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June 14, 2019

Paulette Gaynor, Ph. D
GRAS Notification Program
Office of Food Additive Safety
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

Re: GRAS Notice-Exemption Claim for *Bifidobacterium animalis ssp. lactis* UABla-12™

Dear Dr. Gaynor:

On behalf of my client UAS Laboratories, LLC, and in accordance with FDA's final rule of August 17, 2016 (81 FR 54960) and 21 CFR §170.225(c)(1), please accept submission of notice of a GRAS exemption claim for the above referenced substance, *Bifidobacterium animalis ssp. lactis* UABla-12™for use in conventional foods that are compatible with the addition of live, safe and suitable food microbial cultures in accordance with cGMP levels of 10^9 to 10^{11} cell forming units (CFU) per serving.

UAS Laboratories, LLC certifies that to the best of UAS Laboratories, LLC knowledge this GRAS notice is a complete, representative, and balanced submission, which contains all information known to the company that is pertinent to the evaluation of the safety and GRAS status of the substance.

This GRAS notice is submitted on CD-ROM containing: a GRAS notice exemption claim; detailed information on the notified substance; and attachments containing further referenced and substantiating information on the substance.

Please promptly contact me should you have any questions regarding the submitted notice. I look forward to receiving acknowledgment of receipt of this notice and to a timely response regarding the noticed substance. Thank you.

P. Gaynor Ph. D
OFAS/GNP
June 14, 2019

Sincerely,

Kevin O. Gillies

Enc.

Cc: Gregory Leyer, Ph. D, UAS Laboratories, LLC

KOG/Kg

Bifidobacterium animalis ssp. *lactis* UABla-12™

Generally Recognized as Safe Notice

UAS Laboratories, LLC
4375 Duraform Lane,
Windsor, Wisconsin 53598
USA

Submitted
June 2019

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21 CFR 570. 225; Part 1: Generally Recognized as Safe (GRAS) Notice - Exemption Claim

1.1 Exemption Claim for *Bifidobacterium animalis ssp. lactis* UABla-12™

Paulette Gaynor, Ph. D.
GRAS Notification Program
Office of Food Additive Safety
Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

Re: GRAS Notice-Exemption Claim for *Bifidobacterium animalis ssp. lactis* UABla-12™1

Dear Dr. Gaynor:

On behalf of my client UAS Laboratories LLC (hereinafter UASLabs) located at 4375 Duraform Lane, Windsor, Wisconsin 53598 USA, and in accordance with FDA's final rule of August 17, 2016 (81 FR 54960) and 21 CFR §170.225(c)(1) relating to the filing of generally recognized as safe (GRAS) notices, please accept this claim and the attached information, submitted on a CD-ROM disk, for that purpose as it relates to the use of *Bifidobacterium animalis ssp. lactis* UABla-12™as an ingredient in conventional foods that are compatible with the addition of live, safe and suitable food microbial cultures.

Specifically, UASLabs has concluded that *Bifidobacterium animalis ssp. lactis* UABla-12™ is Generally Recognized as Safe (GRAS) by scientific procedures in accordance with both 21 CFR 170.30(a) and (b) and is thereby exempt from pre-market approval requirements of the Food, Drug and Cosmetic Act. The species *Bifidobacterium animalis ssp. lactis,* in general, and the strain *Bifidobacterium animalis ssp. lactis* UABla-12™, in particular, have a documented safe history of use in fermented food and other traditional uses and this claim essentially extends the uses of strain *Bifidobacterium animalis ssp. lactis* UABla-12™ that are considered GRAS.

In concluding that *Bifidobacterium animalis ssp. lactis* UABla-12™ is GRAS, UASLabs has consulted the documented safe history of use, scientific literature, safety indications reported in human clinical trials and rigorously performed specific safety testing as recommended by Pariza et al. (2015) for the determination of the safety of microbial food cultures for the uses described herein.

