James T. Heimbach, Ph.D., F.A.C.N.
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Port Royal VA  22535
USA
Tel:  (+1) 804-742-5543
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Email:  jh@jheimbach.com

---

**From:** Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
**Sent:** Friday, December 18, 2020 3:20 PM
**To:** jh@jheimbach.com; 'jheimbach@va.metrocast.net' <jheimbach@va.metrocast.net>
**Subject:** RE: GRN 000953 - Questions for Notifier

Dear Dr. Heimbach,

During our review of GRAS Notice No. 000953, we noted further questions that need to be addressed and are attached to this email.

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

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

**Stephanie Hice, PhD**
*Staff Fellow (Biologist)*
*Division of Food Ingredients*
**Center for Food Safety and Applied Nutrition**
**Office of Food Additive Safety**
**U.S. Food and Drug Administration**
stephanie.hice@fda.hhs.gov


JHeimbach LLC

December 23, 2020

Stephanie Hice, Ph.D.
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
U.S. Food & Drug Administration

Dear Dr. Hice:

In your e-mail to me of December 18, you reported that the FDA review team had four additional questions concerning GRN 953 and asked us to respond to them. Following are our responses to the questions.

1. In their December 16, 2020 amendment, the notifier states that the method used to determine bacterial count is International Standards Organization (ISO) 15787:2009-09; however, in the provided references, the method is cited as a method from the British Standards Institute, and not ISO. We note that ISO 15787 corresponds to Technical Product Documentation – Heat-Treated Ferrous Parts – Presentation and Indications. For the administrative record, please clarify this discrepancy.

> This error on the specifications was corrected in March of 2020. Included with the correspondence is a letter of confirmation showing the update to "ÖNORM EN 15787:2009" as the method. The December 16, 2020, response incorrectly gave the method citation as from the British Standards Institute instead of the Austrian Standards Institute.
>
> Austrian Standards Institute, Animal Feeding Stuffs - Isolation and Enumerations of *Lactobacillus* spp. EN 15787. Vienna. 2009.

2. On page 19 of the notice, the notifier states "All testing is done using up-to-date analytical methods that have [been] validated for their specific [a]pplications".

a. In their December 16, 2020 amendment, the notifier states that the method used to detect *Listeria monocytogenes* is ISO 11290-1:2005-01. We note that this method has been revised and replaced by ISO 11290-1:2017, which corresponds to Microbiology of the Food Chain – Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes* and *Listeria* spp. – Part 1: Detection Method. Please make a statement that corrects this reference.

> This specification was updated in March of 2020. Included with this correspondence is a letter of confirmation showing the update to "ISO 11290-1:2017" as the method.

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b. In their December 16, 2020 amendment, the notifier states that the method used to detect *Salmonella* serovars is ISO 6579:2002. We note that this method has been revised and replaced by 6579-1:2017, which corresponds to Microbiology of the Food Chain – Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. Please make a statement that corrects this reference.

> This specification was updated in March of 2020. Included with this correspondence is a letter of confirmation showing the update to "ISO 6579-1:2017" as the method.

c. In their December 16, 2020 amendment, the notifier states that method PNTA0193 is used to analyze for heavy metals. Please clarify whether this analytical method has been validated for its intended purpose. In addition, the notifier states that testing for heavy metals is performed by Mérieux NutriSciences lab using ICP-MS with ISO 17025:2005.We note that this standard has been revised by ISO/IEC 17025:2017. Please indicate that the current standard is being met.

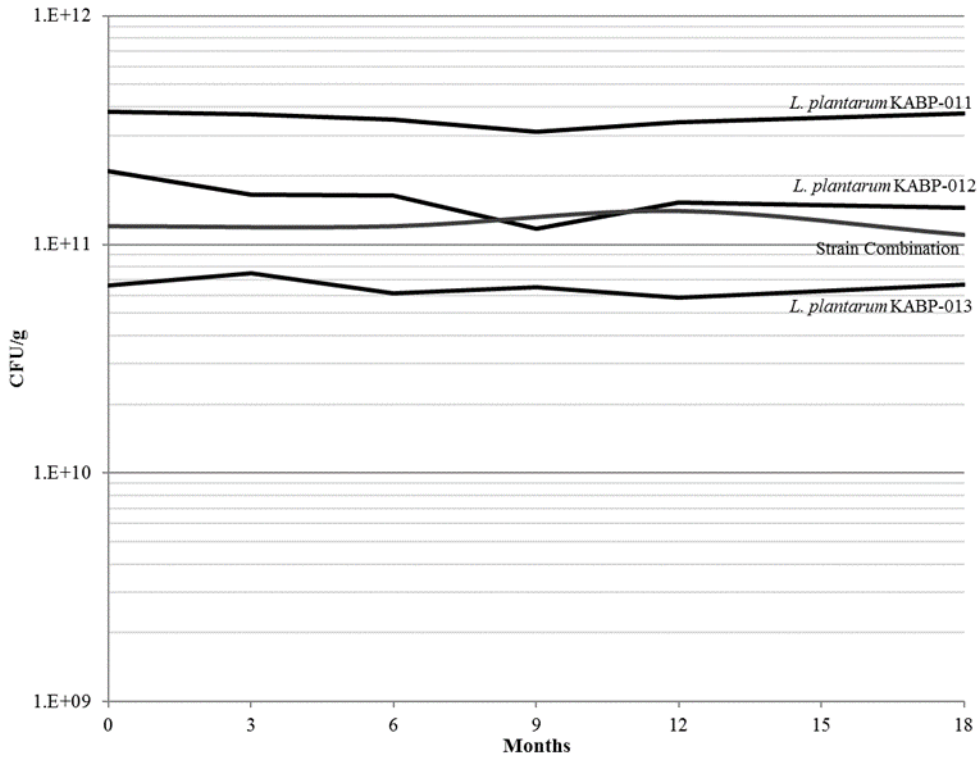
> Included with this correspondence is the current certification meeting UNE-EN ISO/IEC 17025:2017. PNTA0193 is also listed in the certification as validated for its intended purpose.

3. We understand that the stability of the live microorganisms in conventional foods is dependent on the food matrix and the conditions in which the food is stored. For the administrative record, please clarify if the shelf life of the individual strains or a 1:1:1 combination of the strains is 18 months when stored at -20°C.

> As shown in the figure on the next page, the shelf life both of the individual strains and of a 1:1:1 combination of the strains is 18 months when stored at -20°C.

4. In the December 16, 2020 amendment, the notifier provides an updated literature search that is limited to human clinical trials. For the administrative record, please confirm that all relevant publicly-available information was reviewed, and no additional safety concerns were noted, and provide the date (month and year) the literature search was performed.

> The literature search was performed on December 15, 2020. All relevant publicly-available information was reviewed and no additional safety concerns were noted.




I hope these responses provide satisfactory answers to your questions. Please let me know if you have further questions.

Sincerely,

[redacted]

James T. Heimbach, Ph.D., F.A.C.N.
President

LACTOSAN
GmbH & Co. KG, Industriestrasse West 5, 8605 Kapfenberg

AB-BIOTICS, S.A.
Edifici Esade Creapolis
Av. Torre Blanca, 57-3B06
08172 Sant Cugat
Barcelona

## testing methods

Hereby Lactosan GmbH & CO.KG confirms that all testing on AB-Biotics products is performed as followed:

| | |
|---|---|
| active substance: | Meth. LAC AA 3.11.1.42 based on ISO 29981:2010, ÖNORM EN 15786:2009 and ÖNORM EN 15787:2009 |
| yeasts and molds: | Meth. LAC AA 3.11.1.42 according to ISO 6611:2004 |
| enterobacteria: | Meth. LAC AA 3.11.1.42 according to ISO 21528-2:2017 |
| salmonella: | Meth. LAC AA 3.31.1.1 according to ISO 6579-1:2017 |
| listeria monocytogenes: | Meth. LAC AA 3.31.1.2 according to ISO 11290-1:2017 |
| e.coli: | Meth. LAC AA 3.31.1.3 according to Pharm Eu 2.6.13 |
| coag. positive staphylococci: | Meth. LAC AA 3.31.1.4 according to ISO 6888-1:1999/ Amd 2:2018, Agar according to Pharm Eu 2.6.13 |
| bacillus cereus: | Meth. LAC AA 3.11.1.42 according to DIN 10198:2010-07 |

Kapfenberg, 30.03.2020

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DI Violeta Cakic, QM

# SILLIKER IBÉRICA, S.A. (Unipersonal)

Dirección/*Address*: C/ Longitudinal 8, nº26, Mercabarna; 08040 Barcelona
Norma de referencia/*Reference Standard*: **UNE-EN ISO/IEC 17025:2017**
Actividad/*Activity*: **Ensayo/Test**
Acreditación/*Accreditation* nº: **257/LE413**
Fecha de entrada en vigor/*Coming into effect*: 02/03/2001

## ALCANCE DE LA ACREDITACIÓN/*SCHEDULE OF ACCREDITATION*

(Rev./*Ed.* 38 fecha/*date* 08/07/2020)

**Instalaciones donde se llevan a cabo las actividades cubiertas por esta acreditación:**
***Locations where the activities covered by accreditation are performed:***

| |
|---|
| C/ Longitudinal 8, nº26, Mercabarna; 08040 Barcelona (Laboratorio Central) |
| C/ Dos de mayo, nº 273-275; 08025 Barcelona (Centro de Análisis Sensorial) |

