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BIOSEARCH LIFE

October 23, 2018

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition,
Food and Drug Administration
5001 Campus Drive
College Park - MD 20740
USA

Dear Office of Food Additive Safety,

Please accept these documents as submission for Generally Recognized as Safe (GRAS) evaluation of the probiotic, *Lactobacillus fermentum* CECT5716, according to the Final Rule 21 CFR Parts 20, 25, 170, 184, 186 and 570.

Do not hesitate to contact me for further clarification or to request additional i.

Thank you for your time.

BIOSEARCH, S.A
C.I.F. A-18550111
Camino de Purchil, nº 66
18004 GRANADA

Laura Macho Valls
Regulatory Affairs Manager
Biosearch Life.

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FOOD ADDITIVE SAFETY

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GRAS ASSESSMENT

Lactobacillus fermentum CECT5716

Version 1

BIOSEARCH LIFE

Madrid, 23rd October 2018.

Content

Part 1 Signed statements and certification

1.1 GRAS notice according to 21 CFR § 170.225

In accordance with the US Food and Drug Administration's (FDA) Substances Generally Recognized as Safe; Final Rule, (81 FR 54959) relating to the filing of notices for substances that are considered to be generally recognized as safe (GRAS), please accept this claim and attached information, for that purpose as it relates to the use of Lactobacillus fermentum CECT5716 in foods for the general population. Specifically, we, Biosearch S.A. claim that the use of Lactobacillus fermentum CECT5716 in foods for the general population is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such uses are GRAS. This conclusion was made in concert with a Panel of Experts who is qualified by scientific training and experience.

No information used in this part of this notification is trade secret or confidential commercial information. In accordance with the requirements outlined in 21 CFR 170, Subpart E of the final rule, the following information is included with this exemption claim.

1.2 Name and address of notifier.

Notifier

Biosearch S.A.
Camino de Purchil, 66
18004 Granada – Spain
Contact Person: Laura Macho
Telephone: +34-913802973 Ext. 12045
Fax: +34-913802279
E-mail: lmacho@biosearchlife.com

1.3 Name of the notified substance.

Lactobacillus fermentum CECT5716, which is traded under the commercial name Hereditum® LC40.

1.4 Conditions of use.

The intended uses of *Lactobacillus fermentum* CECT5716 are to enrich foods in these beneficial bacteria, so-called probiotics; this term refers to microorganisms that confer a health benefit to the host when administered in adequate amounts. For this reason the technical effect that covers the use of this *Lactobacillus* strain presented herein can be defined by the purpose of providing a benefit to the consumer of the food, or, in other words, a food supplement (definition #20 of the Code of Federal Regulations 21 CFR 170.3) not to be confused with a dietary supplement.

The food category to which *Lactobacillus fermentum* CECT5716 is intended to be added is presented in Table I. The maximum levels are also presented in this Table I for each category. Food category was selected from the 43 food categories as defined in the Code of Federal Regulations 21 CFR 170.3. The use of Lactobacillus fermentum is intended to be for the general population.

Table I. Food categories in which *Lactobacillus fermentum* is intended to be added.

FOOD CATEGORY	Recommended Levels of *Lactobacillus fermentum CECT5716*
(1) Baked goods and baking mixes, including all ready-to-eat and ready-to bake products, flours, and mixes requiring preparation before serving.	3E+09 cfu/day
(3) Beverages and beverage bases, nonalcoholic, including only special or spiced teas, soft drinks, coffee substitutes, and fruit and vegetable flavored gelatin drinks.	3E+09 cfu/day
(4) Breakfast cereals, including ready-to-eat and instant and regular hot cereals.	3E+09 cfu/day
(5) Cheeses, including curd and whey cheeses, cream, natural, grating, processed, spread, dip, and miscellaneous cheeses.	3E+09 cfu/day
(6) Chewing gum, including all forms.	3E+09 cfu/day
(7) Coffee and tea, including regular, decaffeinated, and instant types.	3E+09 cfu/day
(9) Confections and frostings, including candy and flavored frostings, marshmallows, baking chocolate, and brown, lump, rock, maple, powdered, and raw sugars.	3E+09 cfu/day

(10) Dairy product analogs, including nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products.	3E+09 cfu/day
(12) Fats and oils, including margarine, dressings for salads, butter, salad oils, shortenings and cooking oils.	3E+09 cfu/day
(16) Fresh fruits and fruit juices, including only raw fruits, citrus, melons, and berries, and home-prepared ''ades'' and punches made therefrom.	3E+09 cfu/day
(20) Frozen dairy desserts and mixes, including ice cream, ice milks, sherbets, and other frozen dairy desserts and specialties.	3E+09 cfu/day
22) Gelatins, puddings, and fillings, including flavored gelatin desserts, puddings, custards, parfaits, pie fillings, and gelatin base salads.	3E+09 cfu/day
(23) Grain products and pastas, including macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables.	3E+09 cfu/day
(25) Hard candy and cough drops, including all hard type candies.	3E+09 cfu/day
(30) Milk, whole and skim, including only whole, low-fat, and skim fluid milks.	3E+09 cfu/day
(31) Milk products, including flavored milks and milk drinks, dry milks, toppings, snack dips, spreads, weight control milk beverages and other milk origin products.	3E+09 cfu/day
(33) Plant protein products, including the National Academy of Sciences/National Research Council ''reconstituted vegetable protein'' category, and meat, poultry, and fish substitutes, analogs, and extender products made from plant proteins.	3E+09 cfu/day
(35) Processed fruits and fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, ''ades'', and drink substitutes made therefrom.	3E+09 cfu/day
(36) Processed vegetables and vegetable juices, including all commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends.	3E+09 cfu/day
(37) Snack foods, including chips, pretzels and other novelty snacks.	3E+09 cfu/day
(38) Soft candy, including candy bars, chocolates, fudge, mints, and other chewy or nougat candies.	3E+09 cfu/day
(39) Soups, home-prepared, including meat, fish, poultry, vegetable, and combination home-prepared soups.	3E+09 cfu/day
(40) Soups and soup mixes, including commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes.	3E+09 cfu/day
(43) Sweet sauces, toppings, and syrups, including chocolate, berry, fruit, corn syrup, and maple sweet sauces and toppings.	3E+09 cfu/day

1.5 and 1.6 Basis of GRAS status and exemption from premarket notification.

Biosearch S.A. submitted in July 2014 a GRAS Notification describing the use of *Lactobacillus fermentum* CECT5716 in powdered milk-based infant formula. FDA´s evaluation of GRN No. 531 was completed in March 2015 and responded with a no questions letter. *Lactobacillus fermentum* CECT5716 has also obtained the registration in China in 2016 for intended use in infant formula and an NDI no objections letter for its use in dietary supplements.

1.7 Data/information availability statement

The data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Biosearch S.A., C/ Cabeza Mesada 5, 5ª Planta, 28031 Madrid, Spain or in the Manufacturing Site in Granada, Camino de Purchil 66, 18004 Granada, Spain, Telephone: +34 91 380 29 73, email: lmacho@biosearchlife.com or will be sent to the FDA upon request.

1.8 Exempt from disclosure statement

No data or information contained in parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.
If applicable and necessary, as required by §170.270 we authorize FDA to send any trade secrets to the Food Safety Inspection Service (FSIS) to the U.S. Department of Agriculture.

1.9 GRAS Notice Certification

Biosearch S.A. certifies that to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance collected under the trade name *Lactobacillus fermentum* CECT5716 (Hereditum® LC40).

Jose Mª Roset Monrós

CEO, Biosearch S.A.

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

2.1. Identity of substance

Lactobacillus fermentum CECT5716 is a proprietary strain, isolated from human breast milk, in collaboration with the University Complutense of Madrid, Spain

Morphological Characteristics:

Gram positive, rod-shaped bacteria, producing smooth, white and round colonies.

Physiological characteristics:

Facultative anaerob, heterofermentative, acid tolerant, catalase negative, oxidase negative, beta- glucuronidase activity negative, production of biogenic amines negative, mucin degradation negative.

Genetic stability:

Genetically stable, contain no extrachromosomal DNA

Other characteristics:

It is not genetically modified

It is not associated to toxins or virulence factors

Antibiotic production is not known for *Lactobacillus* genus

It does not harbor transmissible drug resistance genes encoding resistance to clinically used drugs.

The taxonomical classification of this strain was based on the analysis of the SDS_PAGE 1D protein profiling and their 16S rRNA sequence, and in order to discriminate between this strain and other *Lactobacillus* species, RAPD-PCR analysis was performed using two specific primers for lactobacilli (ArgDei and OPL5). (Martin et al 2005)

Besides the genetic patterns, the strain was characterized by its carbohydrate metabolic pattern (9API 50, BioMerieux), enzymatic activities (APIZYM: BioMerieux) and antibiotic resistance. (Martin et al 2005 and Lara-Villoslada 2009)

The strain has been deposited according to the Budapest Agreement at the Spanish Culture Collection (CECT) with the following accession number: The total genome has been sequenced and is available in GenBank/EMBL under accession number CP002033.

Phenotypic characterization

Biological origin: human milk

Taxonomy: *Lactobacillus fermentum* (identified by 16S sequence)

Fermentation capability of carbohydrates:

Fermentation capability of the main source of carbohydrates of *Lactobacillus fermentum* CECT5716 (Table II).

Table II: Carbohydrates Fermentation Pattern

	***L.fermentum* CECT5716**
Glucose	+++
Lactose	+++
Lactulose	-
Maltodextrin	-
Polydextrose	+
Fructooligosaccharides	+
Fructooligosaccharides/inulin	+
Inulin	-
Galactooligosaccharides/inulin	+++
Galactooligosaccharides	+++

The enzymatic activities displayed by *L. fermentum* CECT5716 is shown in Table II. The strain shows a wide spectrum of enzymatic activities and none with harmful activity.

Table II: Enzymatic activities pattern

Enzyme	***L.fermentum* CECT5716**
Alkaline phosphatase	-
Esterase (C4)	+
Esterase lipase (C8)	+
Lipase (C14)	-
Leucine arilamidase	+
Valine arilamidase	-
Cistine arilamidase	-
Trypsin	-
α-quimiotrypsin	-
Acid phosphatase	-
Naftol-AS-BI-phosfohydrolase	+
α-galactosidase	+

β-galactosidase	+
β-glucuronidase	-
α-glucosidase	+
β-glucosidase	-
N-acetil-β-glucosaminidase	-
α-mannosidase	-
α-fucosidase	-

Minimal inhibitory concentration (MIC) of antibiotics

The assays for MIC determination were carried out by the broth dilution method following the procedure described in the ISO standard ISO 10932:2012 "Milk and milk products: Determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB)". Inhibitory experiments were repeated in two independent assays and with, at least, two replicates in every assay. The type strain of *Lactobacillus paracasei* subs *paracasei* (CECT 4022) was included in each assay as control strain. Mode of the data was used to calculate MIC values, summarized in Table III.