In conformity with the requirements outlined in the rule, the following information is included with this exemption claim:

1.1 Information about Notifier

Notifier:

UAS Laboratories, LLC
4375 Duraform Lane
Windsor, Wisconsin 53598
USA

Contact person for this file:

See agent below

Agent who is authorized to act on behalf of the Notifier:

Kevin O. Gillies
Kevin O. Gillies Consulting Services, LLC
1759 Grape St.
Denver, Colorado 80220

1.2 Basis for safety determination:

Scientific procedures supported by safe history of use in food and dietary supplements.

1.3 Intended use

As an ingredient in all foods that are compatible with the addition of live, safe and suitable food microbial cultures in accordance with cGMP levels of 10^9 to 10^{11} cell forming units (CFU) per serving.

1.5 Availability of Information

Data and information relevant to this GRAS notice is available to FDA during customary business hours upon request.

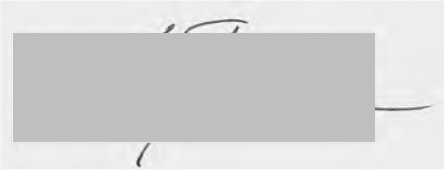
1.6 Confidential Commercial Information

UASLabs further provides notice to FDA that this GRAS Notification does not contain data and information that are exempt from disclosure under FOIA (*e.g.,* as trade secret or as commercial or financial information that is privileged or confidential pursuant to 21CFR170.225(c)(8)).

1.7 Certification Statement

UASLabs further certifies in accordance with 21CFR570.225(c)(9) that, to the best of their knowledge, the GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to UASLabs and pertinent to the evaluation of the safety and GRAS status of the use of *Bifidobacterium animalis ssp. lactis* UABla-12™.

1.8 Signature of Responsible Party or Agent



Kevin O. Gillies Consulting Services, LLC (member)
June 13, 2019

UASLabs
4375 Duraform Lane
Windsor, Wisconsin 53598, USA

21CFR570.230; Part 2: Identity, Manufacturing, Specifications, Use

2.1 Identity of the Substance

- Common and Usual Name of the Substance: *Bifidobacterium animalis* ssp. *lactis* UABla-12™
- Chemical name: None
- Empirical formula: None
- Structural formula: None
- Quantitative formula: None

B. animalis ssp. lactis is a member of the Phylum Actinobacteria (see Fig. 1 below) in bacterial taxonomy. *B. animalis* ssp*. lactis* is a gram-positive lactic acid bacterium commonly found in the gut of healthy individuals and has been identified in the human infant gut biota, particularly in ileal, fecal, and mucosal samples (Barangou 2009) (Turroni 2009) (Wall 2008). *B. animalis* ssp*. lactis* is a well-characterized, non-pathogenic, non-toxigenic, homogeneous species grouping. There are no known pathogenic or toxigenic members of the species (Appendix 2) and the species is listed as a BioSafety Level 1 organism according to the U.S. Public Health Service Guidelines (https://www.cdc.gov/biosafety/publications/bmbl5/index.htm; accessed Feb.20, 2018).

Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes are dominant microbial phyla widely distributed in diverse ecosystems on the planet. Metagenomic analyses of the microbial landscape inhabiting various mammalian environments, notably the human gastrointestinal tract (GIT) (Turroni 2009) and skin, have specifically identified Actinobacteria as an important and occasionally dominant phylum. Among the members of the large, diverse, and dynamic microbial community residing in the human GIT, *Bifidobacterium* is a dominant genus considered beneficial to humans and includes probiotic strains (Barangou 2009).

Fig. 1 (below) describes the current phylogenetic description of strain UABla-12™.