**PROGRAMA DE ACREDITACIÓN: "ENSAYOS PARA INFORMACIÓN NUTRICIONAL" (NT-70.01)*:**
***ACCREDITATION PROGRAMME: "TEST FOR NUTRITION INFORMATION" (NT-70.01)****

- **Ensayos para información nutricional obligatoria y completa conforme al Reglamento CE nº 1169/2011, en alimentos:**
  ***Test for mandatory and full nutrition declaration in accordance with Regulation EC No. 1169/2011, in foods***
  - Valor energético / *Energy value*
  - Grasas / *Fat*
  - Ácidos grasos saturados / *Saturated fatty acids*
  - Hidratos de carbono / *Carbohydrates*
  - Azúcares / *Sugars*
  - Proteínas / *Protein*
  - Sal (determinación de sodio) / *Salt (Deteminatiosn of Sodium)*
  - Ácidos grasos monoinsaturados y poliinsaturados / *Mono-unsaturates and polyunsaturates fatty acids*
  - Almidón / *Starch*
  - Fibra alimentaria / *Fibre*
  - Vitaminas (Vitamina A, Vitamina D, Vitamina E, Vitamina K, Vitamina C, Tiamina, Riboflavina, Niacina, Vitamina B6, Ácido fólico, Vitamina B12, Biotina y Ácido pantoténico) / *Vitamins (Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin C, Thiamin, Riboflavin, Niacin, Vitamin B6, Folic acid, Vitamin B12, Biotin y Pantothenic acid)*
  - Minerales (Cloruro, Calcio, Magnesio, Zinc, Cobre, Hierro, Fósforo, Potasio) / *Minerals (Chloride, Calcium, Magnesium, Zinc, Copper, Iron, Phosphorus, Potassium)*

**PROGRAMA DE ACREDITACIÓN: "ENSAYOS MICROBIOLÓGICOS DE ALIMENTOS" (NT-70.02)*:**
***ACCREDITATION PROGRAMME: "MICROBIOLOGICAL FOOD TESTING" (NT-70.02)* :***

- **Ensayos para el cumplimiento de los criterios microbiológicos de los alimentos:**
  ***Tests for compliance with microbiological criteria for food:***
  - *Listeria monocytogenes* / *Listeria monocytogenes*
  - Salmonella / *Salmonella*
  - *Escherichia coli* / *Escherichia coli*
  - Recuento de colonias aerobias / *Aerobic colony count*
  - Enterobacteriáceas / *Enterobacteriaceae*
  - Estafilococos coagulasa positivos / C*oagulase-positive Staphylococci*
  - Presunto *Bacillus cereus* / *Presumptive Bacillus cereus*
  - Enterotoxinas estafilocócicas / *Staphylococcal enterotoxins*

**PROGRAMA DE ACREDITACIÓN: "ENSAYOS DE GLUTEN Y ALÉRGENOS EN ALIMENTOS" (NT-70.03)*:**
***ACCREDITATION PROGRAMME: "TEST OF GLUTEN AND ALLERGEN IN FOOD" (NT-70.03)*:***

- **Ensayos para la información sobre sustancias o productos que causan alergias o intolerancias:**
  ***Tests for information on substances or products causing allergies or intolerances:***
  - Gluten / *Gluten*
  - Huevo / *Egg*
  - Cacahuetes / *Peanuts*
  - Soja / *Soybean*
  - Leche (proteínas) / *Milk (proteins)*
  - Dióxido de azufre y sulfitos / *Sulphur dioxide and sulphites*
  - Almendra / *Almond*
  - Avellana / *Hazelnut*
  - Nuez / *Walnut*

***Disponibles en la página web de ENAC**

* ***Available on the ENAC website***

**Categoría 0 (Ensayos en el laboratorio permanente)**
***Category 0 (Test in the permanent laboratory)***

**LABORATORIO CENTRAL**

**AREA DE FÍSICO-QUÍMICA/ PHYSICAL-CHEMICAL AREA**

Análisis mediante métodos basados en técnicas gravimétricas y volumétricas
*Analysis by gravimetric and titrimetric methods*

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Valor energético (por cálculo)<br>*Energy value (by calculation)* | PNTA0136<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 1169/2011*<br>*Regulation (EC) nº 1169/2011* |
| Alimentos sólidos (excepto leche y productos lácteos)<br>*Solid foodstuffs (except milk and milk products)* | Grasa por gravimetría<br>*Fat by gravimetry* | PNTA0133 Rev. 11<br>*Método interno*<br>*In-house method* |
| Alimentos líquidos (excepto leche), bebidas, salsas líquidas<br>*Liquid food (except milk), beverages, liquid sauces* | | PNTA0172 Rev. 2<br>*Método interno*<br>*In-house method* |
| Yogures, postres lácteos, quesos frescos<br>*Yogurt, milk desserts, fresh cheeses* | Grasa por gravimetría (método Weibull-Berntrop)<br>*Fat by gravimetry (Weibull-Berntrop method)* | PNTA0132<br>*Método interno basado en*<br>*In-house method based on*<br>*ISO 8262* |
| Queso<br>*Cheese* | Grasa por gravimetría (método Schmid-Bondzynski-Ratzlaff)<br>*Fat by gravimetry (Schmid-Bondzynski-Ratzlaff method)* | ISO 1735/IDF5 |
| Leche líquida<br>Leche concentrada<br>Leche condensada<br>Leche en polvo y productos lácteos en polvo<br>Nata<br>*Liquid milk*<br>*Concentrated milk*<br>*Condensed milk*<br>*Milk powder and powdered milk products*<br>*Cream* | Grasa por gravimetría (método Röse-Gottlieb)<br>*Fat by gravimetry (Röse-Gottlieb method)* | ISO 1211 / IDF1<br>ISO 1737 / IDF13<br>ISO 1736 / IDF9<br>ISO 2450 / IDF16 |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Proteína/nitrógeno mediante volumetría (método Kjeldahl)<br>*Protein / Nitrogen by titration (Kjeldahl method)* | PNTA0100 Rev. 14<br>*Método interno*<br>*In-house method* |
| Leche y productos lácteos<br>*Milk and milk products* | | |
| Alimentos<br>*Food* | Fibra alimentaria (fracciones de alto peso molecular) por método enzimático-gravimétrico<br>*Dietary fiber (high molecular weight fractions) by enzymatic-gravimetric method)* | PNTA0066<br>*Método interno basado en In-house method based on AOAC 991.43* |
| | Hidratos de carbono (por cálculo)<br>*Carbohydrates (by calculation)* | PNTA0136<br>*Método interno basado en In-house method based on Food energy-methods of analysis and conversion factors, FAO Food and Nutrition Paper* |
| | Humedad por gravimetría<br>*Moisture by gravimetry* | PNTA0081 Rev. 16<br>*Método interno*<br>*In-house method* |
| Cacao y productos a base de cacao<br>*Cocoa and cocoa based products* | | IOCCC 3-E |
| Aceites y grasas<br>*Oils and fats* | Humedad y materias volátiles por gravimetría<br>*Moisture and volatile material by gravimetry* | BOE-A-1977-16116<br>Anexo I Num. 9 (a) |
| Leche<br>Leche concentrada<br>Nata<br>*Milk*<br>*Concentrated milk*<br>*Cream* | Materia seca por gravimetría<br>*Dry matter by gravimetry* | ISO 6731/IDF 21 |
| Leche condensada<br>*Condensed milk* | | ISO 6734/IDF 15 |
| Leche en polvo<br>*Milk powder* | | PNTA0024<br>*Método interno basado en In-house method based on FIL 26A:1993* |

*Accreditation will remain valid until notification to the contrary. This accreditation is subject to modifications, temporary suspensions and withdrawal. Its validity can be confirmed at www.enac.es*

| PRODUCTO/MATERIAL A ENSAYAR<br>*PRODUCTS/MATERIALS TESTED* | ENSAYO<br>*TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO<br>*STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Queso<br>*Cheese* | Sólidos totales por gravimetría<br>*Total solids by gravimetry* | ISO5534/IDF4 |
| Alimentos<br>*Food* | Cloruros por volumetría (valoración potenciométrica)<br>*Chlorides by potentiometric titration* | PNTA0078<br>*Método interno basado en In-house method based on AOAC 986.26* |
| | *Cenizas por gravimetría*<br>*Ashes by gravimetry* | PNTA0083 Rev. 9<br>*Método interno*<br>*In-house method* |
| Leche<br>Leche en polvo<br>Nata<br>Queso<br>*Milk*<br>*Dried milk*<br>*Cream*<br>*Cheese* | | PNTA0027<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 945.46*<br>*AOAC 930.30*<br>*AOAC 935.42*<br>*AOAC 920.108* |
| Cerveza<br>*Beer* | | PNTA0073<br>*Método interno basado en*<br>*In-house method based on*<br>*BOE-A-1985-21911 Anexo I Num. 8* |
| Alimentos<br>*Food* | Dióxido de azufre y sulfitos por volumetría<br>*Sulphur dioxide and sulfites by titration*<br>*(≥ 5 mg/kg)* | PNTA0067<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC962.16* |
| Alimentos envasados<br>*Packed food* | Peso neto y escurrido por gravimetría<br>*Net weight and drained weight by gravimetry* | PNTA0098<br>*Método interno conforme a*<br>*In-house method according to*<br>*BOE-A-1984-26465* |
| Aceites y grasas<br>*Oils and fats* | Índice de peróxidos por volumetría<br>*Peroxide index by titration* | ISO 3960 |