Table III. MIC results for *L. fermentum* CECT 5716

Antibiotic	MIC
Ampicillin	1
Gentamycin	<2
Kanamycin	64
Streptomycin	32
Erythromycin	0,25
Clindamycin	<0,03
Tetracycline	8
Chloramphenicol	4

Biogenic amines production and mucin degradation

The ability to form biogenic amines (tyramine, histamine, putrescine and cadaverine) and to degrade mucins was studied by Martín et al. (2005) and Cardenas et al. (2015). Amines production was determined using the decarboxylase broth and the method described by Bover-Cid and Holzapfel (1999). Briefly, *L. fermentum* CECT 5716 was streaked onto different decarboxylase medium plates, containing the precursor amino acids (tyrosine, histidine, ornithine or lysine, respectively) and incubated for 4 days at 37 °C under aerobic and anaerobic conditions. A positive result was indicated by a change of the medium color to purple in response to the pH shift caused by the production of the more alkaline biogenic amine from the amino acid initially included in the medium. The plate procedure developed by Zhou et al. (2001) was employed to study the potential of *L. fermentum* CECT 5716 to degrade gastric

mucins. Agar plates containing 0.5 % and 1.5 % (w/v) hog gastric mucins were incubated for 72 h at 37 ºC and anaerobiosis. Lysis of mucins was visualized by amido black staining. Experiments carried out to detect the production of tyramine, histamine, putrescine or cadaverine by *L. fermentum* CECT 5716 did not detect the formation of these compounds. On the other hand, the results of the *in vitro* assays showed that the microorganism studied was not able to degrade mucins under the experimental conditions employed.

Molecular identification/detection

DNA fragments isolated from *L. fermentum* CECT 5716 by subtractive suppressive hybridization (Biosearch, S.A.; unpublished data) were employed to design the strain-specific primers described in this document.

The assay for strain identification is based on amplification and detection of a specific DNA fragment by a TaqMan-based real-time PCR procedure. Oligonucleotides to be used as primers and probe, and reaction profile are shown in Table IV. These primers are specific for this strain, and as PCR is done, no other strains are amplified. The purity of this strain can be followed without contamination problems with other *Lactobacillus* strains.

Table IV. Real-time PCR conditions

Primers		
Id	**Sequence (5'-3')**	**Working conc.**
L40C126_D (Forward)	TCAACGGCCCCTTCAATACA	500 nM
L40C126_R (Reverse)	GACCTAATTCACGTCAAACATATTTCA	500 nM
L40C126_P (Probe)	JOE-AGTGGTGAGATGCCCAGTGTTCCCG-BHQ3	200-250 nM
Thermal profile		
Stage	**Cycles**	**Time**	**Temp**
Initial denaturing	1	3-5 min	95 ºC
Amplification	40	15 s	95 ºC
		60 s	60 ºC

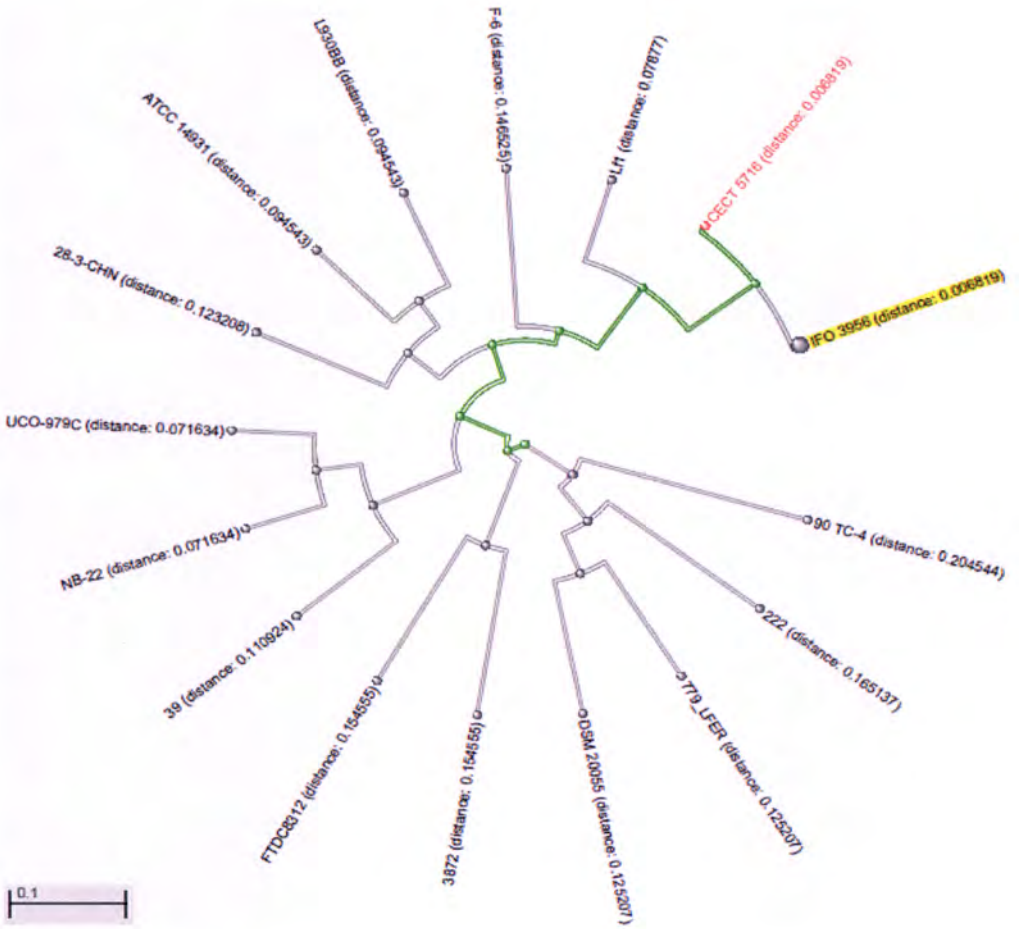
Amplification reactions with positive detection of *L. fermentum* CECT5716 show a Ct value < 35 cycles in the appropriate fluorescence reading channel (520/548 nm excitation/emission respective wavelengths), and an amplicon of 75 bp, resolved by PAGE.

Genetic analysis of L. fermentum CECT5716

The genome of *L. fermentum* CECT 5716 was sequenced by Jiménez et al (2010) and its relationship with some phenotypic characteristics studied by Cárdenas et al (2015). The genomic data of this strain and the genomic sequences, available in the GenBank, of

representative microorganisms of the same species show that *L. fermentum* CECT 5716 is closely related to *L. fermentum* IFO 3956; strain from which its genome has been chosen as representative of the species (Figure I)

Figure I. Dendogram based on genomic BLAST obtained from the NCBI web site. 16 different *L. fermentum* strains were sorted according to the distance between their genomes. *L. fermentum* CECT 5716 (pink) shows the lowest distance from the IFO 3956 strain (yellow; representative genome of the species).



2.2 Manufacturing process

Introduction

Lactobacillus fermentum CECT5716 is grown by standard fermentation techniques and under conditions that are suitable for human food use. All raw materials used meet specifications of the Food Chemical Codex. The culture media consist of a sugar as carbon and energy source, a complex nitrogen source (yeast extract) and minerals.

In order to grow the selected strain as a pure culture, all required measurements well known in fermentation technology are applied to avoid contamination by foreign microorganisms present in the environment.

The manufacturing plant producing the ingredient object of this request is located in Granada (Spain) next to one of the milk processing plants of Puleva Food (Lactalis Group). Because of its location the manufacturing plant receives all industrial utilities (hot and cold water, electricity and steam) from Puleva Food. The manufacturing plant has been operational since June, 2003.

Strain maintenance

Biosearch Life maintains a cell bank system of all its strains. This system is based on a Master Cell Bank (MCB) of each strain and Working Cell Banks (WCB) that is prepared when needed, starting from a vial of the Master Cell Bank. The Master Cell Bank consists of several frozen vials stored at -80°C.

Each time a WCB has to be prepared, a vial of the MCB is plated and one isolated colony is grown in a non-selective culture media. After 16h of growth, the broth is centrifuged and the biomass is resuspended in the same volume of media plus 30% glycerol. This cell suspension is divided into portions and stored at -80°C.

Before the new WCB is liberated to be used as starting material for production, it is checked in terms of potency, purity and identity.

Identification is performed by phenotypic tests followed by genetic identification.

Identity is confirmed by:

- Colony appearance and cell morphology under microscopic observation.
- qPCR with strain-specific oligonucleotides and probes for each strain.

Fermentation

Lactobacillus fermentum CECT5716 is grown using standard fermentation techniques and under conditions that are suitable for human food use. Raw materials are mixed to grow the strain as a pure culture; all required measurements are applied to avoid contamination by foreign microorganism present in the environment.

Concentration and Freeze-drying

When fermentation is over, cells are cooled and harvested, the biomass is concentrated by centrifugation and sent to a tank with a solution of cryoprotectant (maltodextrin, sucrose and sodium ascorbate). Cells are mixed with the cryoprotectant solution and then frozen in trays as the first step in the freeze-drying process. When the process is over, the material is harvested in an ultra-clean room, milled and filled in sterile bags.
Samples are analyzed to determine potency, identity and the absence of microbial contamination and the product is packed in aluminum laminated sachets at pre-defined potency per sachet.