Fig. 1

Family: Bacteria
Phylum: Actinobacteria
Order: Bifidobacteriales
Family: Bifidobacteriaceae
Genus: *Bifidobacterium*
Species: *animalis*
subspecies: lactis
Strain: UABla-12™

B. animalis ssp*. lactis* strains in addition to their common occurrence as native gut microflora are intentionally introduced in the food chains, particularly in fermented dairy products and applied as probiotics. As such, the species has been approved for use by competent authorities world-wide (See Section 6.2 below for details). We note, specifically, that these regulatory agencies, recognize the inherent safety of these human commensal organisms for use in food and supplements without restriction as to consumer demographic groups, food type or usage rates. Because a bacterial species is not an individual entity, but a taxonomic grouping of like organisms, in practice, the approval of a species means the approval of individual members or strains of the species unless otherwise noted.

B. animalis ssp*. lactis* is listed in the International Dairy Federation (International Dairy Federation, Other publication 18/2012. Safety Demonstration of Microbial Food Cultures (MFC) in Fermented Food Products http://www.fil-idf.org; International Dairy Federation, Bulletin No. 377/2002. Inventory of Microorganisms with a Documented History of Use in Food) list of strains with a documented safe history of use in food (Bourdichon 2012) (Morgensen 2002).

The European Food Safety Authority has published in the Agency's Qualified Presumption of Safety (QPS) documentation the results of its comprehensive safety evaluation of the IDF listed organisms with some modifications. EFSA found that members of *B. animalis* ssp*. lactis* species are safe for general use in foods without restriction as to food category or usage rate and exempt from requirements for pre-market approval of use in food and feed in the European Union (EFSA; Appendix 2).

In the US, prior sanctions were granted by the FDA for the use of harmless lactic acid producing bacteria, such as *B. animalis* ssp*. lactis*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (21 CFR 131.112), sour cream (21 CFR 131.160), cottage cheese (21CFR 133.128), and yogurt (21 CFR 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt (https://www.fda.gov/food/ingredientspackaginglabeling/gras/microorganismsmicrobialderivedingredients/default.htm; last accessed Feb. 20, 2018).
Moreover, in addition to *Lactobacillus acidophilus*, *B. animalis* ssp. *lactis* may be the most widely used probiotic culture in food and supplements in the US and EU (Barangou 2009).

The International Food Additives Council has determined that the strains listed on the IDF list, the EFSA QPS list and the subject of regulatory approval worldwide are GRAS for their traditional food uses by scientific procedures (Stevens 2009).

2.1.1 Genetic Identification of *B. animalis* ssp*. lactis* UABla-12™

Because, the regulatory approvals for the species *B. animalis* ssp. *lactis* are implicit approvals for individual isolates of the species, it is essential that the identity of a strain proposed for application in food, including supplements be characterized using state of the art methodologies.

In accord with the safety decision tree of Pariza, et al. (Pariza 2015) (Appendix 1) and EFSA recommendations (European Food Safety Authority n.d.), UASLabs has confirmed by state of the art sequencing methods that the strain *Bifidobacterium animalis* ssp*. lactis* UABla-12™ is (1) a member of the *B. animalis* ssp*. lactis* species and thereby a member of the group with a safe history of use in food; (2) does not contain gene sequences encoding known toxins; and (3) is free of known virulence genes (Section 2.1.2).

UABla-12™ has also been confirmed as an isolate of *B. animalis* ssp*. lactis* by ribotype 5s, 16s, and 23s rDNA genetic homology method (Appendix 4). The RiboPrinter® system is an automated southern blotting (ribotyping) platform for microbial identification and characterization. It automates restriction fragment length polymorphism (RFLP) analysis and targets the RNA-coding region of the bacterial genome. Restriction enzymes, such as *Eco*RI or *Pvu*II, cut bacterial DNA into fragments that are then separated via gel electrophoresis, and hybridized with labeled DNA probes derived from regions encoding the 5S, 16S and 23S sequences, as well as the spacer regions and flanking genes on either side processed to form a characteristic banding pattern or "fingerprint." This pattern can then be compared to a reference database of over 1,700 microbial species patterns from historic samples. This rich depth of information is what allows highly precise differentiation among strains of the same species, even those with the same 16S sequence. Because sequencing methods are time consuming and expensive, it is important to have methods such as ribotyping for quality control use.