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Leche<br>*Milk* | Acidez por volumetría<br>*Acidity by titration* | PNTA0020<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 947.05* |
| Mantequilla<br>*Butter* | | ISO 1740 / IDF6 |
| Cerveza<br>*Beer* | Acidez por volumetría (valoración potenciométrica)<br>*Acidity by potentiometric titration* | PNTA0073<br>*Método interno basado en*<br>*In-house method based on BOE-A-1985-21911 Anexo I Num. 5* |

Análisis mediante métodos basados en técnicas electroanalíticas
*Analysis by electroanalytic methods*

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Nitrógeno / Proteína por conductividad térmica (método Dumas)<br>*Protein / Nitrogen by thermal conductivity (Dumas method)* | PNTA0135<br>*Método interno basado en*<br>*In-house method based on*<br>*ISO 16634* |
| Cacao y productos a base de cacao<br>*Cocoa and cocoa based products* | pH por potenciometría<br>*pH by potentiometry*<br>*(3,00 – 8,00 pH units)* | IOCCC 9-E |
| Alimentos<br>*Food* | | PNTA0062 Rev. 10<br>*Método interno*<br>*In-house method* |
| | Actividad de agua por resistividad<br>*Water activity by resistivity* | ISO 18787 |

Análisis físico-químicos
*Physico-chemical analysis*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/TEST PROCEDURE*** |
|---|---|---|
| Cerveza<br>*Beer* | Grado alcohólico por densimetría<br>*Alcoholic degree by densimetry* | PNTA0073<br>*Método interno basado en*<br>*In-house method based on*<br>*EBC 9.2.1* |
| | Extracto real, extracto seco primitivo y extracto aparente por densimetría<br>*Real extract by densimetry* | PNTA0073<br>*Método interno basado en*<br>*In-house method based on*<br>*EBC 9.4* |
| | Densidad por densimetría<br>*Density by densimetry* | PNTA0073<br>*Método interno basado en*<br>*In-house method based on*<br>*EBC 9.43.2* |
| | Grado de fermentación (por cálculo)<br>*Degree of fermentation (by calculation)* | PNTA0073<br>*Método interno basado en*<br>*In-house method based on*<br>*BOE-A-1985-21911 Anexo I Num. 3* |
| Alimentos líquidos (excepto vinos y cervezas)<br>*Liquid food (except wine and beer)* | Densidad por densimetría electrónica<br>*Density by densimetry*<br>*(0,900 – 1,500 g/ml)* | PNTA0194<br>*Método interno basado en método fabricante Mettler Toledo equipo DE40 Density Meter*<br>*In-house method based on manufacturer Mettler Toledo equipment DE40 Density Meter* |
| Envases<br>*Packaging* | Oxígeno y dióxido de carbono en el envase por célula electroquímica e IR<br>*$O_2$ and $CO_2$ in packaging by electrochemical cell and IR*<br>*(0-60%)* | PNTA0195<br>*Método interno basado en método fabricante WITT equipo Analizador OXYBABY® M+i*<br>*In-house method based on manufacturer WITT equipment Analizador OXYBABY® M+i* |

Análisis mediante métodos basados en técnicas de espectroscopía molecular
*Analysis by molecular spectroscopy methods*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/TEST PROCEDURE*** |
|---|---|---|
| Alimentos<br>*Food* | Almidón por espectrofotometría UV-VIS<br>*Starch by UV-VIS spectrophotometry* | PNTA0190<br>*Método interno basado en In-house method based on AOAC920.83* |
| Mantequillas<br>*Butter* | Índice de peróxidos por espectrofotometría UV-VIS<br>*Peroxide* index *by UV-VIS spectrophotometry* | FIL 74A:1991 |
| Cerveza<br>*Beer* | Dióxido de azufre y sulfitos por espectrofotometría UV-VIS<br>*Sulphur dioxide and sulfites by UV-VIS spectrophotometry*<br>*(≥ 0,5 mg/l)* | PNTA0073<br>*Método interno basado en In-house method based on BOE-A-1985-21911 Anexo I Num. 10* |
| | Color por espectrofotometría UV-VIS<br>*Colour by UV-VIS spectrophotometry*<br>*(4 – 200 EBC)* | PNTA0073<br>*Método interno basado en In-house method based on EBC 9.6* |
| | Amargor por espectrofotometría UV-VIS<br>*Bitter by UV-VIS spectrophotometry*<br>*(1 – 40 IBU)* | PNTA0073<br>*Método interno basado en In-house method based on EBC 9.8* |