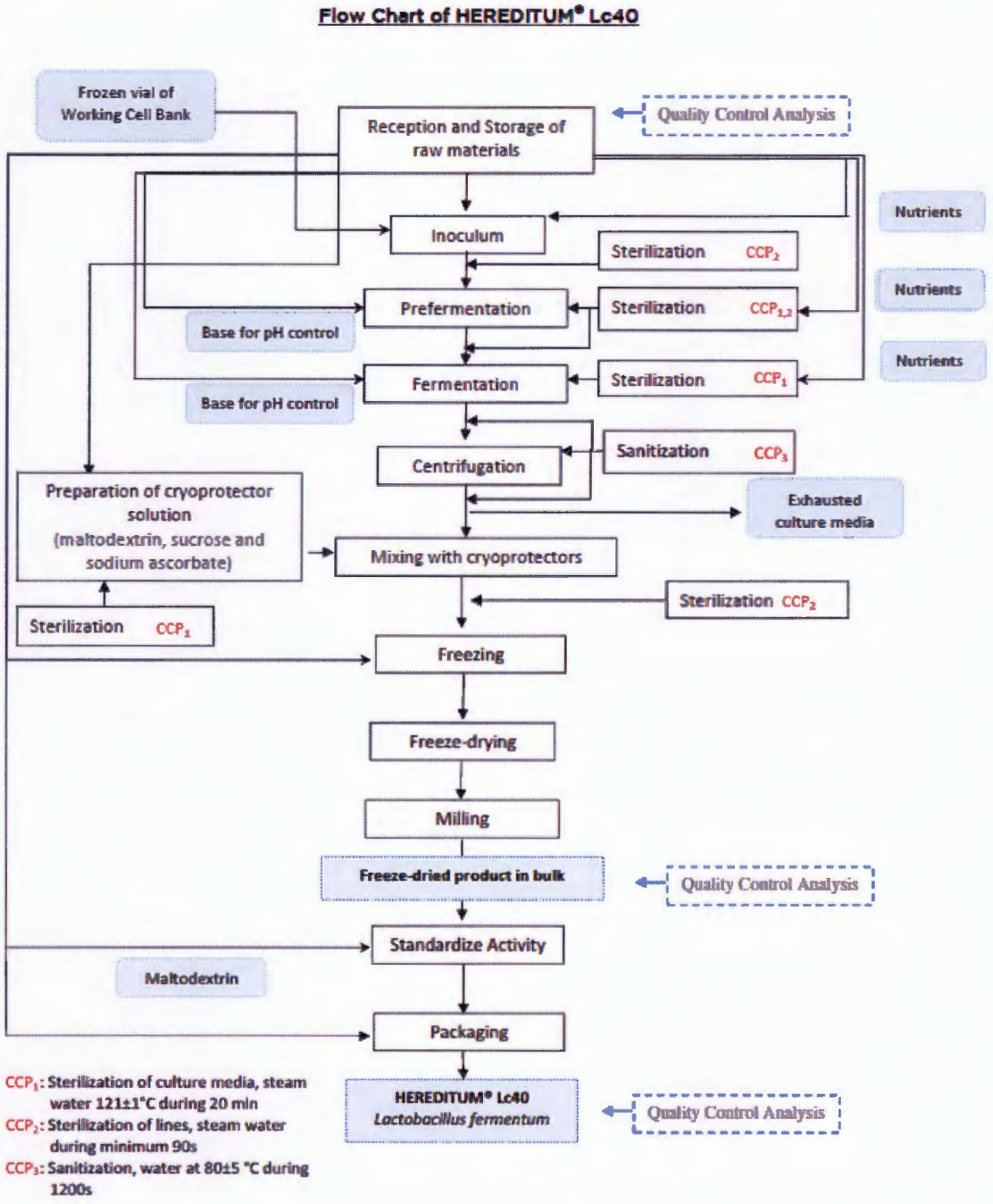
Blend preparation.

The required amount of the freeze dried strain, according to its viability, is mixed with maltodextrin to achieve the cell concentration that complies with the product specification.

Storage and transport

The aluminum – laminated sachets containing the product are stored at -30 °C until delivery. The bags are transported in special boxes containing frozen elements to maintain a cool environment.

Scheme I Schematic representation of the manufacturing process of *Lactobacillus fermentum* CECT5716



2.3. Product specifications

Biosearch Life has developed specifications for *Lactobacillus fermentum* CECT5716 to demonstrate that this product is food grade. These specifications are listed below, together with the standard operating procedures used by Biosearch Life for determining compliance with each specification. Each batch is checked for identity by qPCR using strain-specific oligonucleotides and probes in order to confirm the identity of the strain and the absence of other strains.

Product: **HEREDITUM® Lc40**

Composition: *Lyophilized culture of Lactobacillus fermentum lactic acid bacteria (CECT5716), maltodextrin, sucrose and sodium ascorbate.*

Product Code: **HER002**

Appearance: *Slightly yellowish lyophilized powder.*

PARAMETER	SPECIFICATION	UNITS	METHOD
The following parameters are analyzed on all final product batches.			
Cell content *L. fermentum*	*minimum* **1.0E+11**	cfu/g	Internal Method according to ISO 15214:1998 and ISO 20128:2006
Enterobacteriaceae	*< 10*	cfu/g	ISO 21528-1:2004
Yeast and moulds	*maximum* **50**	cfu/g	Internal Method according to ISO 6611:2004
Listeria monocytogenes	*Not detected*	cfu/25g	ISO 11290-1:2004
Salmonella spp.	*Not detected*	cfu/25g	ISO 6579:2003
S. aureus	*< 10*	cfu/g	ISO 6888-1:1999 / Amd 1:2003
B. cereus	*maximum* **500**	cfu/g	Internal Method according to UNE-EN ISO 7932:2004
E. coli	*Not detected*	cfu/g	ISO 7251:2005
Cronobacter spp.	*Not detected*	cfu/10g	ISO 22964:2006
Moisture	*maximum* **10**	g/100g	Internal Method according to ISO 6731:2010 (FIL-IDF 21:2010)
a_w - water activity	*maximum* **0.25**	-	ISO 21807:2004

Batch analysis results

To demonstrate agreement with the proposed specifications listed above, the analytical results of five different lots of *Lactobacillus fermentum CECT5716* are presented in Table V. The Certificates of analysis of these non-consecutive batches of finished product are included in Annex F. These indicate that the manufacturing process consistently meets product specifications.

Table V Analytical results of five different nonconsecutive batches of Lactobacillus fermentum CECT5716

Parameter	BATCH NUMBER				
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Cell Content ***L. fermentum*** **cfu/g**	1.5E+11	1.3E+11	1.3E+11	1.5E+11	1.3E+11
B. Cereus **cfu/g**	<10	<10	<10	<10	<10
Yeasts and moulds cfu/g	Not detected	Not detected	Not detected	Not detected	Not detected
Cronobacter spp. **cfu/10g**	Not detected	Not detected	Not detected	Not detected	Not detected
Enterobacteriaceae **cfu/g**	Not detected	Not detected	Not detected	<10	Not detected
E. coli **cfu/g**	Not detected	Not detected	Not detected	Not detected	Not detected
S. aureus **cfu/g**	<10	<10	<10	<10	<10
L. monocytogenes **cfu/25g**	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella **cfu/25g**	Not detected	Not detected	Not detected	Not detected	Not detected
Moisture g/100g	4	3	3	4	5
aw – water activity	0.12	0.08	0.08	0.15	0.16

Analytical methods

The analytical procedures used to confirm the quality of every batch are based on the methods presented in table VI.

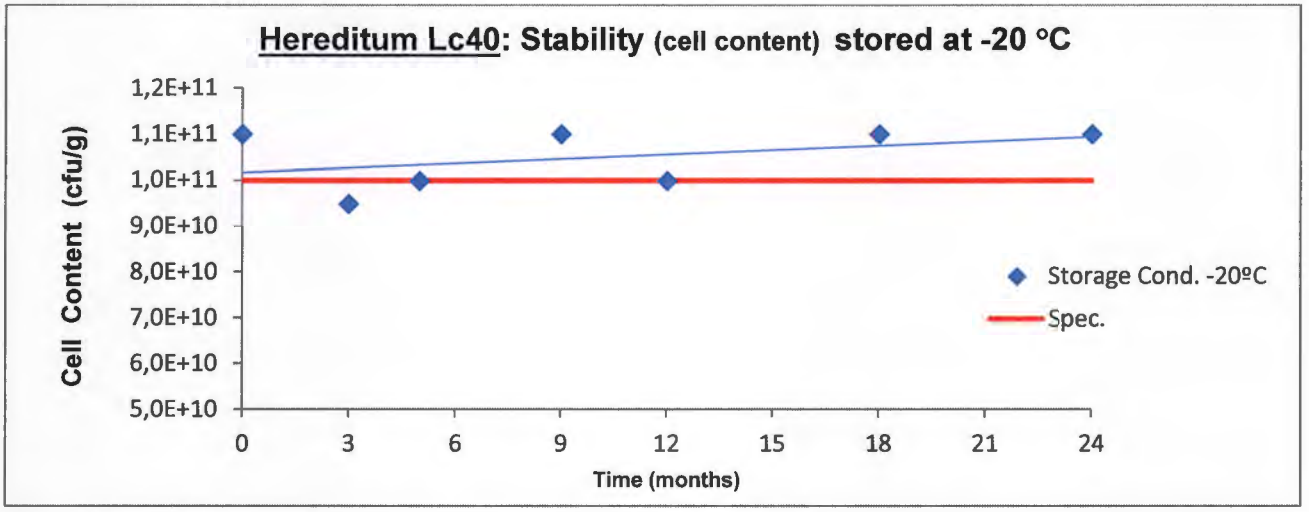
Table VI Analytical methods used to analyze each batch of Lactobacillus fermentum CECT5716.

Parameter	Analytical Method
L. fermentum (cfu/g)	ISO 15214 (1998) Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony – count technique at 30 °C
B. cereus (cfu/g)	ISO 7932:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Bacillus cereus – Colony-count technique at 30°C
Yeasts and Moulds (cfu/g)	ISO 6611 IDF 94 (2004) Milk and milk products – Enumeration of colony forming units of yeasts and/or moulds – Colony count technique at 25°C
Cronobacter spp.(cfu/10g)	ISO 22964:2006
Enterobacteriaceae (cfu/g)	ISO 21528 – 1 (2004) Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 1: Detection and enumeration by MPN technique with pre-enrichment
S. aureus (cfu/g)	ISO 6888 – 1:1999/Amd 1:2003 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase – positive staphylococci (Staphylococcus aureus and other species) Part 1: Technique using Baird-Parker agar medium
E. coli (cfu/g)	ISO 7251:2005 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of presumptive Escherichia coli – Most probable number technique
L. monocytogenes (cfu/25g)	ISO 11290-1:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of Listeria monocytogenes – Part 1: Detection method
Salmonella (cfu/25g)	ISO 6579 (2003) Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.
Moisture (g/100g)	In house method
aw – water activity	Internal method according to ISO 6731:2010 (FIL-IDF 21:2010)

Stability data

The stability of *Lactobacillus fermentum* CECT5716 was determined at -20 ºC over a 24 – month period by monitoring viable cell counts at regular intervals (Figure 2). The product is stable under these conditions. The results of a typical lot are represented in Figure 2.

Figure 2. Stability of *Lactobacillus Fermentum* CECT5716. Hereditum LC40



GMO status

Biosearch S.A. certifies that *Lactobacillus fermentum* CECT5716 is non-GMO. Any culture strain used in the manufacture of these products or any culture strain contained as part of this product has itself not been genetically modified according to Directive 2001/18/EC neither subject to the labeling requirement of (EC) 1830/2003 nor to the authorization procedure of Regulation (EC) 1829/2003 (Annex D).

Allergens

The *Lactobacillus fermentum* CECT5716 is free from wheat, rye, oats, barley, spelt, kamut, nuts, nut oil, peanuts, fish, soybeans, egg, crustaceans, mollusks, mustard, celery, sesame seeds, lupin and products thereof and sulfites or sulfur dioxide (Annex E).

It may contain traces of milk (Explanation and analysis in milk proteins of one batch, also in Annex E).