A robust identification of the strain is important as the determination of the strain identity ties the strain directly to the history of safe use and safety determinations discussed above. Thus, UABla-12™ has a safe history of use in food, is listed on the EFSA QPS list, is allowed for use in certain standardized food in the US via prior sanction listing, etc.

2.1.2 Absence of Toxin and other virulence genes

Both EFSA (European Food Safety Authority n.d.) and Pariza et al. (Pariza 2015) recommend that new strains of known safe genus/species be analyzed for gene encoded traits that could impact the health of consumers. Determining the absence of these gene-encoded factors that have the potential to negatively impact consumer health has been accomplished using whole gene sequencing and bioinformatic annotation methods described below.

Prior to gene sequencing, the genetic homogeneity of UABla-12™ was assessed. The culture was initially plated on the appropriate solid medium to assess colony morphology consistency. Five (5) distinct, well-defined colonies were picked from the plate for further genetic evaluation by two independent PCR-based fingerprinting protocols, which showed unambiguously the same profiles for all five isolates. Based on these results, strain UABla-12™ was judged to be homogeneous and a pure culture and the five isolated colonies represented *B. animalis* ssp*. lactis* UABla-12™. One of the five (5) colonies was picked for further sequence analysis.

The whole genome sequence of *B. animalis* ssp. *lactis* UABla-12™ was determined by combining Illumina HiSeq™ and PacBio™ platforms, to ensure high nucleotide sequence fidelity and long-reads-based high assembly completeness, respectively. Prior to sequencing, culture homogeneity was assessed by two independent PCR-based fingerprinting protocols and a batch of high quality and high integrity DNA was purified from the pure culture.

The genome of *B. animalis* ssp*. lactis* UABla-12™ was confirmed to be free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity, as assessed by a BLAST-based tool against the Virulence Factor DataBase (VFDB) (Chen L. H. 2016). The VFBD is an integrated comprehensive online resource for curating information about virulence factors of bacterial pathogens and includes 30,178 genes related to 1,796 virulence factors of 74 pathogenic genera.

The genome was found to not comprise any complete prophage, as analyzed by PHASTER (Arndt 2016), the most recent upgrade of the PHAGE Search Tool (PHAST). PHAST is an integrated search and annotation tool that combines genome-scale ORF prediction and translation (via GLIMMER), protein identification (via BLAST matching and annotation by homology), phage sequence identification (via BLAST matching to a phage-specific sequence database), tRNA identification, attachment site recognition and gene clustering density measurements using density-based spatial clustering of applications with noise (DBSCAN) and sequence annotation text mining.

In addition, no extra-chromosomal elements were identified. The combination of the lack of prophage and extra-chromosomal DNA indicate an absence of genetic mechanisms that are known to facilitate horizontal gene transfer.

2.1.3 Absence of acquired anti-microbial resistance genes

The Pariza et al. (2015) decision tree and the European Food Safety Authority (EFSA Panel on Additives and Products or Substances used in Animal Feed (Gueimonde M. 2010) recommend that microbial strains used in food applications must not harbor acquired antimicrobial resistance genes to clinically relevant antimicrobials. Analysis of the UABla-12™ antimicrobial susceptibility phenotype indicates that UABla-12™ does

not express acquired antimicrobial resistance factors to known antibiotics of clinical significance with the exception of Tetracycline (Table 1).

In order to assess antibiotic resistance of *B. animalis* ssp*. lactis*, a Minimum Inhibitory Concentration (MIC) analysis was performed applying the ISO10932/IDF223 (https://www.iso.org/standard/46434.html). The strain was analyzed against relevant antibiotics according to EFSA guidelines (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP 2012), including Ampicillin, Vancomycin, Gentamicin, Kanamycin, Streptomycin, Erythromycin, Clindamycin, Tetracycline and Chloramphenicol. strain UABla-12™ was determined to be sensitive to all relevant tested antibiotics according to EFSA guidelines (EFSA Journal 2012), with MIC values at or below the reported species characteristics (cut-off values) for all antibiotics tested with the exception of Tetracycline.