Análisis mediante métodos basados en técnicas cromatográficas
*Analysis by chromatographical methods*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Grasa extraída de alimentos<br>Aceites y grasas<br>*Fat extracted from foodstuffs*<br>*Oils and fats* | Composición relativa de ácidos grasos por cromatografía de gases con detector de ionización de llama (CG-FID)<br>*Relative composition of fatty acids by gas chromatography with flame ionization detector (GC-FID)*<br>Ácido Butírico — *Butyric acid*<br>Ácido Caproico — *Caproic acid*<br>Ácido Heptanoico — *Heptanoic acid*<br>Ácido Caprílico — *Caprylic acid*<br>Ácido Cáprico — *Capric acid*<br>Ácido Undecanoico — *Undecanoic acid*<br>Ácido Láurico — *Lauric acid*<br>Ácido Tridecanoico — *Tridecanoic acid*<br>Ácido Mirístico — *Myristic acid*<br>Ácido Miristoleico — *Myristoleic acid*<br>Ácido Pentadecanoico — *Pentadecanoic acid*<br>Ácido Pentadecenoico — *Pentadecenoic acid*<br>Ácidos grasos C15 ramificados (iso y ante-iso) — *C15 branched-chain fatty acids (iso & ante-iso)*<br>Ácido Palmítico — *Palmitic acid*<br>Ácido Palmitoleico — *Palmitoleic acid*<br>Ácidos grasos C16 ramificados (iso) — *C16 branched-chain fatty acids (iso)*<br>Ácido Heptadecanoico — *Heptadecanoic acid*<br>Ácido Heptadecenoico — *Heptadecenoic acid*<br>Ácidos grasos C17 ramificados (iso y ante-iso) — *C17 branched-chain fatty acids (iso & ante-iso)*<br>Ácido Esteárico — *Stearic acid*<br>Ácidos Trans-oleicos — *Trans-oleic acids*<br>Ácido Cis-oleico — *Cis-oleic acid*<br>Ácidos Trans-linoleicos — *Trans-linoleics acids*<br>Ácido Cis,cis-linoleico — *Cis-cis-linoleic acid*<br>Ácido Gamma-linolénico — *Gamma-linolenic acid*<br>Ácidos Trans-linolénicos — *Trans-linolenic acids*<br>Ácido Cis,cis-linolénico — *Cis,cis-linolenic acid*<br>Ácidos grasos C18 ramificados (iso y ante-iso) — *C18 branched-chain fatty acids (iso & ante-iso)*<br>Ácido Aráquico — *Arachic acid*<br>Ácido Gadoleico — *Gadoleic acid*<br>Ácido Heneicosanoico — *Heneicosanoic acid*<br>Ácido Eicosadienoico — *Eicosadienoic acid*<br>Ácido Behénico — *Behenic acid*<br>Ácido Dihommo-gammalinolénico — *Dihomo-gamma-linolenic acid*<br>Ácido Eicosatrienoico — *Eicosatrienoic acid*<br>Ácido Araquidónico — *Arachidonic acid*<br>Ácido Erúcico — *Erucic acid*<br>Ácido Tricosanoico — *Tricosanoic acid*<br>Ácido Docosadienoico — *Docosadienoic acid*<br>Ácido Eicosapentanoico — *Eicosapentanoic Acid*<br>Ácido Lignocérico — *Lignoceric acid*<br>Ácido Nervónico — *Nervonic acid*<br>Ácido Docosapentanoico — *Docosapentaenoic acid*<br>Ácido Docosahexanoico — *Docosahexaenoic acid* | PNTA0129 Rev. 13<br>*Método interno*<br>*In-house method* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Leche y productos lácteos sin lactosa<br>Bebidas de soja, bebidas de cereales, bebidas de frutos secos<br>*Milk and milk products without lactose*<br>*Soya drinks, cereal drinks, dry fruits drink* | Lactosa por cromatografía líquida con detector de pulso amperométrico (LC-PAD)<br>*Lactose by liquid chromatography with amperometric pulse detector (LC-PAD)*<br>*(≥ 0,01 %)* | PNTA0149<br>*Método interno basado en*<br>*In-house method based on*<br>*DIONEX 87238-AN218-IC Lactose Free PAD_LPN2507* |
| Alimentos<br>Cerveza<br>*Food*<br>*Beer* | Azúcares por cromatografía líquida con detector de pulso amperométrico (LC-PAD)<br>*Sugars by liquid chromatography with pulsed amperometric detector (LC-PAD)*<br>Fructosa/*Fructose*<br>Glucosa/*Glucose*<br>Sacarosa/*Saccharose*<br>Maltosa/*Maltose*<br>Lactosa monohidratada/ *Monohydrated lactose*<br>Galactosa/*Galactose*<br>*(≥ 0,05 %) Alimentos/Food*<br>*(≥ 0,01 %) Cerveza/Beer* | PNTA0179<br>*Método interno basado en*<br>*In-house method based on*<br>*115482-Man-065495-01-CarboPac-PA10* |
| Alimentos<br>*Food* | Lactosa por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Lactose by liquid chromatography with mass spectrometry detector (LC-MS/MS)*<br>*(≥ 20 mg/kg)* | PNTA0189 Rev.5<br>Método interno<br>*In-house method* |
| | Fibra alimentaria (fracciones de alto y bajo peso molecular) por método enzimático-gravimétrico y cromatografía líquida con detector de índice de refracción (LC-RID)<br>*Dietary fiber (high and low molecular weight fractions) by enzymatic-gravimetric method and liquid chromatography with refraction index detector (LC-RID)* | PNTA0151<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 2009.01* |
| | Polialcoholes por cromatografía líquida con detector de índice de refracción (LC-RID)<br>*Polyols by liquid chromatography with refractive index detector (LC-RID)*<br>Maltitol/*Maltitol*<br>Lactitol/*Lactitol*<br>Mannitol/*Mannitol*<br>Xilitol/*Xilitol*<br>Sorbitol/*Sorbitol*<br>Isomaltitol/*Isomaltitol*<br>Eritritol/*Eritritol*<br>*(≥ 0,5%)* | PNTA0177<br>*Método interno basado en*<br>*In-house method based on*<br>*Supelco bulletin 887B* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Azúcares por cromatografía líquida con detector de índice de refracción (LC-RID)<br>*Sugars by liquid chromatography with refraction index detector (LC-RID)*<br>Fructosa/*Fructose*<br>Glucosa/*Glucose*<br>Sacarosa/*Saccharose*<br>Maltosa/*Maltose*<br>Lactosa monohidratada/ *Monohydrated lactose*<br>*(≥ 0,5%)* | PNTA0101<br>*Método interno basado en*<br>*In-house method based on*<br>*IOCCC 117* |
| | Glicerina por cromatografía líquida con detector de índice de refracción (LC-RID)<br>*Glycerol by liquid chromatography with refraction index detector (LC-RID)*<br>*(≥ 0,5%)* Productos líquidos/*Liquid products*<br>*(≥ 0,2 g/l)* Productos sólidos/*Solid products* | PNTA0205<br>*Método interno basado en*<br>*In-house method based on*<br>*Amer. Journal of Enology and Viticulture, Vol.2, 2007, 279-282* |
| | Vitamina B1 (tiamina) y vitamina B2 (riboflavina) por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Vitamin B1 (Thiamine) and vitamin B2 (riboflavin) by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,1 mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 0,2 mg/kg)* Productos sólidos/*Solid products* | PNTA0128<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 14122*<br>*EN 14152* |
| | Vitamina B6 (Piridoxina) por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Vitamin B6 (Pyridoxine) by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,2 mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 0,4 mg/kg)* Productos sólidos/*Solid products* | PNTA0056<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 14164* |
| | Biotina por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Biotin by liquid chromatography with mass spectrometer detector (LC-MS/MS)*<br>*(≥ 20 µg/kg)* | PNTA0164<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 15607* |
| | Vitamina C (ácido ascórbico + dehidroascórbico) por cromatografía líquida con detector ultravioleta (LC-UV)<br>*Vitamin C (ascorbic acid + dehydroascorbic acid) by liquid chromatography with UV detector (LC-UV)*<br>*(≥ 5 mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 20 mg/kg)* Productos sólidos/*Solid products* | PNTA0127<br>*Método interno basado en*<br>*In-house method based on*<br>*NF V03-135* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Vitamina B12 (cianocobalamina) por cromatografía líquida con detector de diodos en serie (CL-DAD)<br>*Vitamin B12 (cyanocobalamin) by liquid chromatography with diode array detector (CL-DAD)*<br>*(≥ 1 µg/kg)* | PNTA0137<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 2011.08* |
| | Ácido fólico por cromatografía líquida con detector de diodos en serie (CL-DAD)<br>*Folic acid by liquid chromatography with diode array detector (LC-DAD)*<br>*(≥ 10 µg/kg)* | PNTA0138<br>*Método interno basado en*<br>*In-house method based on*<br>*r-Biopharm EASI-EXTRACT FOLIC ACID P81* |
| | Vitamina A (retinol) por cromatografía líquida con detector ultravioleta (LC-UV)<br>*Vitamin A (retinol) by liquid chromatography with UV detector (LC-UV)*<br>*(≥ 0,1 mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 0,5 mg/kg)* Productos sólidos/*Solid products* | PNTA0145<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 12823-1* |
| | Vitamina E (alfa-tocoferol) por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Vitamin E (alpha-tocopherol) by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,3 mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 1,5 mg/kg)* Productos sólidos/*Solid products* | PNTA0145<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 12822* |
| | Vitamina D3 (colecalciferol) por cromatografía líquida con detector ultravioleta (LC-UV)<br>*Vitamin D3 (cholecalciferol) by liquid chromatography with UV detector (LC-UV)*<br>*(≥ 1 µg/kg)* Productos líquidos /*Liquid products*<br>*(≥ 5 µg/kg)* Productos sólidos/*Solid products* | PNTA0146<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 12821* |
| | Vitamina B3 (niacina) por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Vitamin B3 (niacin) by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 2 mg/kg)* | PNTA0147<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 15652* |
| | Vitamina B5 (Ácido pantoténico) por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Vitamin B5 (pantothenic acid) by liquid chromatography with mass spectrometer detector (LC-MS/MS)*<br>*(≥ 0,5 mg/kg)* | PNTA0148<br>*Método interno basado en*<br>*In-house method based on*<br>*J AOAC Int. 2012 Jan-Feb;95(1):143-8* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Vitamina K1 (filoquinona) por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Vitamin K1 (Phylloquinone) by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 1 µg/kg)* Productos líquidos /*Liquid products*<br>*(≥ 10 µg/kg)* Productos sólidos/*Solid products* | PNTA0178<br>*Método interno basado en*<br>*In-house method based on*<br>*EN 14148* |
| | Vitamina B9 (ácido fólico y folatos) por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Vitamin B9 (folic acid and folates) by liquid chromatography with mass spectrometer detector (LC-MS/MS)*<br>*(≥ 10 µg/kg)* | PNTA0192<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 2011.06* |
| | Vitamina D2 y Vitamina D3 por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Vitamin D2 and vitamin D3 by liquid chromatography with mass spectrometer detector (LC-MS/MS)*<br>*(≥ 1 µg/kg)* Productos líquidos /*Liquid products*<br>*(≥ 5 µg/kg)* Productos sólidos/*Solid products* | PNTA0202<br>*Método interno basado en*<br>*In-house method based on*<br>*ISO 20636* |
| | Colesterol por cromatografía de gases con detector de ionización de llama (CG-FID)<br>*Cholesterol by gas chromatography with flame ionization detector (GC-FID)*<br>*(≥ 10 mg/kg)* | PNTA0093<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 994.10* |
| Café verde, café tostado<br>Cacao<br>Cereales y productos elaborados a base de cereal<br>Alimentos infantiles<br>*Green coffee, roasted coffee*<br>*Cocoa*<br>*Cereals and cereal based products*<br>*Baby food* | Ocratoxina A por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Ochratoxin A by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,5 µg/kg)* (excepto alimentos infantiles ≥ 0,25 µg/kg)<br>*(≥ 0,5 µg/kg) (except baby food ≥ 0,25 µg/kg)* | PNTA0077<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |
| Frutos secos<br>Especias<br>Cereales y productos elaborados a base de cereal<br>*Nuts*<br>*Spices*<br>*Cereals and cereal based products* | Aflatoxinas por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Aflatoxins by liquid chromatography with fluorescence detector (LC-FLD)*<br>Aflatoxinas B1 y G1/ *Aflatoxins B1 and G1*: *(≥ 0,5 µg/kg)*<br>Aflatoxinas B2 y G2/ *Aflatoxins B2 and G2*: *(≥ 0,2 µg/kg)* | PNTA0053<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos infantiles<br>*Baby food* | Aflatoxina B1 por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Aflatoxin B1 by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,05 µg/kg)* | PNTA0053<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |
| Cereales y productos elaborados a base de cereal Alimentos infantiles<br>*Cereals and cereal based products*<br>*Baby food* | Zearalenona por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Zearalenone by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 20 µg/kg)* | PNTA0122<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |
| Cereales y productos elaborados a base de cereal Alimentos infantiles<br>*Cereals and cereal based products*<br>*Baby food* | Deoxynivalenol por cromatografía líquida con detector de diodos en serie (LC-DAD)<br>*Deoxynivalenol by liquid chromatography with diode array detector (LC-DAD)*<br>*(≥ 100 µg/kg)* | PNTA0121<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |
| | Fumonisinas B1 y B2 por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Fumonisins B1 and B2 by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 100 µg/kg)* | PNTA0123<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |
| Leche y productos lácteos Alimentos infantiles<br>*Milk and dairy products*<br>*Milk – based baby food* | Aflatoxina M1 por cromatografía líquida con detector de fluorescencia (LC-FLD)<br>*Aflatoxin M1 by liquid chromatography with fluorescence detector (LC-FLD)*<br>*(≥ 0,010 µg/kg)* Leche líquida y alimentos infantiles/*Milk and milk – based baby food*<br>*(≥ 0,050 µg/kg)* Leche condensada/*Condensed milk*<br>*(≥ 0,10 µg/kg)* Productos lácteos/*Dairy products* | PNTA0170<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (CE) nº 401/2006 y sus posteriores modificaciones*<br>*Regulation (EC) nº 401/2006 and its subsequent amendments* |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Panes y productos de bollería/pastelería, bebidas<br>*Bread and bakery products, beverages* | Ácido benzoico y sórbico por cromatografía líquida con detector de diodos en serie (LC-DAD)<br>*Sorbic acid and benzoic acid by liquid chromatography with diode array detector (LC-DAD)*<br>*(≥ 2,5mg/kg)* Productos líquidos/*Liquid products*<br>*(≥ 5 mg/kg)* Productos sólidos/*Solid products* | PNTA0063<br>*Método interno basado en*<br>*In-house method based on*<br>*ISO 9231* |
| Bebidas no alcohólicas<br>Batidos de cacao o chocolate<br>Chocolate blanco o con leche y productos que contienen cacao, chocolate o café<br>Chocolate negro<br>Café descafeinado<br>Cacao<br>Café<br>Té<br>*Non-alcoholic beverages*<br>*Cocoa or chocolate drinks*<br>*White or milk chocolate, other products containing cocoa, chocolate or coffee*<br>*Black chocolate*<br>*Decaffeinated coffee*<br>*Cocoa*<br>*Coffee*<br>*Tea* | Cafeína y teobromina por cromatografía líquida con detector de array de diodos (LC-DAD)<br>*Caffein and theobromin by liquid chromatography with diode array detector (LC-DAD)*<br>*(≥ 1 mg/l)* Bebidas no alcohólicas/*Non-alcoholic beverages*<br>*(≥ 5 mg/kg)* Batidos de cacao o chocolate/*Cocoa or chocolate drinks*<br>*(≥ 50 mg/kg)* Chocolate blanco o con leche y productos que contienen cacao, chocolate o café/*White or milk chocolate and other products containing cocoa, chocolate or coffee*<br>*(≥ 100 mg/kg)* Chocolate negro, Café descafeinado/*Black chocolate, Decaffeinated coffee*<br>*(≥ 150 mg/kg)* Cacao/*Cocoa*<br>*(≥ 200 mg/kg)* Café/*Coffee*<br>*(≥ 500 mg/kg)* Té/*Tea* | PNTA0169<br>*Método interno basado en*<br>*In-house method based on*<br>*AOAC 980.14* |
| Patatas y productos a base de patatas<br>Cereales y productos a base de cereales, incluyendo galleteria, bolleria, panes;<br>Alimentos infantiles a base de cereales<br>*Potato and potato based products;*<br>*Cereals and cereal based products, including cookies; bakery products and Bread*<br>*Cereal based baby food* | Acrilamida por cromatografía líquida con detector de espectrometría de masas (LC-MS/MS)<br>*Acrylamide by liquid chromatography with mass spectrometer detector (LC-MS/MS)*<br>*(≥ 20 µg/kg)* | PNTA0171<br>*Método interno conforme a*<br>*In-house method according to*<br>*Reglamento (UE) nº 2017/2158*<br>*Regulation (EU) nº 2017/2158* |