Biosearch is going to perform analysis in aleatory batches of *Lactobacillus fermentum* CECT5716 for an extended period of time so we can conclude, if none of these batches contains milk proteins, milk is not an allergen in this product.

Part 3 Dietary exposure

3.1 Current dietary exposure of *L. fermentum* CECT5716

Lactobacillus fermentum CECT5716 is intended to be used in food for the general population (Table I). Uses are limited to foods that can sustain living *Lactobacillus fermentum* CECT5716 during shelf life. It is intended to be added to foods at initial levels as high as $1x10^{10}$ CFU/serving (100-120 mg of product/serving) to ensure at least 3 x 10^9 CFU/serving throughout the shelf life of the product. For example, 1-5 x10E+10 CFU/ 125g (yogurt) or 1-5 x10E+10 CFU/ 200g (juice, dairiy product, etc).

The function of *Lactobacillus fermentum* CECT5716 is to serve as a probiotic microorganism to be consumed by the general population.

3.2 Intended human food uses (estimated daily intake)

Lactobacillus fermentum CECT5716 will not proliferate in the food but instead will decline over the shelf-life. At the maximum daily intake in the food, the total estimated consumption would be 1 x 10^{10} CFU/person/day. Food will be labeled recommending a single daily dose. In the unlikely case of a consumer ingests 10 servings of the product containing *L.fermentum* CECT5716, the estimated consumption would be 1 x 10^{11} CFU/person/day. No maximum level of *Lactobacillus fermentum* CECT5716 of even other probiotic strain could be defined. The administration of 5 $x10^{11}$ CFU/kg body weight of *Lactobacillus fermentum* CECT5716 daily for 4 weeks was totally safe (Lara-Villoslada et. al 2009). Thus, higher consumption than recommended would continue to be safe.

Part 4 Self-limiting levels of use

There are no self-limiting intake levels.

Part 5 Experience based on common use in food before 1958

The statutory basis for our conclusions of GRAS status in the notice is not based on common use in foods. It is based on scientific procedures in accordance with § 170.30 (a) and (b).

Part 6 Narrative

6.1 Introduction.

Probiotic bacteria are commonly defined as viable microorganisms that, when ingested in adequate amounts, exhibit a benefit for the host health. An important aspect of probiotic strains is their capabilities to colonize the human gut. *Lactobacillus fermentum* CECT5716 shows a high rate of adhesion to intestinal cells and is able to survive the human gut transit (Martín et al 2005). *Lactobacillus fermentum* CECT5716 also produces compounds that help to maintain the integrity of the human gut epithelium. This strain induces the production of mucins that may protect the intestinal epithelium from physical, chemical and bacteriological damage (Olivares et al 2006). The consumption of *Lactobacillus fermentum* CECT5716 increases the production of short chain fatty acids (SCFAs) that constitute an important energy source for the intestinal cells (Perán et al 2006). *Lactobacillus fermentum* CECT5716 is endowed of an anti-infectious character since it produces antimicrobial substances, such as lactic acid, which inhibit the growth of pathogenic bacteria such as *E. coli, Salmonella* spp., *Listeria* spp., *Staphylococcus aureus*, etc., and inhibit pathogens' adhesion to the gut mucosa favoring the elimination of pathogens with the fecal bulk. The anti-infectious activity has been revealed by the capability of *Lactobacillus fermentum* CECT5716 to protect against gastrointestinal infections caused by *Salmonella choleraesuis* in an in vivo murine infection model (Olivares et al 2006).

In addition to their probiotic effect on intestinal function, *Lactobacillus fermentum* CECT5716 also possesses an immunoregulatory activity (Díaz-Ropero et al 2007). The consumption of this strain boosts the natural and adaptive immunological responses in healthy adults (Olivares et al 2007). Therefore, *Lactobacillus fermentum* CECT5716 is a *Lactobacillus* strain with a potent probiotic character that makes its use interesting for human nutrition.

6.2. Safety of the intended use of *Lactobacillus fermentum* CECT5716.

Although it has been reported that lactobacilli and bifidobacteria may invade the host body by bacterial translocation and other routes (Berg et al 1992), it seems probable that for these bacteria to cause infection both bacterial and host factors have to be involved. In fact, the isolation of probiotic bacteria from infections is likely to be the result of opportunistic infections. All cases of probiotic sepsis have occurred in immune compromised patients, with a chronic disease or debilitation. There is no report on probiotic sepsis in healthy individuals. In a review by Boyle et al. (2006) the known risks of probiotic treatments are revised. These authors

reported 12 cases of bacterial sepsis temporally related to probiotic use in humans, nine cases of bacteremia, two cases of endocarditis and one case of liver abscess. All of them were associated with different risk factors such as diabetes mellitus, short gut syndrome, central nervous catheter and antibiotic diarrhea among others. *Lactobacillus rhamnosus* LGG and *Bacillus subtilis* were the most frequent isolated strains, which might be due, at least in the case of LGG, to the widespread use of these strains. However, it has been reported that, in spite of the marked increase in the use of the probiotic *L.rhamnosus GG* in Finland since 1990, no significant increase in *Lactobacillus bacteremia* attributable to probiotic strains has been observed in southern Finland (Saxelin et al 1996). Although *L. fermentum* has a long history of use in traditional foods, only one report has been found in the literature about adverse effects or infections caused by this species of microorganism and occurred in a patient with a chronic disease and debilitation (Chery et al 2013). The infection was a documented cholecystitis which occurred in an 87 years old man, with medical history of obesity, diabetes mellitus, hypertension, dislipemia and coronary artery disease.

In the selection of new probiotic strains safety criteria are applied such as a long history of use in humans and the absence of pathogenic mechanisms (Collins et al. 1998). Thus, safety assessments are recommended for probiotic strains that are aimed to be incorporated into food products (Conway 1996). These safety criteria have been applied for *Lactobacillus fermentum* CECT5716.

6.3 Origin of *Lactobacillus fermentum* CECT5716.

It has been showed that breast milk is an important source of lactic acid bacteria for infants (Martín et al 2003). It is estimated that breast milk contains about 103 colony forming units of commensal bacteria per milliliter. *Lactobacillus fermentum* CECT5716 was originally isolated from human milk of healthy mothers (Martín et al. 2005b).

6.4. Identification of *Lactobacillus fermentum* CECT5716.

The taxonomic classification of probiotic bacteria must be accurate since this aspect can involve connotations of safety and regulation (O´Brien et al 1999). The joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) expert Consultation on Health and Nutritional properties of powder milk with live lactic acid bacteria recommended that strains should be named according to the International Code of Nomenclature, be deposited in an internationally recognized culture collection, and that strain identification be performed by phenotypic tests followed by genetic identification with methods

such as DNA/RNA hybridization and 16sRNA sequencing. *Lactobacillus fermentum* CECT5716 was identified by 16S rRNA sequencing and species-specific polymerase chain reaction (PCR) (Martín et al 2003; Martín et al 2005. This strain was deposited in the Spanish culture collection (CECT) with the code CECT5716. The strain has not been genetically modified thus is not under the special regulation of GMO.

Furthermore, the complete genome of *Lactobacillus fermentum* CECT5716 was recently sequenced (Jimenez et al, 2010). The complete genome of *Lactobacillus fermentum* CECT5716 consists of a circular chromosome of 2,100,449 bp, with a GC content of 51.49%, and has no plasmids. The complete sequence is available in GenBank/EMBL under accession no. CP002033.

6.5 Metabolic activities.

Probiotic bacteria could convert food components or biological secretions into secondary substances potentially harmful for the host. Production of amines during digestion of food proteins by Lactobacilli and Bifidobacteria has been proposed as a test to assess detrimental effects of probiotics (Ishibashi and Yamazaki 2001). The production of biogenic amines was evaluated by incubating *Lactobacillus fermentum* CECT5716 in the presence of amino acids in decarboxylase broth by the method of Bover-Cid and Holzapfel (Bover-Cid and Holzapfel 1999). The strains tested did not produce biogenic amines, suggesting a low deaminase activity (Martín et al 2005). Also it is recommended not to use strains with beta-glucuronidase activity since this activity could deconjugate toxic substances bound to glucuronic acid releasing toxins that could be absorbed. *Lactobacillus fermentum* CECT5716 is not endowed with such an enzymatic activity (Martín et al 2005). It has been suggested that the ability of some bacteria to degrade mucins may facilitate the translocation of normal intestinal bacteria into extra intestinal tissues. *Lactobacillus fermentum* CECT5716 is not able to degrade gastric mucins in vitro (Martín et al 2005).

6.6 Determination of antibiotic resistance patterns.

The emergence and the spread or resistance to antimicrobials in bacteria pose a threat to human and animal health and present a major financial cost. As with any other bacteria, antibiotic resistance exists among some lactic acid bacteria, including probiotic microorganisms (Salminen et al 1998). The joint FAO/WHO expert Consultation on Health and Nutritional properties of powder milk with live lactic acid bacteria suggested in 2001 that further research

must be done related to the antibiotic resistance of lactobacilli and bifidobacteria (FAO/WHO 2001). The Consultation recommended that probiotic bacteria should not harbor transmissible drug resistance genes encoding resistance to clinically used drugs. FAO/WHO convened in 2002 a Working Group to generate guidelines and recommend criteria and methodology for the evaluation of probiotics in foods. The Working Group recommended the determination of antibiotic resistance patterns of the probiotic bacteria strains as a requirement for proving the safety of these bacteria (FAO/WHO 2002). A list of antibiotics was proposed for which the minimal inhibitory concentration (MIC) should be tested, and also MIC breakpoints for *Enterococcus faecium, E. faecalis, Pediococcus, Lactobacillus and Bacillus* categorizing a bacterial strain as resistant to an antibiotic. These criteria have been applied to *Lactobacillus fermentum* CECT5716 in order to discard the presence of non-desirable resistances. Minimum inhibitory concentrations (MICs) to 18 antimicrobial agents were calculated. The antimicrobial agents tested included amoxicillin/clavulanic acid, ampicillin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fosfomycin, gentamycin, imipenem, linezolid, mupirocin, oxacillin, penicillin, quinupristin/dalfopristin, rifampicin, teicoplanin, tetracycline, trimethoprim–sulfamethoxazole and vancomycin.

The values of MICs for the tested antibiotics are below those breakpoints proposed (Lara-Villoslada et al., 2009).