Table 1. Antimicrobial Resistance (Rep A and Rep B)

Antibiotic Tested	EFSA cut-off value (mg/L)	MIC Value (mg/L) Rep-A	MIC Value (mg/L) Rep-B	Resistance Profile
Ampicillin	2	0.125	0.25	Sensitive
Vancomycin	2	0.5	0.5	Sensitive
Gentamicin	64	64	64	Sensitive
Streptomycin	128	64	64	Sensitive
Erythromycin	1	0.125	0.25	Sensitive
Clindamycin	1	<0.032	<0.032	Sensitive
Tetracycline	8	32	32	Resistent

As *B. animalis* ssp*. lactis* does not show any sign of acquired antibiotic resistance, i.e. MIC values over the threshold cut-off value, with the exception of resistance to Kanamycin, UAS Labs investigated the origin of the resistance at the genetic level to assess the potential for horizontal gene transfer.

The identification of tetracycline resistance can be explained by the presence of tetW (COAIJIPA_00636, tetracycline resistance protein TetW). Tetracycline resistance is well conserved across a range of *Bifidobacterium* strains, including GRAS ingredient *B.*

animalis subsp. *lactis* BB-12 (GRN 49) (Gueimonde M. 2010) (Delcour J. 1999) and has previously been shown to correlate directly with the presence of tetW (Delcour J. 1999). Analysis of the UABla-12™ genome indicates that the tetW gene in is located in a highly-conserved region of the genome and is unlikely to be involved in horizontal gene transfer.

2.1.4 Absence of genetic determinants for biogenic amine production

Many lactic acid bacteria exhibit amino acid decarboxylase activity. Histamine, tyramine, putrescine and cadaverine are generated by decarboxylation of histidine, tyrosine, ornithine and lysine, respectively (Landete JM 2007) (Romano A 2013) (Diaz M 2015) (Gardini F 2016). Moreover, the deimination of agmatine can also form putrescine via N-carbymoyl putrescine (Coton M 2010). Reports of toxicity from the consumption of biogenic amines are rare, and when they occur are usually associated with histamine, and to a lesser extent tyramine exposure. It should be emphasized however, that exposure to these compounds is expected on a daily basis as the gastrointestinal tract contains numerous microorganisms with active amine degradation enzymatic capacity, and the presence of biogenic amines in wine, cider, cheeses, and cured meats due to the presence of lactic acid fermenting bacteria is common (Landete JM 2007) (Ferreira IMPLVO 2006) (Garai G 2006) (Suzzi G 2003).

An *in-silico* analysis was performed to identify possible genetic determinants for the synthesis of biogenic amines within the genome of *B. lactis* UABla-12™. The bioinformatics analysis was adapted from a PCR based genetic screen reported by Li et al. (2018). The nucleotide sequence of biogenic amine related genes, from the NCBI database, were aligned against the genome of *B. lactis* UABla-12™ using BLASTx. No relevant matches were detected within the *B. lactis* UABla-12™ genome for histidine decarboxylase, tyrosine decarboxylase, lysine decarboxylase, ornithine decarboxylase or agmatine deiminase, which are involved in the generation of histamine, tyramine, cadaverine, putrescine or N-carbamoyl putrescine, respectively. (Appendix 5).

2.2 Intended Use

B. animalis ssp. *lactis* UABla-12™1 is intended to be used as an ingredient in conventional foods that are compatible with the addition of live, safe and suitable food microbial cultures including but not limited to dairy products, beverages, nutritional powders, juices, bars, confections, and cereals at levels of 10^9 to 10^{11} per serving of food products intended for children and adults with the exception of infant formula.

2.3 Manufacturing; production and release specifications

The UASLabs facility at 4375 Duraform Lane, Windsor, Wisconsin 53598 USA is a FDA-regulated and inspected purpose-built food microorganism production facility including seed preparation, fermentation, and post-fermentation processing

capabilities. All raw materials used in the production of *B. animalis* ssp. *lactis* UABla-12™ are safe and suitable GRAS or approved food additives for the use and all product contact surfaces are approved for food contact use.