Análisis mediante métodos basados en técnicas de espectrometría atómica
*Analysis by atomic absorption spectrometry methods*

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Aceites y grasas<br>Alimentos estimulantes (cacao y productos a base de cacao, café, té)<br>Alimentos infantiles<br>Bebidas alcohólicas/no alcohólicas<br>Carne y productos cárnicos<br>Cereales y productos elaborados a base de cereal<br>Comidas preparadas<br>Frutas y vegetales<br>Frutos Secos<br>Leche y productos lácteos<br>Ovoproductos<br>Pastelería y repostería<br>Productos de la bollería<br>Productos de la pesca<br><br>*Oils and fats*<br>*Stimulating food (Cocoa and cocoa based products, coffee, tea)*<br>*Baby food*<br>*Alcoholic/Non-Alcoholic beverages*<br>*Meat and meat products*<br>*Cereals and cereal based products*<br>*Ready- to-eat food*<br>*Fruits and vegetables*<br>*Nuts*<br>*Milk and milk products*<br>*Egg products*<br>*Confectionery*<br>*Bakery products*<br>*Fish products* | Calcio, Magnesio y Zinc por espectrometría de absorción atómica (atomización por llama)<br>*Calcium, Magnesium and Zinc by Flame Atomic Absorption Spectroscopy (FAAS)*<br><br>Cerveza/*Beer*<br>Calcio/*Calcium* *(≥ 10 mg/kg)* Zinc/*Zinc* *(≥ 0,1 mg/kg)*<br>Magnesio/*Magnesium* *(≥ 5 mg/kg)*<br><br>Productos sólidos y aceites y grasas/*Solid products and oils and fats*<br>Calcio/*Calcium* *(≥ 50 mg/kg)* Zinc/*Zinc* *(≥ 5 mg/kg)*<br>Magnesio/ *Magnesium* *(≥ 25 mg/kg)*<br><br>Productos líquidos (excepto cerveza)/*Liquid products (except beer)*<br>Calcio/*Calcium* *(≥ 10 mg/kg)* Zinc/*Zinc* *(≥ 1 mg/kg)*<br>Magnesio/*Magnesium* *(≥ 5 mg/kg)* | PNTA0016<br><br>*Método interno conforme a In-house method according to EN 13804* |
| | Sodio por fotometría de llama<br><br>*Sodium by flame photometry*<br><br>*(≥ 5 mg/kg)* Productos sólidos y aceites y grasas/*Solid products and oils and fats*<br><br>*(≥ 1 mg/kg)* Productos líquidos/*Liquid products* | |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/TEST PROCEDURE* |
|---|---|---|
| Aceites y grasas<br>Alimentos estimulantes (cacao y productos a base de cacao, café, té)<br>Alimentos infantiles<br>Azúcar y derivados<br>Bebidas alcohólicas/no alcohólicas<br>Carne y productos cárnicos<br>Cereales y productos elaborados a base de cereal<br>Comidas preparadas<br>Frutas y vegetales<br>Frutos Secos<br>Leche y productos lácteos<br>Ovoproductos<br>Pastelería y repostería<br>Productos de la bollería<br>Productos de la pesca<br><br>*Oils and fats*<br>*Stimulating food (Cocoa and cocoa based products, coffee, tea)*<br>*Baby food*<br>*Sugar and derivatives*<br>*Alcoholic/Non-Alcoholic beverages*<br>*Meat and meat products*<br>*Cereals and cereal based products*<br>*Ready- to-eat food*<br>*Fruits and vegetables*<br>*Nuts*<br>*Milk and milk products*<br>*Egg products*<br>*Confectionery*<br>*Bakery products*<br>*Fish products* | Arsénico, Cadmio, Cobre, Hierro y Plomo por espectrometría de absorción atómica (atomización electrotérmica)<br>*Arsenic, Cadmium, Copper, Iron and Lead by Electrothermal Atomic Absorption Spectroscopy (ETAAS)*<br><br>Aceites y grasas/*Oils and fats*<br>Arsénico/*Arsenic* *(≥ 0,050 mg/kg)* Hierro/*Iron* *(≥ 0,20 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,020 mg/kg)* Plomo/*Lead* *(≥ 0,020 mg/kg)*<br>Cobre/*Copper* *(≥ 0,20 mg/kg)*<br><br>Azúcar y derivados/*Sugar and derivatives*<br>Arsénico/*Arsenic* *(≥ 0,050 mg/kg)* Plomo/*Lead* *(≥ 0,050 mg/kg)*<br><br>Carne y productos cárnicos, vegetales /*Meat and meat products, vegetables*<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,010 mg/kg)* Plomo/*Lead* *(≥ 0,020 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br><br>Cereales y productos de molienda, Legumbres /*Cereals and products of the milling, legumes*<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,020 mg/kg)* Plomo/*Lead* *(≥ 0,040 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br><br>Leche líquida y alimentos infantiles reconstruídos/*Liquid milk and baby foods reconstructed*<br>Arsénico/*Arsenic* *(≥ 0,020 mg/kg)* Hierro/*Iron* *(≥ 0,10 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,010 mg/kg)* Plomo/*Lead* *(≥ 0,008 mg/kg)*<br>Cobre/*Copper* *(≥ 0,10 mg/kg)*<br><br>Alimentos infantiles líquidos base láctea/*Dairy liquid baby foods*<br>Arsénico/*Arsenic* *(≥ 0,020 mg/kg)* Hierro/*Iron* *(≥ 0,10 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,002 mg/kg)* Plomo/*Lead* *(≥ 0,008 mg/kg)*<br>Cobre/*Copper* *(≥ 0,10 mg/kg)*<br><br>Alimentos infantiles sólidos/*Solid baby foods*<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,004 mg/kg)* Plomo/*Lead* *(≥ 0,050 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br><br>Productos de la pesca /Fish products<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,010 mg/kg)* Plomo/*Lead* *(≥ 0,050 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br><br>Cacao y derivados /*Cocoa and cocoa based products*<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,020 mg/kg)* Plomo/*Lead* *(≥ 0,050 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br><br>Resto productos líquidos/*Other liquid products*<br>Arsénico/*Arsenic* *(≥ 0,020 mg/kg)* Hierro/*Iron* *(≥ 0,10 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,010 mg/kg)* Plomo/*Lead* *(≥ 0,010 mg/kg)*<br>Cobre/*Copper* *(≥ 0,10 mg/kg)*<br><br>Resto productos sólidos/*Other solid products*<br>Arsénico/*Arsenic* *(≥ 0,10 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,050 mg/kg)* Plomo/*Lead* *(≥ 0,050 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)* | PNTA0017<br><br>*Método interno conforme a In-house method according to EN 13804* |