6.7 Animal studies.

Toxicity studies in animals have been considered unnecessary for lactobacilli species which have a long history of use and lacking pathogenic mechanisms. However, such studies were carried out for *Lactobacillus fermentum* CECT5716 with the aim to reinforce the data of safety of the strain. Although extrapolation of results obtained from animal studies to human has limited validity, toxicity studies in experimental animals are generally accepted as a reliable tool to assess acute toxicity. These studies, conducted using the same procedures used for testing toxicity of chemicals, have also been performed for the evaluation of probiotic safety and tolerance of *Lactobacillus fermentum* CECT5716 (Lara-Villoslada et al., 2009). This strain was daily administered for 1 month with doses of 10^{10}CFU mouse/day, doses about 10 thousand times higher than normally consumed by humans when body weight is taken into account. Results showed that oral administration of *Lactobacillus fermentum* CECT5716 to mice had no adverse effects on body weight or food intake. No bacteremia was present in liver or spleen and there was no treatment-associated bacterial translocation into these tissues. Liver glutathione content, as well as plasma malondialdehyde concentration, was not statistically different in probiotic-treated mice when compared to control mice. Probiotic treatment did not cause

changes in the biochemical and hematological parameters analyzed. Therefore the acute toxicity study demonstrated the safety of *Lactobacillus fermentum* CECT5716 under these conditions (Lara-Villoslada et al, 2009).

6.8 Human studies.

Two human studies where performed in which *Lactobacillus fermentum* CECT5716 was orally administered to infants. In these studies no adverse effects related to the strains were reported. The summary of these clinical studies were submitted in the *Lactobacillus Fermentum* CECT5716 GRAS notification, number 000531 (Pages 20-25) for intended use in Infant Formula, answered with a no questions letter by FDA:

1. *Clinical study in infants from 1 to 6 months of age using Lactobacillus fermentum* CECT5716 *(Gil-Campos et al., 2012).*

CONCLUSIONS: The consumption from 1 to 6 months of life of an infant formula enriched with the probiotic strain *Lactobacillus fermentum* CECT5716 is well tolerated and safe. Furthermore, the consumption of this formula improved the health of the infants reducing the incidence of gastrointestinal infections.

2. *Clinical study in infants from 6 to 12 months of age using Lactobacillus fermentum* CECT5716 *(Maldonado et al., 2012)*

CONCLUSIONS: Administration of a follow-on formula with *Lactobacillus fermentum* CECT5716 is safe and well-tolerated by 6 months old infants, and may be useful for the prevention of community-acquired GI and upper respiratory infections.

Four more studies have been performed, in which *Lactobacillus fermentum* CECT5716 was orally administered to adults. In these studies no adverse effects related to the strains were reported. Below a summary of these 4 clinical studies is given.

1. *Clinical study in healthy adults using Lactobacillus fermentum CECT5716 (Olivares et al., 2007).*

OBJECTIVE: To evaluate the ability of the probiotic strain *L. fermentum* CECT5716 to enhance the immune response against the influenza vaccine.

METHODS AND DOSE: A randomized, double-blinded, placebo-controlled human clinical trial including 50 volunteers (31 male and 19 female) was performed to address the

immunologic effects of an intramuscular anti-influenza vaccine in adults (33.0 ± 7.7 years old). Fifty percent of volunteers received an oral daily dose of methylcellulose (placebo) or probiotic bacteria (1 x 10^{10} cfu/d) 2 weeks before vaccination and 2 weeks after vaccination.

RESULTS: No adverse effects associated with the intake of the probiotic strain were observed during the treatment or follow-up periods. The results showed, two weeks after vaccination, an increase in NK cells proportion in the group consuming probiotic, but not in the placebo group. The vaccination induced an increase in Th1 cytokine concentrations, and in T helper and T cytotoxic proportions in both groups; however, the group consuming probiotic bacteria showed a significant higher induction in some of these parameters. Regarding the humoral effects, induction of antibody response in the placebo group could not be detected. In the case of the probiotic group a significant increase in anti-influenza specific IgAs was detected. Although an increase in total IgM was observed, changes in anti-influenza specific IgMs were not observed. Finally, the incidence of influenza like illness during 5 months after vaccination (October-February) was lower in the group consuming *Lactobacillus fermentum* CECT5716.

CONCLUSIONS: Daily administration of *Lactobacillus fermentum* CECT5716 during 1 month is safe and well tolerated in adults. Furthermore, the oral administration of the strain potentiates the immunological response of an anti-influenza vaccine and may provide enhanced systemic protection from infection by increasing Th1 response and virus neutralizing antibodies.

2. *Clinical study in women with infectious mastitis during lactation. (Arroyo et al 2010).*

OBJECTIVE: Mastitis is a common infectious disease during lactation, and the main etiological agents are staphylococci, streptococci, and/or *Corynebacterium*. The efficacy of oral administration of *Lactobacillus fermentum* CECT5716 or *Lactobacillus salivarius* CECT5713, two lactobacilli strains isolated from breast milk, to treat lactational mastitis was evaluated and was compared with the efficacy of antibiotic therapy.

METHODS AND DOSE: In this study, 352 women with infectious mastitis were randomly assigned to 3 groups. Women in groups A (n=124) and B (n=129) ingested daily 9 log10 colony-forming units (CFU) of L. fermentum CECT5716 or L. salivarius CECT5713, respectively, for 3 weeks, whereas those in group C (n=101) received the antibiotic therapy prescribed in their respective primary care centers.

RESULTS: On day 0, the mean bacterial counts in milk samples of the 3 groups were similar (4.35–4.47 log10 CFU/mL), and lactobacilli could not be detected. On day 21, the mean bacterial counts in the probiotic groups (2.61 and 2.33 log10 CFU/mL) were lower than that of the control group (3.28 log10 CFU/mL). *L. fermentum* CECT5716 and *L. salivarius* CECT5713 were isolated from the milk samples of women in the probiotic groups A and B, respectively. Women assigned to the probiotic groups improved more and had lower recurrence of mastitis than those assigned to the antibiotic group.

CONCLUSIONS: The use of *L. fermentum* CECT5716 or *L. salivarius* CECT5713 appears to be an efficient alternative to the use of commonly prescribed antibiotics for the treatment of infectious mastitis during lactation.

3. *Clinical study in lactating mothers suffering breast pain (MaldonadoLobon 2015).*

OBJECTIVE: To evaluate the ability of the probiotic strain *Lactobacillus fermentum* CECT5716 to reduce the *Staphylococcus* load in lactating mothers, the main outcome. Secondary outcomes were *Streptococcus*, *Lactobacillus*, and total bacteria counts, immunoglobulin A (IgA) and interleukin 8 (IL-8) concentrations in breastmilk, and breast pain scores.

METHODS AND DOSE: A randomized double-blinded controlled study with four study groups was performed. 113 women participated.
Three groups received the probiotic strain for 3 weeks at doses of 3E+09 colony-forming units (CFU)/day, 6E+09 CFU/day, or 9E+09 CFU/day. The fourth group received a placebo of maltodextrin. The main outcome of the study was *Staphylococcus* counts in breastmilk. The secondary outcomes were *Streptococcus*, *Lactobacillus*, and total bacteria counts in breastmilk, immunoglobulin A and interleukin 8 concentrations in breastmilk, and breast pain scores.

RESULTS: At the end of the study, a significant decrease in the *Staphylococcus* load was observed in the probiotic groups compared with the baseline loads ($p = 0.045$), whereas the control group maintained similar levels over time.
A significant difference in the pain score was observed among the groups receiving the three probiotic doses compared with the control group ($p = 0.035$, $p = 0.000$, and $p = 0.028$, respectively). A dose– response effect could not be observed because the three doses tested induced similar effects, and no significant differences were detected.

CONCLUSIONS: *L. fermentum* CECT5716 is an efficient treatment for breast pain during lactation associated with a high level of *Staphylococcus* in breastmilk from a dose of $3x10^9$ cfu/day.

4. *Clinical study of oral administration of L. fermentum to nursing women prevents lactational mastitis development. (Hurtado et al 2017).*

OBJECTIVE: The objective of this study is to evaluate the preventive effect of oral administration of *Lactobacillus fermentum* CECT5716 on mastitis incidence in lactating women.

METHODS: A randomized double-blinded controlled trial that included 625 women was conducted. Women who received a preventive dose of antibiotic in the context of delivery were recruited 1–6 days after childbirth and randomly assigned to a group. Probiotic group received 1 capsule/day containing *L. fermentum* $3 \cdot 10^9$ CFU, control group received 1 placebo capsule/day containing maltodextrin. The intervention period was 16 weeks.
The primary outcome of the study was the incidence of clinical mastitis defined as at least two out of the three breast symptoms (pain, redness, and lump) and at least one of fever or flu-like symptoms (shivering, hot sweats, or aches).

RESULTS: Two hundred ninety-one women completed 16 weeks of treatment. Sixteen women in the probiotic group developed mastitis versus 30 women in the control group (odds ratio = 0.531; p = 0.058). Incidence rate of mastitis in the probiotic group was significantly lower than that in the control group (IR = 0.130 in the probiotic group versus IR = 0.263 in the control group; p = 0.021). Therefore, the oral administration of *L. fermentum* CECT5716 during lactation decreased by 51% the incidence rate of clinical mastitis. Staphylococcus spp. load at the end of intervention was significantly lower in breast milk of women in the probiotic group than in breast milk of women in the control group (p = 0.025).

CONCLUSION: Consumption of the probiotic strain *L. fermentum* CECT5716 might be used during breastfeeding as an efficient strategy to prevent development of lactational mastitis in women.

6.9 Alternative rationale for reasonable expectation of safety based on other evidence of safety.

In the European Union a system was proposed for a pre-market safety assessment of selected groups of microorganisms leading to a "Qualified Presumption of Safety (QPS)".
In essence a safety assessment of a defined taxonomic group (e.g. genus or group of related species) was made based on four pillars: establishing identity, body of knowledge including history of use, 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 qualification), the group was granted QPS status. Thereafter, any strain of microorganism the identity of which could be unambiguously established and assigned to a QPS group would be free from the need for further safety assessment other than satisfying any qualifications specified. The QPS status refers to the micro-organisms and is not restricted to a specific application allowing the development of novel products.
Microorganisms not considered suitable for QPS would remain subject to a full safety assessment in Europe.

The EFSA Scientific Committee recommended that this QPS system for microorganisms should be introduced and implemented across EFSA as an assessment tool within the framework of the current and proposed legislation for all safety considerations of microorganisms intentionally added to the food chain, regardless of purpose (EFSA 2007). The Scientific Committee elaborated in 2007 a list of microorganisms considered suitable for QPS status (EFSA 2007). The list is annually updated and currently contains 79 species of microorganisms including 35 species of *Lactobacillus* and 5 of *Bifidobacterium* (EFSA 2012). *Lactobacillus fermentum* is included in this list as QPS microorganism, and, therefore, is considered safe.

6.10 Inconsistent Information.

Biosearch S.A. and the convened expert panel has reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with this conclusion of GRAS status.