All UASLabs microbial products are manufactured in accordance with current Good Manufacturing Practices as specified in 21CFR110, 21CFR111, and 21CFR117.

Fig. 2 and Table 2. provide a description of the production process. Included are references to critical control points identified by UASLabs.

Fig. 2. Production flow diagram

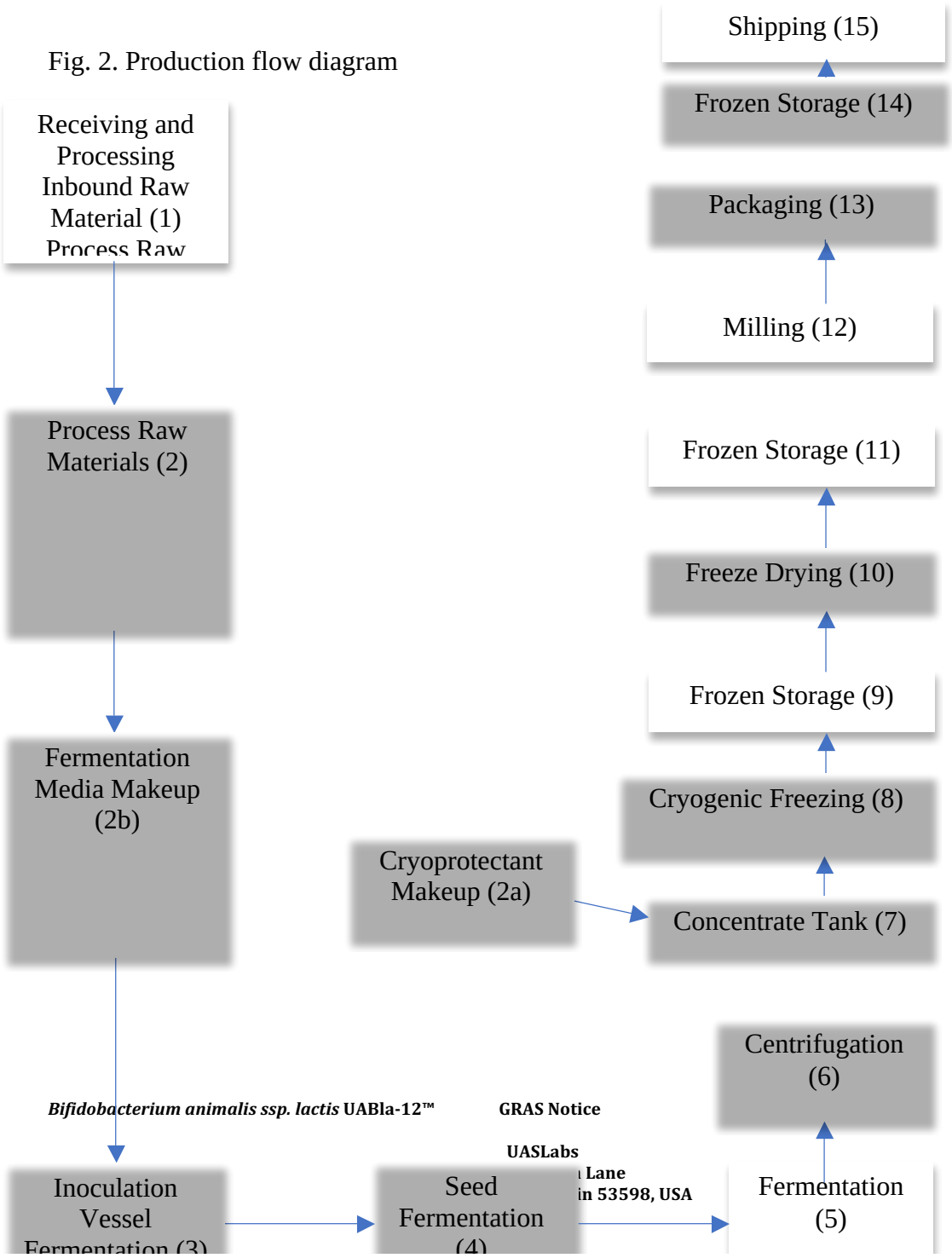


Table 2. Production Process Description

Production Process Step Name	Step Number	Description of Process and Controls
Processing Incoming Raw Material	1	Required documents and forms are completed for all materials received into the facility. All materials are reconciled, and documents are completed before the raw materials can be accepted into the system
Process Raw Materials	2	Materials utilized in production processes are weighed and stored as appropriate
Cryoprotectant Makeup	2a	Cryoprotectant is used to protect the organisms during any freezing steps
Media Makeup	2b	Media is a nutritional source for the organisms during the three stages of fermentation
10L Seed Can Fermentation	3	Inoculation of the bacteria into a 10L canister that contains nutritional media using a frozen culture. This process takes place in the controlled seed room located within the production laboratory.
400L Fermentation	4	The product is moved through the closed system into a larger 400L vessel that contains a new nutritional media
20,000L Fermentation	5	The product is moved through the closed system into a larger 20,000L vessel that contains a new nutritional media
Centrifuge	6	Product is moved into the centrifuge where the heavy phase and light phase are separated out and the desired portion is moved into the concentrate tank for further processing
Concentrate (Add Cryoprotectant 2a)	7	After the centrifuge process, the cryoprotectant that was made in step 5a is added into the concentrate tank to mix
Cryogenic Processing	8	The stabilized product is sent through the cryogenic processing unit in a solid state
Storage in -60˚C Freezer	9	The frozen pellets are stored in a -60˚C freezer until they are released by Quality Control to move on to the next step