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** **TYPE OF TEST** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Aceite<br>Alimentos dietéticos<br>Alimentos infantiles<br>Azúcar<br>Bebidas no alcohólicas<br>Cacao y productos a base de cacao<br>Cereales y productos elaborados a base de cereal<br>Cervezas<br>Golosinas, caramelos y otros derivados de azúcar<br>Leche y productos lácteos<br>Productos cárnicos cocidos<br>Productos cárnicos curados<br><br>*Oil*<br>*Dietary products*<br>*Baby food*<br>*Sugar*<br>*Non-alcoholic beverages*<br>*Cocoa and cocoa based products*<br>*Cereals and cereal based products*<br>*Beer*<br>*Candies, sweeties and other sugar derivatives*<br>*Milk and milk products*<br>*Cooked meat products*<br>*Cured meat products* | Calcio, Cobre, Fósforo, Hierro, Magnesio, Potasio, Sodio y Zinc por espectroscopia de emisión atómica con plasma de acoplamiento inductivo (ICP-AES)<br>*Calcium, Copper, Phosphorus, Iron, Magnesium, Potassium, Sodium and Zinc by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)*<br><br>Aceite/*Oil*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)* Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 25 mg/kg)* Sodio/*Sodium* *(≥ 5 mg/kg*<br><br>Cerveza/B*eer*<br>Calcio /*Calcium* *(≥ 5 mg/kg)* Magnesio/*Magnesium* *(≥ 5 mg/kg)*<br>Cobre/*Copper* *(≥ 0,10 mg/kg)* Potasio/*Potassium* *(≥ 5 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 5 mg/kg)* Sodio/*Sodium* *(≥ 1 mg/kg)*<br>Hierro/*Iron* *(≥ 0,10 mg/kg)* Zinc /*Zinc* *(≥ 0,1 mg/kg)*<br><br>Golosinas, caramelos y otros derivados de azúcar/*Candies, sweeties and other sugar derivatives*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)* Sodio/*Sodium* *(≥ 5 mg/kg)*<br><br>Leche y productos lácteos/*Milk and milk products*<br>Productos sólidos/ *Solid products* — Productos líquidos/ *Liquid products*<br>Calcio /*Calcium* *(≥ 25 mg/kg)* *(≥ 5 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg* *(≥ 0,10 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 25 mg/kg)* *(≥ 5 mg/kg)*<br>Hierro/*Iron* *(≥ 0,50 mg/kg)* *(≥ 0,10 mg/kg)*<br>Magnesio /*Magnesium* *(≥ 25 mg/kg)* *(≥ 5 mg/kg)*<br>Potasio/*Potassium* *(≥ 25 mg/kg)* *(≥ 5 mg/kg)*<br>Sodio/*Sodium* *(≥ 5 mg/kg)* *(≥ 1 mg/kg)*<br>Zinc /*Zinc* *(≥ 5 mg/kg)* *(≥ 1 mg/kg)*<br><br>Resto productos sólidos/Other s*olid products*<br>Calcio /*Calcium* *(≥ 25 mg/kg)* Magnesio /*Magnesium* *(≥ 25 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)* Potasio/*Potassium* *(≥ 25 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 25 mg/kg)* Sodio/*Sodium* *(≥ 5 mg/kg)*<br>Hierro/*Iron* *(≥ 0,50 mg/kg)* Zinc /*Zinc* *(≥ 5 mg/kg)*<br><br>Resto productos líquidos/*Other liquid products*<br>Calcio /*Calcium* *(≥ 5 mg/kg)* Magnesio /*Magnesium* *(≥ 5 mg/kg)*<br>Cobre/*Copper* *(≥ 0,10 mg/kg)* Potasio/*Potassium* *(≥ 5 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 5 mg/kg)* Sodio/*Sodium* *(≥ 1 mg/kg)*<br>Hierro/*Iron* *(≥ 0,10 mg/kg)* Zinc /*Zinc* *(≥ 1 mg/kg)* | PNTA0141<br><br>*Método interno conforme a*<br>*In-house method according to EN 13804* |

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** **TYPE OF TEST** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Alimentos<br>Cerveza<br><br>*Food*<br>*Beer* | Elementos por espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS)<br>*Elements by mass-spectrometry with Inductively Coupled Plasma (ICP-MS)*<br><br>Alimentos (excepto aceites y grasas)/*Food (except Oils and fats)*<br><br>Aluminio/*Aluminium* *(≥ 5,0 mg/kg)*<br>Arsénico/*Arsenic* *(≥ 0,020 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,005 mg/kg)*<br>(excepto alimentación infantil/*except baby food*: *≥0,002 mg/kg*)<br>Calcio/*Calcium* *(≥ 10mg/kg)*<br>Cobalto/*Cobalt* *(≥ 0,50 mg/kg)*<br>Cobre/*Copper* *(≥ 0,50 mg/kg)*<br>Cromo/*Chromium* *(≥ 0,050 mg/kg)*<br>Estaño/*Tin* *(≥ 0,050 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 20mg/kg)*<br>Hierro/*Iron* *(≥ 0,50 mg/kg)*<br>Magnesio/*Magnesium* *(≥ 10 mg/kg)*<br>Manganeso/*Manganese* *(≥ 0,050 mg/kg)*<br>Mercurio/*Mercury* *(≥ 0,005 mg/kg)*<br>(excepto alimentación infantil/*except baby food*: *≥0,004 mg/kg*)<br>Molibdeno/*Molybdenum* *(≥ 0,050 mg/kg)*<br>Níquel/*Nickel* *(≥ 0,50 mg/kg)*<br>Plomo/*Lead* *(≥ 0,005 mg/kg)*<br>Potasio/*Potasium* *(≥ 10mg/kg)*<br>Selenio/*Selenium* *(≥0,050 mg/kg)*<br>Sodio/*Sodium* *(≥ 10mg/kg)*<br>Zinc/*Zinc* *(≥0,50 mg/kg)*<br><br>Aceites y grasas/*Oils and fats*<br><br>Aluminio/*Aluminium* *(≥ 10,0 mg/kg)*<br>Arsénico/*Arsenic* *(≥ 0,040 mg/kg)*<br>Cadmio/*Cadmium* *(≥ 0,010 mg/kg)*<br>Calcio/*Calcium* *(≥ 20mg/kg)*<br>Cobalto/*Cobalt* *(≥ 1,0 mg/kg)*<br>Cobre/*Copper* *(≥ 1,0 mg/kg)*<br>Cromo/*Chromium* *(≥ 0,10 mg/kg)*<br>Estaño/*Tin* *(≥ 0,10 mg/kg)*<br>Fósforo/*Phosphorus* *(≥ 40mg/kg)*<br>Hierro/*Iron* *(≥ 1,0 mg/kg)*<br>Magnesio/*Magnesium* *(≥ 20mg/kg)*<br>Manganeso/*Manganese* *(≥ 0,10 mg/kg)*<br>Mercurio/*Mercury* *(≥ 0,010 mg/kg)*<br>Molibdeno/*Molybdenum* *(≥ 0,10 mg/kg)*<br>Níquel/*Nickel* *(≥ 1,0 mg/kg)*<br>Plomo/*Lead* *(≥ 0,010 mg/kg)*<br>Potasio/*Potasium* *(≥ 20mg/kg)*<br>Selenio/*Selenium* *(≥0,10 mg/kg)*<br>Sodio/*Sodium* *(≥ 20mg/kg)*<br>Zinc/*Zinc* *(≥ 1,0 mg/kg)*<br><br>Cerveza/*Beer*<br><br>Aluminio/*Aluminiun* *(≥ 0,10 mg/l)*<br>Arsénico/*Arsenic* *(≥ 0,005 mg/l)*<br>Cadmio/*Cadmium* *(≥ 0,001 mg/l)*<br>Calcio/*Calcium* *(≥ 10 mg/l)*<br>Cobalto/*Cobalt* *(≥ 0,010 mg/l)*<br>Cobre/*Copper* *(≥ 0,10 mg/l)*<br>Cromo/*Chromium* *(≥ 0,050 mg/l)*<br>Estaño/*Tin* *(≥ 0,010 mg/l)*<br>Fósforo/*Phosphorus* *(≥ 20 mg/l)*<br>Hierro/*Iron* *(≥ 0,10 mg/l)*<br>Magnesio/*Magnesium* *(≥ 10 mg/l)*<br>Manganeso/*Manganese* *(≥ 0,050 mg/l)*<br>Mercurio/*Mercury* *(≥ 0,001 mg/l)*<br>Molibdeno/*Molybdenum* *(≥ 0,050 mg/l)*<br>Níquel/*Nickel* *(≥ 0,10 mg/l)*<br>Plomo/*Lead* *(≥ 0,001 mg/l)*<br>Potasio/*Potasium* *(≥ 20 mg/l)*<br>Selenio/*Selenium* *(≥ 0,050 mg/l)*<br>Sodio/*Sodium* *(≥ 10 mg/l)*<br>Zinc/*Zinc* *(≥ 0,10 mg/l)* | PNTA0193<br><br>*Método interno conforme a In-house method according to EN 13804* |