6.11 Expert Panel Evaluation.

Biosearch S.A. has concluded that *Lactobacillus fermentum* CECT5716 is GRAS for use in conventional foods on the basis of scientific procedures. This GRAS conclusion is based on the

totality of evidence generally available in the public domain pertaining to the safety of *Lactobacillus fermentum* CECT5716, as discussed herein, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of infant formula ingredients and food ingredients. The Expert Panel consisted of the following qualified scientific experts: D. Angel Gil (University of Granada), D. Andreu Palou Oliver (University of Illes Balears) and D. Luis Moreno Aznar (University of Zaragoza). The Expert Panel convened by Biosearch S.A. independently and critically evaluated all data and information presented herein, and concluded that *Lactobacillus fermentum* CECT5716 is GRAS for use in conventional foods based on scientific procedures.

6.12 Final Conclusion.

Based on scientific procedures, the above data, and the information presented herein, Biosearch S.A. has concluded the intended uses of *Lactobacillus fermentum* CECT5716 are GRAS when consumed in conventional foods at 3E+09 cfu/day

General recognition of Biosearch´ s GRAS determination is supported by the unanimous consensus rendered by an independent Expert Panel, qualified by experience and scientific training to evaluate the proposed uses of *Lactobacillus fermentum* CECT5716.

This declaration is made in accordance with FDA´s standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

Part 7 List of supporting data and information

All data and information used in accordance with the above document are generally available.

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Annex A.

BIOSEARCH LIFE

Hereditum® PROBIOTICS

Food Grade Statement

To whom it may concern:

Hereby, Biosearch S.A. declares that we have implemented an HACCP system in accordance with the Regulation (EC) No 852/2004 for the manufacturing and/or processing, packing and distribution of microbial culture. As a result of the implementation of the HACCP system, we are registered in the Spanish Ministry of Health with number 31.0002208/GR for manufacturing activities carried out at our site.

According to the certificates provided by our suppliers, all ingredients and packaging materials used in the manufacturing process of **Hereditum® Lc40**. (*Lactobacillus fermentum* Lc40 (CECT5716)) are food grade.

Granada, 5th September 2017

María Martínez Santos
Quality Assurance Dept.

Annex B.

BIOSEARCH LIFE

Hereditum PROBIOTICS

CONTAMINANTS STATEMENT

To whom it may concern:

According to the HACCP system implemented in our site, Biosearch S.A. is able to certify that the investigation for the following contaminants:

- Heavy metals
- Mycotoxines
- PAHs
- Residual Solvents
- Others

is not applicable to the final product **Hereditum® Lc40** (***Lactobacillus fermentum Lc40 (CECT5716)***) due to the fact that each raw material we use is controlled in advanced, in accordance with the *REGULATION (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.*

We can also assure the process of manufacturing of *Lactobacillus fermentum* does not increase those contaminants.

Granada, 5th September 2017

María Martínez Santos
Quality Assurance Dept.

Annex C.

BIOSEARCH LIFE

Hereditum® PROBIOTICS

BSE/TSE Statement
(according to EMEA 410/01)

According to the certificates provided by our suppliers, Biosearch S.A., is able to certify that:

Hereditum® Lc40 does not contain risk ingredients derived from Beef, Sheep or Goat.

Therefore, we can certify that Hereditum® Lc40 is Bovine Spongiform Encephalopathy / Transmissible Spongiform Encephalopathies Free (BSE/TSE-Free).

Granada, 5th September 2017

María Martínez Santos
Quality Assurance Dept.

Rev.02

Annex D.

BIOSEARCH LIFE

Hereditum PROBIOTICS

Non-GMO Statement

(according to EU Regulation 1829/2003 and 1830/2003)

According to the certificates of our suppliers, Biosearch S.A., states that the following products:

Hereditum® Lc40
Hereditum® Lc40 Infant
Hereditum® MIX
Hereditum® BfM26
Hereditum® Breastcare
Hereditum® Breastcare Capsules
Paradens
Hereditum® Digestiplus
Hereditum® K8 Lyophilized
Hereditum® Lc9 Lyophilized
Hereditum® MIX Neo

Do not contain genetically modified DNA and/or proteins. Neither have these products been produced from raw materials or additives derived from genetically modified organisms.

During the manufacturing process, these products do not come into contact with genetically modified organisms, or with any other raw material or additive derived from genetically modified organisms.

Therefore, these products do not need to comply with the requirements for labeling and traceability of foods produced from genetically modified organisms, as defined under EU Regulation 1829/2003 and 1830/2003.

Granada, 15th January 2017

María Martínez Santos
Quality Assurance Manager

Rev.08

Annex E.

BIOSEARCH LIFE

Hereditum PROBIOTICS

Allergen Statement

(according to Regulation (EU) 1169/2011 and Directive 2007/68/EC)

The product **Hereditum® Lc40** may contain traces of **milk**.

This product is free from:	
Wheat and products thereof	Soybeans and products thereof
Rye and products thereof	Egg and products thereof
Oats and products thereof	Crustaceans and products thereof
Barley and products thereof	Molluscs and products thereof
Spelt and products thereof	Mustard and products thereof
Kamut and products thereof	Celery and products thereof
Nuts, nut oil and products thereof	Sesame seeds and products thereof
Peanuts and products thereof	Lupin and products thereof
Fish and products thereof	Sulphites or sulphur dioxide

Granada, 5th September 2017

María Martínez Santos
Quality Assurance Dept.

BIOSEARCH LIFE

BIOSEARCH LIFE

Hereditum® PROBIOTICS

Hereditum® Lc40 Allergen Declaration

To whom it may concern,

After carrying out an evaluation of possible risks derived from the use of Ingredients with allergens in the production line of Probiotics, Biosearch declares that *MRS Broth* (19% of peptone of casein), *peptone of casein*, *lactose* and *skimmed milk* are ingredients of different culture media.

From the point of view of Allergen Ingredients, the only ingredient in the above list used in the manufacture of **Hereditum® Lc40** is MRS Broth. This raw material is culture media rich in nutrients necessary to initiate our fermentation process since peptone of casein represents a readily available source of nitrogen and carbon for microorganisms.
MRS Broth is one of the minor ingredients in the composition of the fermentation culture media and that once it is exhausted is rejected. After that, the strain is introduced in a crioprotectant solution and standardized with maltodextrin.

On the other hand, from a possible cross-contamination with other allergen ingredients used during other strains production process, Biosearch has implemented a strict cleaning system between productions in order to guarantee that the cross-contamination risk is minimized.

For all the above mentioned Biosearch considers that the probability of presence of milk proteins or lactose in the final product is very low, and if it were, it would be in proportion of traces which are in the quantification limit according to analytical technique (milk proteins < 2.5 mg/kg and Lactose <0.01 g/100g).

Granada, 18th September 2017

Maria Martinez Santos
Quality Assurance Dept.

Rev.01

External laboratory testing of one aleatory lot for the presence/absence of milk proteins.

MERIEUX NutriSciences

Barcelona, 15/11/2017

Informe Nº : 20171106496
Revisión Nº : 0

Fecha recepción : 14/11/2017
Fecha inicio de análisis: 14/11/2017
Fecha finalización de análisis : 15/11/2017

BIOSEARCH LIFE, S.A.
Camino de Purchil, 66
18004-GRANADA
Granada

A/a. Sra. Luisa Pérez Vázquez

MUESTRA

Referencia del cliente: **Hereditum Lc40 FF Lote: 92810**

Descripción : Polvo, en envase de plástico transparente.

ENSAYOS REALIZADOS	*RESULTADOS OBTENIDOS*
Detección de proteínas totales de leche (en leche desnatada en polvo) *PNTA0144 (ELISA)*	**Ausencia o inferior a 2,5 mg/kg**

José Mestres
Director

Laura Miralles
Técnico Responsable

SILLIKER
Ceinal
ENAC
Miembro de: eurolab
AELI

Los resultados del presente informe corresponden únicamente a la muestra recibida y analizada.
Este informe no puede reproducirse parcialmente sin autorización explícita de SILLIKER Ibérica S.A. Soc. Unipersonal.
La muestra ha sido remitida por el cliente, salvo que el informe indique lo contrario.
La incertidumbre asociada a las determinaciones está a disposición del cliente.
Los ensayos y demás actividades marcadas con un asterisco no están amparadas por la acreditación de ENAC.

Pág. 1/1

SILLIKER Ibérica S.A.U.

Sede Barcelona
Longitudinal 8, 26
Mercabarna
08040 Barcelona
Tel. 932 632 454
Fax. 932 632 646
silliker.es@mrons.com

www.merieuxnutrisciences.es

Annex F.

BIOSEARCH LIFE

Quality Control - BIOSEARCH S.A.
Camino de Purchil, 66
18004 -GRANADA (Spain)
Phone.: 609 805 091 Fax: 958 240 160

Certificate of Analysis

Product	Batch	Manufacturing Date	Best Before
HEREDITUM® Lc40 Lyophilised probiotics culture Lactobacillus fermentum (CECT5716)	[redacted]	20.October.2017	20.October.2019

Results

Parameters Tested	Units	Method	Results	Specification
Cell content L. fermentum	cfu/g	PTE-ANL-49	1.5E+11	Min. 1.0E+11
B. cereus	cfu/g	PTE-ANL-46	<10	Max. 500
Yeasts and moulds	cfu/g	PTE-ANL-43	Not detected	Max. 50
Cronobacter	cfu/10 g	ISO 22964:2006	Not detected	Not detected
Enterobacteriaceae	cfu/g	ISO 21528-2:2004	Not detected	< 10
E. coli*	cfu/g	ISO 7251:2005	Not detected	Not detected
S. aureus*	cfu/g	ISO 6888-1:1999	<10	< 10
Listeria monocytogenes*	cfu/25 g	ISO 11290-1:2004	Not detected	Not detected
Salmonella spp.*	cfu/25 g	ISO 6579:2003	Not detected	Not detected
Moisture	g/100 g	PTE-ANL-34	4	Max. 10
aw – water activity		PTE-ANL-58	0.12	Max. 0.25

*The microbiological analysis of, Listeria monocytogenes and S. aureus and have been carried out by an external Laboratory, Silliker, with the method mentioned in this report.

Name: L. Pérez Vázquez
Quality Control
[redacted]

Name: V. Martín del Castillo
Quality Assurance
[redacted]

Batch conforms to manufacturing controlled procedures and analyzed according to validated methods referred in this certificate.

Date: 13.November.2017

End of report

BIOSEARCH LIFE
Quality Control - BIOSEARCH S.A.
Camino de Purchil, 66
18004 -GRANADA (Spain)
Phone.: 609 805 091 Fax: 958 240 160

Certificate of Analysis

Product	Batch	Manufacturing Date	Best Before
HEREDITUM® Lc40 Lyophilised probiotics culture *Lactobacillus fermentum* (CECT5716)	[redacted]	09.November.2017	09.November.2019

Results

Parameters Tested	Units	Method	Results	Specification
Cell content *L. fermentum*	cfu/g	PTE-ANL-49	1.3E+11	Min. 1.0E+11
B. cereus	cfu/g	PTE-ANL-46	<10	Max. 500
Yeasts and moulds	cfu/g	PTE-ANL-43	Not detected	Max. 50
Cronobacter	cfu/10 g	ISO 22964:2006	Not detected	Not detected
Enterobacteriaceae	cfu/g	ISO 21528-2:2004	Not detected	< 10
*E. coli**	cfu/g	ISO 7251:2005	Not detected	Not detected
*S. aureus**	cfu/g	ISO 6888-1:1999	<10	< 10
*Listeria monocytogenes**	cfu/25 g	ISO 11290-1:2004	Not detected	Not detected
*Salmonella spp.**	cfu/25 g	ISO 6579:2003	Not detected	Not detected
Moisture	g/100 g	PTE-ANL-34	3	Max. 10
aw – water activity		PTE-ANL-58	0.08	Max. 0.25

**The microbiological analysis of, Listeria monocytogenes and S. aureus and have been carried out by an external Laboratory, Silliker, with the method mentioned in this report.*

Name: L. Pérez Vázquez
Quality Control
[redacted]

Name: M. Martinez
Quality Assurance
[redacted]

Batch conforms to manufacturing controlled procedures and analyzed according to validated methods referred in this certificate.

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Date: 17.November.2017

End of report

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Camino de Purchil, 66
18004 -GRANADA (Spain)
Phone.: 609 805 091 Fax: 958 240 160

Certificate of Analysis

Product	Batch	Manufacturing Date	Best Before
HEREDITUM® Lc40 Lyophilised probiotics culture *Lactobacillus fermentum* (CECT5716)	[redacted]	13.November.2017	13.November.2019

Results

Parameters Tested	Units	Method	Results	Specification
Cell content *L. fermentum*	cfu/g	PTE-ANL-49	1.3E+11	Min. 1.0E+11
B. cereus	cfu/g	PTE-ANL-46	<10	Max. 500
Yeasts and moulds	cfu/g	PTE-ANL-43	Not detected	Max. 50
Cronobacter	cfu/10 g	ISO 22964:2006	Not detected	Not detected
Enterobacteriaceae	cfu/g	ISO 21528-2:2004	Not detected	< 10
*E. coli**	cfu/g	ISO 7251:2005	Not detected	Not detected
*S. aureus**	cfu/g	ISO 6888-1:1999	<10	< 10
*Listeria monocytogenes**	cfu/25 g	ISO 11290-1:2004	Not detected	Not detected
*Salmonella spp.**	cfu/25 g	ISO 6579:2003	Not detected	Not detected
Moisture	g/100 g	PTE-ANL-34	3	Max. 10
aw – water activity		PTE-ANL-58	0.08	Max. 0.25

**The microbiological analysis of, Listeria monocytogenes and S. aureus and have been carried out by an external Laboratory, Silliker, with the method mentioned in this report.*

Name: L. Pérez Vázquez
Quality Control
[redacted]

Name: M. Martinez
Quality Assurance
[redacted]

Batch conforms to manufacturing controlled procedures and analyzed according to validated methods referred in this certificate.

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Date: 11.December.2017

End of report

BIOSEARCH LIFE
Quality Control - BIOSEARCH S.A.
Camino de Purchil, 66
18004 -GRANADA (Spain)
Phone.: 609 805 091Fax: 958 240 160

Certificate of Analysis

Product	Batch	Manufacturing Date	Best Before
HEREDITUM® Lc40 F.F. Lyophilised probiotics culture *Lactobacillus fermentum* (CECT5716)	89596	05.April.2017	05.april.2019

Results

Parameters Tested	Units	Method	Results	Specification
Cell content *L. fermentum*	cfu/g	PTE-ANL-49	1.5E+11	Min. 1.0E+11
B. cereus	cfu/g	PTE-ANL-46	<10	Max. 500
Yeasts and moulds	cfu/g	PTE-ANL-43	Not detected	Max. 50
Cronobacter	cfu/10 g	ISO 22964:2006	Not detected	Not detected
Enterobacteriaceae	cfu/g	ISO 21528-2:2004	<10	< 10
*E. coli**	cfu/g	ISO 7251:2005	Not detected	Not detected
*S. aureus**	cfu/g	ISO 6888-1:1999	< 10	< 10
*Listeria monocytogenes**	cfu/25 g	ISO 11290-1:2004	Not detected	Not detected
*Salmonella spp.**	cfu/25 g	ISO 6579:2003	Not detected	Not detected
Moisture	g/100 g	PTE-ANL-34	4	Max. 10
aw – water activity		PTE-ANL-58	0.15	Max. 0.25

**The microbiological analysis of Salmonella spp., Listeria monocytogenes, S. aureus and E. coli have been carried out by an external Laboratory, Silliker, with the method mentioned in this report.*

Name: L. Pérez Vázquez
Quality Control

Name: M. Martinez
Quality Assurance

Batch conforms to manufacturing controlled procedures and analyzed according to validated methods referred in this certificate.

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Date: 19.April.2017

End of report

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Camino de Purchil, 66
18004 -GRANADA (Spain)
Phone.: 609 805 091 Fax: 958 240 160

Certificate of Analysis

Product	Batch	Manufacturing Date	Best Before
HEREDITUM® Lc40 F.F. Lyophilised probiotics culture *Lactobacillus fermentum* (CECT5716)	[redacted]	26.May.2017	26.May.2019

Results

Parameters Tested	Units	Method	Results	Specification
Cell content *L. fermentum*	cfu/g	PTE-ANL-49	1.3E+11	Min. 1.0E+11
B. cereus	cfu/g	PTE-ANL-46	<10	Max. 500
Yeasts and moulds	cfu/g	PTE-ANL-43	Not detected	Max. 50
Cronobacter	cfu/10 g	ISO 22964:2006	Not detected	Not detected
Enterobacteriaceae	cfu/g	ISO 21528-2:2004	Not detected	< 10
*E. coli**	cfu/g	ISO 7251:2005	Not detected	Not detected
*S. aureus**	cfu/g	ISO 6888-1:1999	<10	< 10
*Listeria monocytogenes**	cfu/25 g	ISO 11290-1:2004	Not detected	Not detected
*Salmonella spp.**	cfu/25 g	ISO 6579:2003	Not detected	Not detected
Moisture	g/100 g	PTE-ANL-34	5	Max. 10
aw – water activity		PTE-ANL-58	0.16	Max. 0.25

**The microbiological analysis of Salmonella spp., Listeria monocytogenes, S. aureus and E. coli have been carried out by an external Laboratory, Silliker, with the method mentioned in this report.*

Name: L. Pérez Vázquez
Quality Control
[redacted]

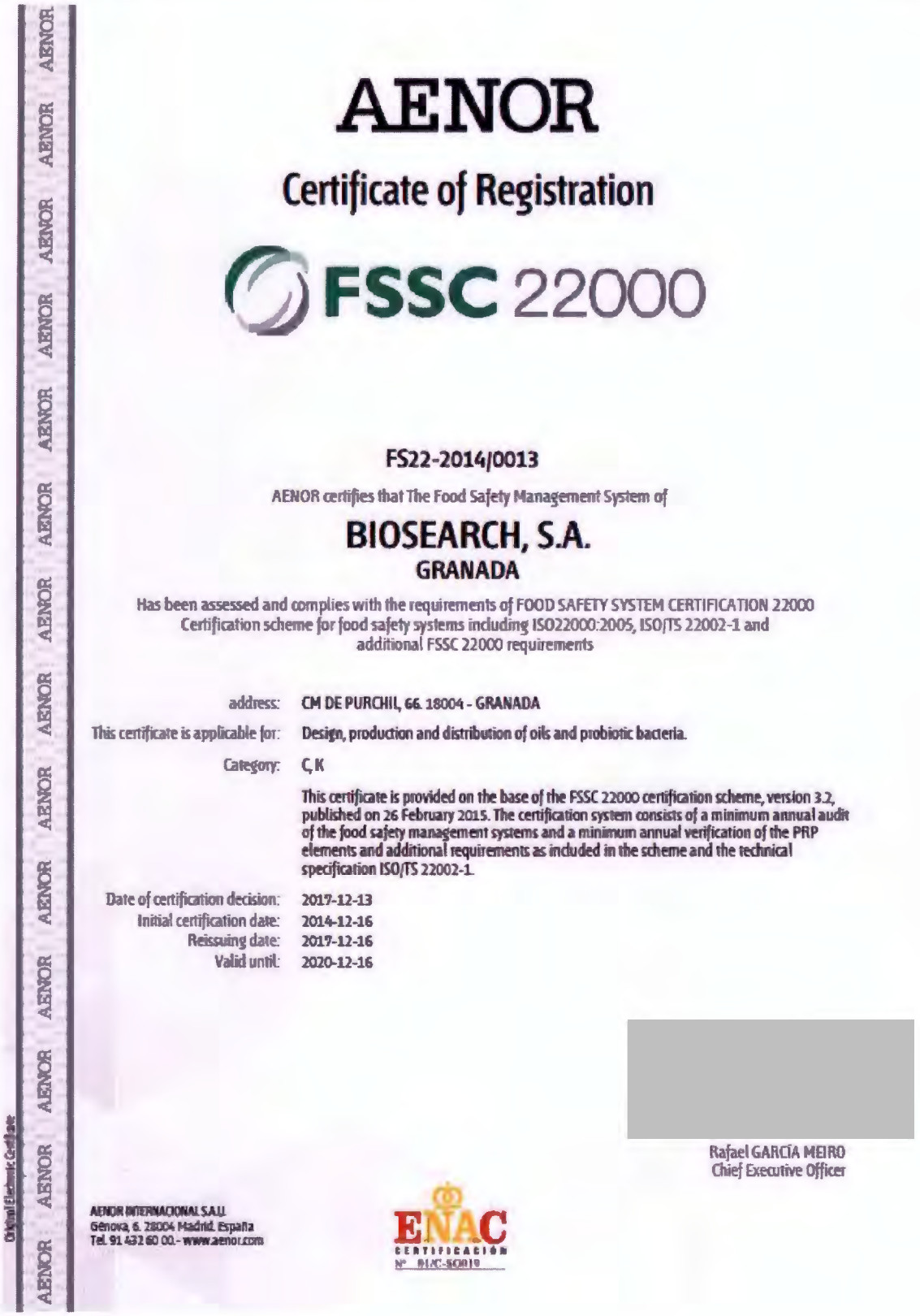
Name: M. Martinez
Quality Assurance
[redacted]

Batch conforms to manufacturing controlled procedures and analyzed according to validated methods referred in this certificate.