Análisis mediante métodos basados en técnicas de enzimoinmunoensayo
*Analysis by enzyme immunoassay methods*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Alimentos hidrolizados y/o fermentados<br>*Hydrolyzed and/or fermented foods* | Determinación del gluten por ELISA Competitivo<br>*Gluten by Competitive ELISA*<br>*(≥ 10 mg/kg)* | AOAC2015.05 |
| Alimentos (excepto productos hidrolizados y/o fermentados) Aguas de proceso<br>*Food (except hydrolyzed and/or fermented products) Process water* | Gluten mediante ELISA sándwich (anticuerpo R5)<br>*Gluten by ELISA sandwich (R5 antibody)*<br>*(≥ 5 mg/kg)*<br>*(≥ 2,5 mg/kg)* | AOAC 2012.01<br>PNTA0207<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Leche por ELISA<br>*Milk by ELISA*<br>*(≥ 2,5 mg/kg) expresado en leche desnatada en polvo/expressed skimmed milk powdered* | PNTA0144<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| Alimentos Aguas de proceso<br>*Food Process water* | Huevo por ELISA<br>*Egg by ELISA*<br>*(≥ 0,1 mg/kg)* expresado en proteína de clara de huevo/*expressed in egg white protein* | PNTA0143<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Soja por ELISA<br>*Soya by ELISA*<br>*(≥ 2,5 mg/kg) expresado en proteína de soja/expressed in soya protein* | PNTA0161<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Almendra por ELISA<br>*Almond by ELISA*<br>*(≥ 2,5 mg/kg)* expresado en almendra/*expressed in almond*<br>*(≥ 0,5 mg/kg)* expresado en proteína de almendra/ *expressed in almond protein* | PNTA0162<br>PNTA0203<br>*Métodos internos basados en kit comercial(*)*<br>*In-house methods based on commercial kit(*)* |

(*) La información sobre el kit concreto usado está disponible en el laboratorio
*(*) Information about the specific kit used is available in the laboratory*

*Accreditation will remain valid until notification to the contrary. This accreditation is subject to modifications, temporary suspensions and withdrawal. Its validity can be confirmed at www.enac.es*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Alimentos<br>Aguas de proceso<br>*Food*<br>*Process water* | Avellana por ELISA<br>*Hazelnut by ELISA*<br>*(≥ 2,5 mg/kg)* expresado en avellana*/expressed in hazelnut* | PNTA0163<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Cacahuete por ELISA<br>*Peanut by ELISA*<br>*(≥ 2,5 mg/kg)* expresado en cacahuete*/expressed in peanut* | PNTA0166<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Nuez de macadamia por ELISA<br>*Macadamia nut by ELISA*<br>*(≥ 2,0 mg/kg)* expresado en nuez de macadamia*/expressed in macadamia nut* | PNTA0204<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| Alimentos<br>*Food* | Nuez por ELISA<br>*Walnut by ELISA*<br>*(≥ 2,0 mg/kg)* expresado en nuez*/expressed in walnut* | PNTA0181<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Lisozima por ELISA<br>*Lysozym by ELISA*<br>*(≥ 0,05 mg/kg)* para vino*/for wine*<br>*(≥ 0,25 mg/kg)* resto de alimentos*/for other foods* | PNTA0167<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| Bebidas de soja<br>*Soy beverages* | Caseína por ELISA<br>*Casein by ELISA*<br>*(≥ 0,3 mg/kg)* | PNTA0165<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| Bebidas de soja<br>*Soy beverages* | Beta lactoglobulina por ELISA<br>*Beta-lactoglobulin by ELISA*<br>*(≥ 0,1 mg/kg)* | PNTA0174<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |

(*) La información sobre el kit concreto usado está disponible en el laboratorio
*(*) Information about the specific kit used is available in the laboratory*

| **PRODUCTO/MATERIAL A ENSAYAR** ***PRODUCTS/MATERIALS TESTED*** | **ENSAYO** ***TYPE OF TEST*** | **NORMA/PROCEDIMIENTO DE ENSAYO** ***STANDARD SPECIFICATIONS/ TEST PROCEDURE*** |
|---|---|---|
| Leche y productos lácteos<br>*Milk and dairy products* | Nuez de Brasil por ELISA (método cualitativo)<br>*Brasil nut by ELISA (Qualitative method)*<br>*(≥ 2,0 mg/kg)* expresado en nuez de Brasil/*expressed in Brasil nut* | PNTA0197<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Nuez pecana por ELISA (método cualitativo)<br>*Pecan nut by ELISA (Qualitative method)*<br>*(≥ 2,0 mg/kg)* expresado en nuez pecana/*expressed in pecan nut* | PNTA0199<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Pistacho por ELISA (método cualitativo)<br>*Pistaccio by ELISA (Qualitative method)*<br>(*≥ 2,0 mg/kg)* expresado en pistacho/*expressed in pistaccio* | PNTA0200<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| | Anacardo por ELISA (método cualitativo)<br>*Cashew by ELISA (Qualitative method)*<br>*(≥ 2,0 mg/kg)* expresado en anacardo/*expressed in cashew* | PNTA0201<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |
| Alimentos<br>*Food* | Huevo por ELISA<br>*Egg by ELISA*<br>*(≥ 1,0 mg/kg)* expresado en proteína de huevo/*expressed in egg protein* | PNTA0196<br>*Método interno basado en kit comercial(*)*<br>*In-house method based on commercial kit(*)* |

(*) La información sobre el kit concreto usado está disponible en el laboratorio
*(*) Information about the specific kit used is available in the laboratory*

## ÁREA DE MICROBIOLOGÍA

Análisis de alimentos mediante métodos basados técnicas de aislamiento en medios de cultivo
*Food analysis by isolation in culture media methods*

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>Piensos<br>Esponjas y escobillones de muestreo de superficies<br>*Food*<br>*Feed*<br>*Sampling sponges and swaps of surfaces* | Recuento en placa de microorganismos aeróbicos a 30 °C<br>*Plate count of aerobic microorganisms (30°C)* | ISO 4833-1 |
| Alimentos<br>Piensos<br>Esponjas y escobillones de muestreo de superficies<br>*Food*<br>*Feed*<br>*Sampling sponges and swaps of surfaces* | Recuento en placa de mohos y levaduras a 25 °C<br>*Plate count of moulds and yeasts (25°C)* | NF V08-059 |
| | Recuento en placa de enterobacterias<br>*Plate count of Enterobacteriaceae* | ISO 21528-2 |
| | Recuento en placa de enterobacterias presuntivas<br>*Plate count of presumptive Enterobacteriaceae* | NF V08-054 |
| Alimentos<br>*Food* | Recuento en placa de *Bacillus cereus* presuntivos<br>*Plate count of presumptive Bacillus cereus* | ISO 7932 |
| | Recuento en placa de bacterias lácticas<br>*Plate count of lactic acid bacteria* | ISO 15214 |
| | Recuento en placa de *Clostridium perfringens*<br>*Plate count of Clostridium perfringens* | ISO 7937 |
| | Recuento en placa de coliformes a 30 °C<br>*Plate count of coliforms (30 °C)* | ISO 4832 |
| | Recuento en placa de *Escherichia coli* β-glucuronidasa positivo<br>*Plate count of Escherichia coli β-glucuronidase positive* | ISO 16649-2 |
| | Recuento en placa de *Listeria monocytogenes*<br>*Plate count of Listeria monocytogenes* | ISO 11290-2 |
| | Recuento en placa de *Listeria* spp.<br>*Plate count of Listeria spp.* | ISO 11290-2 |
| | Recuento en placa de estafilococos coagulasa positivos<br>*Plate count of coagulase-positive staphylococci* | ISO 6888-1 |

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Recuento en placa de mohos y levaduras a 25 °C<br>*Plate count of moulds and yeasts (25°C)* | ISO 21527-1<br>ISO 21527-2 |
| | Recuento en placa de bacterias anaerobias sulfitorreductoras<br>*Plate count of sulfite-reducing anaerobic bacteria* | ISO 15213 |
| | Detección de *Bacillus cereus* presuntivos<br>*Detection of Presumptive Bacillus cereus* | ISO 21871 |
| | Detección de enterobacterias<br>*Detection of Enterobacteriaceae* | ISO 21528-1 |
| | Detección de *Escherichia coli*<br>*Detection of Escherichia coli* | ISO 7251 |
| | Detección de *Salmonella* spp.<br>*Detection of Salmonella spp.* | ISO 6579-1 |
| | Detección de *Salmonella* (móviles)<br>*Detection of Salmonella (mobile)* | PNTA0140<br>*Método interno basado en*<br>*In-house method based on ISO6579-1* |
| Alimentos<br>Esponjas, gasas y escobillones de muestreo de superficies<br>*Food*<br>*Sampling sponges, chiffon and swaps of surfaces* | Detección de *Listeria* spp.<br>*Detection of Listeria spp.* | ISO11290-1 |
| | Detección de *Listeria monocytogenes*<br>*Detection of Listeria monocytogenes* | |
| Alimentos<br>Esponjas, gasas y escobillones de muestreo de superficies<br>*Food*<br>*Sampling sponges, chiffon and swaps of surfaces* | Detección de *Listeria monocytogenes*<br>*Detection of Listeria monocytogenes* | PNTA0153<br>*Método interno basado en*<br>*In-house method based on ALOA® COUNT* |
| Alimentos<br>*Food* | Recuento en placa de estafilococos coagulasa positivo<br>*Plate count of coagulase-positive staphylococci* | ISO 6888-2 |