Date: 06.06.2017

End of report

Annex G. Biosearch's Certifications



AENOR

Certificate of Registration

FSSC 22000

FS22-2014/0013

AENOR certifies that The Food Safety Management System of

BIOSEARCH, S.A.
GRANADA

Has been assessed and complies with the requirements of FOOD SAFETY SYSTEM CERTIFICATION 22000 Certification scheme for food safety systems including ISO22000:2005, ISO/TS 22002-1 and additional FSSC 22000 requirements

address:	CM DE PURCHIL, 66. 18004 - GRANADA
This certificate is applicable for:	Design, production and distribution of oils and probiotic bacteria.
Category:	C, K

This certificate is provided on the base of the FSSC 22000 certification scheme, version 3.2, published on 26 February 2015. The certification system consists of a minimum annual audit of the food safety management systems and a minimum annual verification of the PRP elements and additional requirements as included in the scheme and the technical specification ISO/TS 22002-1.

Date of certification decision:	2017-12-13
Initial certification date:	2014-12-16
Reissuing date:	2017-12-16
Valid until:	2020-12-16

Rafael GARCÍA MEIRO
Chief Executive Officer

AENOR INTERNACIONAL S.A.U.
Génova, 6. 28004 Madrid. España
Tel. 91 432 60 00.- www.aenor.com

ENAC CERTIFICACIÓN Nº 01/C-SC0019

Original Electronic Certificate

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

IQNet and
AENOR
hereby certify that the organization

BIOSEARCH, S.A.

A)
CM DE PURCHIL, 66
18004 - GRANADA

B)
PI ALCANTARILLA S/N
10310 - TALAYUELA
(CACERES)

for the following field of activities

A) Design, production and distribution of oils and probiotic bacteria.
B) Design, production and distribution of herbal extracts.

has implemented and maintains a

Food Safety Management System

which fulfills the requirements of the following standard

ISO 22000:2005

First issued on: 2011-12-22 Last issued: 2017-12-16 Validity date: 2020-12-16

Registration Number: **ES-SA-0035/2011**

IQNet

AENOR

Michael Drechsel
President of IQNet

Rafael GARCÍA MEIRO
Chief Executive Officer

Original Electronic Certificate

IQNet Partners*:
AENOR Spain AFNOR Certification France AIB-Vincotte International Belgium ANCE Mexico APCER Portugal CCC Cyprus
CISQ Italy CQC China CQM China CQS Czech Republic Cro Cert Croatia DQS Holding GmbH Germany
FCAV Brazil FONDONORMA Venezuela ICONTEC Colombia IMNC Mexico Inspecta Certification Finland IRAM Argentina
JQA Japan KFQ Korea MIRTEC Greece MSZT Hungary Nemko AS Norway NSAI Ireland PCBC Poland
Quality Austria Austria RR Russia SII Israel SIQ Slovenia SIRIM QAS International Malaysia
SQS Switzerland SRAC Romania TEST St Petersburg Russia TSE Turkey YUQS Serbia
IQNet is represented in the USA by: AFNOR Certification, CISQ, DQS Holding GmbH and NSAI Inc.
* The list of IQNet partners is valid at the time of issue of this certificate. Updated information is available under www.iqnet-certification.com

AENOR

Certificado del Sistema de Gestión de la Seguridad Alimentaria

AENOR
ER
Seguridad Alimentaria
ISO 22000

SA-0035/2011

AENOR certifica que la organización

BIOSEARCH, S.A.

dispone de un sistema de gestión de la seguridad alimentaria conforme con la Norma UNE-EN ISO 22000:2005

para las actividades: A) Diseño, producción y distribución de aceites y bacterias probióticas.
B) Diseño, producción y distribución de extractos herbales.

que se realizan en: A) CM DE PURCHIL, 66. 18004 - GRANADA
B) PI ALCANTARILLA S/N. 10310 - TALAYUELA (CACERES)

Fecha de primera emisión: 2011-12-22
Fecha de última emisión: 2017-12-16
Fecha de expiración: 2020-12-16

Rafael GARCÍA MEIRO
Director General

AENOR INTERNACIONAL S.A.U.
Genova, 6. 28004 Madrid. España
Tel. 91 432 60 00.- www.aenor.com

ENAC
IQNet

AENOR

Certificado de Conformidad GMP

GMP
Good Manufacturing Practices
FOOD
AENOR conform

GMP-2017/0003

AENOR certifica las GMP de la organización
AENOR certifies Good Manufacturing Practices (GMP) of the organization

BIOSEARCH, S.A.

Con domicilio social/ established in:	CM DE PURCHIL, 66. 18004 - GRANADA
Conforme con/ In accordance with:	Codex Alimentarius CAC/RCP 1-1969, Rev 4 2003 Código Internacional de prácticas recomendadas – principios generales de higiene de los alimentos. Codex Alimentarius CAC/RCP 1-1969, Rev 4 2003 Recommended international code of practice. General principles of food hygiene
para las actividades/ for the activities:	Diseño, producción y distribución de aceites y bacterias probióticas. Design, production and distribution of oils and probiotic bacteria.
que se realizan en/ developed in:	CM DE PURCHIL, 66. 18004 – GRANADA
Sistema de certificación/ Certification scheme:	Para conceder esta certificado, AENOR ha comprobado la prestación del servicio. AENOR realiza estas actividades periódicamente mientras el Certificado no haya sido anulado, según se establece en el RP B13. When issuing this certificate, AENOR has checked the provision of the service. AENOR will periodically check this provision according to Codex Alimentarius CAC/RCP 1-1969 rev.4 unless the certificate is cancelled, as set forth in RP B13
Fecha de primera emisión/ First issued on	2017-05-08
Fecha de modificación/ Renewal date	2017-12-16
Fecha de expiración/ Validity date	2018-12-16

[redacted]
Rafael GARCÍA MEIRO
Chief Executive Officer

AENOR INTERNACIONAL S.A.U.
Génova, 6. 28004 Madrid. España
Tel. 91 432 60 00.- www.aenor.com

Annex H. Expert panel consensus statement concerning the GRAS status of *Lactobacillus fermentum* CECT576

To demonstrate that *Lactobacillus fermentum* CECT5716 is GRAS under their intended conditions of use, the safety of the intake of this bacterial strain is established under its intended conditions of use. This intake of *Lactobacillus fermentum* CECT5716 is determined to be safe by showing that the safety of these levels of intake is generally recognized by experts qualified by scientific training and experienced to evaluate the safety of food substances, and is based on generally available and accepted information.

The publicly available data demonstrating the safety of the proposed uses of Lactobacillus fermentum CECT5716 was reviewed by an Expert Panel consisting of:

Luis Moreno Aznar, PhD Professor University of Zaragoza, Spain
Andreu Palou, PhD Professor University of Islas Baleares, Spain
Angel Gil, PhD Professor University of Granada, Spain

The undersigned, an independent panel of recognized experts, qualified by their scientific capacity and relevant national and international experience to evaluate the safety of food and food ingredients, was convened by Biosearch Life S.A to determine the Generally Recognized As Safe (GRAS) status of *Lactobacillus fermentum* CECT5716 to enrich foods in these beneficial bacteria, so-called probiotics (definition #20 of the Code of Federal Regulations 21 CFR 170.3).

The Expert Panel independently and critically evaluated materials submitted by Biosearch Life S.A and other information deemed appropriate or necessary. Biosearch life accepts responsibility for the GRAS determination that has been made for Lactobacillus fermentum CECT5716 as described herein.

This Expert Panel concluded that:

Lactobacillus fermentum CECT5716 has been sufficiently characterized to ensure that it is a food grade product, and, therefore, Lactobacillus fermentum CECT5716, meeting the food grade specifications described in this notification, is safe and GRAS under their intended conditions of use.

In the opinion of these experts, other qualified and competent scientists reviewing the same publicly available data would reach the same conclusion. It is therefore concluded that, based on scientific procedures, the intended use of Lactobacillus fermentum CECT5716, as shown in Table I, is safe and GRAS. Because Lactobacillus fermentum CECT5716 is GRAS for their proposed uses, it is excluded from the definition of a food additive, and thus may be lawfully marketed and sold for its use in the U.S. without the promulgation of a food additive regulation under 21 CFR.

 D. Angel Gil / University Granada	 D. Andreu Palou Oliver University Illes Balears	 D. Luis Moreno Aznar University Zaragoza

Bonnette, Richard

From: Laura Montes Ordoñez <lmontes@biosearchlife.com>
Sent: Monday, November 19, 2018 3:40 AM
To: Bonnette, Richard
Subject: RE: your submission to the US FDA GRAS notification program for Lactobacillus fermentum

Dear Richard,

My name is Laura Montes and I will be the new person in charge of Regulatory Affairs.
I am very pleased to meet you.

Please, first of all, could you change the contact data? (address is the same):

- Contact person: Laura Montes Ordóñez
- Email: lmontes@biosearchlife.com
- Phone number: +34 913802973 ext 12022

Regarding your questions:

- The statutory basis for our conclusion of GRAS status is through scientific procedures in accordance with §170.30(a) and (b). We forgot to include it in Part 1 of the notification
- Regarding the intended uses for Lactobacillus fermentum CECT5716, USDA-regulated products are excluded from the intended use.

If you need more information, please do not hesitate to contact me

Thank you and best regards,

Laura Montes Ordóñez
Regulatory Affairs

lmontes@biosearchlife.com
+34 913802973 Ext 12022

C/ Cabeza Mesada, 5, 5º plta. 28031 Madrid.

www.biosearchlife.com

BIOSEARCH LIFE | PASSION FOR INNOVATION PASSION FOR LIFE

From: "Bonnette, Richard" <Richard.Bonnette@fda.hhs.gov>
Date: 16 November 2018 at 14:06:53 CET
To: Laura Macho Valls <lmacho@biosearchlife.com>
Subject: your submission to the US FDA GRAS notification program for Lactobacillus fermentum

Dear Ms. Macho,
We have completed a pre-filing evaluation of your GRAS submission regarding *Lactobacillus fermentum* CECT5716 received on October 26, 2018. We have one or two points to confirm with you before we can move forward with the submission.

The submission does not indicate (per 170.225(c)(5)) the statutory basis for the GRAS conclusion. From 170 Subpart E:

"(5) Inform us of the statutory basis for your conclusion of GRAS status (i.e., through scientific procedures in accordance with §170.30(a) and (b) or through experience based on common use in food in accordance with §170.30(a) and (c));"

The content of the submission suggests that it is a "scientific procedures" basis, but that is not indicated in Part 1.

Also, the submission does not indicate whether any of the intended uses will be in products that are under USDA's authority (meat and poultry). For example, the submission lists soups and soup mixes that can contain meat. These foods may be subject to USDA regulation. If the ingredient is intended for use in USDA regulated products, there will be a separate requirement for additional data that USDA will need to evaluate the GRAS notice. If you don't intend for the ingredient to be used in USDA-regulated products, you can simply indicate that USDA-regulated products are excluded from the intended use.

You can reply to this email with the requested information and we'll amend the submission with your response. The notice (along with your clarifying email) will be posted to our online inventory as part of the GRAS notice. If you have any questions, please let me know.

Regards,
Richard

Richard E. Bonnette, M.S.
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1235
richard.bonnette@fda.hhs.gov