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Productos cárnicos<br>Productos lácteos<br>*Meat products*<br>*Milk products* | Recuento en placa de *Pseudomonas* spp.<br>*Plate count of Pseudomonas spp.* | PNTA0009<br>*Método interno basado en*<br>*In-house method based on*<br>*ISO 13720* |
| Preparados lácteos en polvo<br>*Milk preparations powdered* | Detección de *Cronobacter* spp.<br>*Detection of Cronobacter spp.* | ISO 22964 |

Análisis mediante métodos de ensayo basados en inmunofluorescencia (ELFA)
*Analysis by inmunofluorescence methods*

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>Esponjas y escobillones de muestreo de superficies<br>*Food*<br>*Sampling sponges and swaps of surfaces* | Detección de *Listeria monocytogenes* por inmunofluorescencia (ELFA)<br>*Detection of Listeria monocytogenes by inmunofluorescence (ELFA)* | PNTA0118<br>*Método interno basado en*<br>*In-house method based on*<br>*VIDAS® Listeria monocytogenes II (LM02)* |
| | Detección de *Listeria* spp. *y Listeria monocytogenes* por inmunofluorescencia (ELFA)<br>*Detection of Listeria spp. and Listeria monocytogenes by inmunofluorescence (ELFA)* | PNTA0168<br>*Método interno basado en*<br>*In-house method based on*<br>*VIDAS® Listeria Duo (LDUO)* |
| | Investigación de *Listeria* spp. por inmunofluorescencia (ELFA)<br>*Detection of Listeria spp. by inmunofluorescence (ELFA)* | PNTA0175<br>*Método interno basado en*<br>*In-house method based on*<br>*VIDAS® Listeria (LIS)* |
| Alimentos<br>*Food* | Detección de enterotoxina estafilocócica por inmunofluorescencia (ELFA)<br>*Detection of staphylococcal enterotoxins by inmunofluorescence (ELFA)* | European screening method of the European Union Reference Laboratory for Coagulase Positive Staphylcocci (ANSES) |

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>Piensos<br>Esponjas y escobillones de muestreo de superficies<br>*Food*<br>*Feed*<br>*Sampling sponges and swaps of surfaces* | Detección de *Salmonella* spp. por inmunofluorescencia (ELFA)<br>*Detection of Salmonella spp. by inmunofluorescence (ELFA)* | PNTA0160<br>*Método interno basado en*<br>*In-house method based on*<br>*VIDAS® Easy Salmonella* |

Análisis mediante métodos basados en técnicas PCR
*Analysis by PCR methods*

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>Piensos<br>Esponjas y escobillones de muestreo de superficies<br>*Food*<br>*Feed*<br>*Sampling sponges and swaps of surfaces* | Detección de *Salmonella* spp. por PCR a tiempo real<br>*Detection of Salmonella spp. by real-time PCR* | PNTA0139<br>*Método interno basado en*<br>*In-house method based on*<br>*BAX® System Real Time PCR Assay* |

Análisis de *Legionella*
*Legionella analysis*

| PRODUCTO/MATERIAL A ENSAYAR *PRODUCTS/MATERIALS TESTED* | ENSAYO *TYPE OF TEST* | NORMA/PROCEDIMIENTO DE ENSAYO *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Aguas de consumo<br>Aguas continentales tratadas<br>*Drinking waters*<br>*Continental treated waters* | Recuento de *Legionella* spp.<br>*Enumeration of Legionella spp.* | ISO 11731 |
| | Identificación de *Legionella pneumophila* (Aglutinación de látex)<br>*Identification of Legionella pneumophila (Aglutination latex test)* | PNTA0206<br>*Método interno basado en kit comercial (*)*<br>*In-house method based on commercial kit (*)* |

(*) La información sobre el kit concreto usado está disponible en el laboratorio
*(*) Information on the concrete kit used is available in the laboratory*

**FOOD SCIENCE CENTER**

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos elaborados listos para consumo<br>*Ready- to-eat food* | Estudio del potencial de crecimiento (δ) y de la tasa máxima de crecimiento (*μmax*) de *Listeria monocytogenes* mediante la técnica del CHALLENGE TEST<br>*Challenge test (growth potential and maximum growth rate) of Listeria monocytogenes* | Eurl Lm Technical Guidance Document For Conducting shelf-life studies on Listeria monocytogenes in ready-to-eat foods (version 3) |

**CENTRO DE ANÁLISIS SENSORIAL**

**DEPARTAMENTO DE ANÁLISIS SENSORIAL**

Análisis sensorial: pruebas de diferenciación

*Sensory analysis: tests for difference*

| **PRODUCTO/MATERIAL A ENSAYAR** *PRODUCTS/MATERIALS TESTED* | **ENSAYO** *TYPE OF TEST* | **NORMA/PROCEDIMIENTO DE ENSAYO** *STANDARD SPECIFICATIONS/ TEST PROCEDURE* |
|---|---|---|
| Alimentos<br>*Food* | Prueba triangular<br>*Triangle test* | UNE-EN ISO 4120 |

Un método interno se considera que está basado en métodos normalizados cuando su validez y su adecuación al uso se han demostrado por referencia a dicho método normalizado y en ningún caso implica que ENAC considere que ambos métodos sean equivalentes. Para más información recomendamos consultar el Anexo I al CGA-ENAC-LEC.

*An in-house method is considered based on standardized methods when its validity and suitability have been demonstrated against standard reference methods. This will never imply that ENAC considers both methods equivalent. For more information, please consult Annex I to the CGA-ENAC-LEC.*

| | |
|---|---|
| **From:** | jheimbach@va.metrocast.net |
| **To:** | Hice, Stephanie; jh@jheimbach.com |
| **Subject:** | RE: GRN 000953 - Questions for Notifier |
| **Date:** | Monday, January 11, 2021 5:21:57 PM |
| **Attachments:** | image001.png<br>Hice Stephanie 20210111.pdf |

Dear Stephanie—

Here is our response to your question.

HAPPY NEW YEAR!
Jim

James T. Heimbach, Ph.D., F.A.C.N.
JHeimbach LLC
923 Water Street #66
Port Royal VA 22535
USA
Tel: (+1) 804-742-5543
Cell: (+1) 202-320-3063
Email: jh@jheimbach.com

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**From:** Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
**Sent:** Monday, January 11, 2021 4:27 PM
**To:** jh@jheimbach.com; 'jheimbach@va.metrocast.net' <jheimbach@va.metrocast.net>
**Subject:** RE: GRN 000953 - Questions for Notifier

Dear Dr. Heimbach,

During our review of GRAS Notice No. 000953, we noted a further question that needs to be addressed and is attached to this email.

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

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stephanie Hice

**Stephanie Hice, PhD**
*Staff Fellow (Biologist)*
*Division of Food Ingredients*

**Center for Food Safety and Applied Nutrition**
**Office of Food Additive Safety**
**U.S. Food and Drug Administration**
stephanie.hice@fda.hhs.gov


# JHeimbach LLC

January 11, 2021

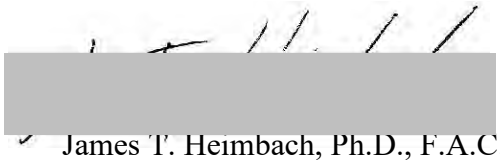
Stephanie Hice, Ph.D.
Staff Fellow (Biologist)
Division of Food Ingredients
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety
U.S. Food & Drug Administration

Dear Dr. Hice:

In the email I received from you earlier today, you stated that FDA reviewers of GRN 000953 had the following question:

> The notifier indicates that the likely maximum ingestion of *Lactiplantibacillus plantarum* strain CECT 7527, *L. plantarum* strain CECT 7528 and *L. plantarum* strain CECT 7529 is less than 1010 CFU/d. In their December 16, 2020 amendment, the notifier indicates that the use level for the combined strains is 1.2 x 109 CFU/serving. However, in the GRAS notice, the notifier indicates a maximum use level for the combined strains of 1.2 x 1010 CFU/serving. Presuming a maximum use level of 1.2 x 1010 CFU/serving and a mean consumption of 20 servings of food/d, the maximum exposure would be less than 1011 CFU/d, not 1010 CFU/d if half the servings of food contained the combined strains. For the administrative record, please confirm that the maximum exposure would be less than 1011 CFU/d.

We agree that the proper statement of the potential exposure is that the maximum exposure would be less than $10^{11}$ cfu/day. We also note that this is an extremely conservative estimate, as it would require an individual to consume ten servings of foods containing the subject bacterial strains at the maximum addition level with no loss of viability during shipping or storage. We thank FDA for correcting our statement.



James T. Heimbach, Ph.D., F.A.C.N.
President

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