Lyophilization	10	The pellets are processed through Lyophilization to remove excess water in the product
Storage in -20˚C Freezer	11	The Lyophilized pellets are stored in a -20˚C freezer until they are released by Quality Control to move on to the next step
Milling	12	The pellets are milled to a powder
Packaging	13	Packaging schedules determine the size and type of package that will be used. An in-line metal detector is utilized prior to packaging the product.
Storage in -20˚C Finished Goods Freezer	14	Finished product is stored in a -20˚C freezer, under quarantine, until it is released by Quality Control after which it may be made available for distribution
Outbound Product	15	Per shipping schedules, finished product is staged and a corresponding COA will be generated for the customer.

The production source organism employed is *B. animalis* ssp*. lactis* UABla-12™. Stock seed cultures are produced and maintained in the secure culture bank of UASLabs as frozen 1m. vials at -80^{0}C under the control of the Quality function. UASLabs verifies the identity of the seed by genetic analysis and purity of each seed culture lot.

The product is manufactured through a specific time and temperature controlled fermentation of safe and suitable food grade ingredients (either GRAS ingredients or approved food additives) inoculated with UABla-12™. The food grade ingredients are used in the fermentation for the sole purpose as substrates for growth of UABla-12™ and no chemical alteration of the resulting fermented product is intended or expected.

Table 3. UASLabs *B. animalis ssp. lactis* UABla-12™ manufacturing specification M-SPEC-60003

UASLabs Testing Requirements		
Parameter	**Specification**	**Standard Test Procedure**
Physical Testing		
Color	Off-White – Cream	Visual
Appearance	Powder	Visual
Identification		
Identification	*B. animalis ssp. lactis*	SOP-J61 or 16s rRNA Gene Sequence
Purity Testing		
Non Lactics	< 5,000 CFU/g	Doc# TBD or ISO 13559
Potency/Strength		
Total Viable Cell Count	NLT 200 Billion CFU/g	M-SOP-Q13 or ISO 7889/IDF 117
Microbial Testing		
Escherichia coli	Negative by Test	TBD or USP <2022> or AOAC
Staphylococcus aureus	Negative by Test	TBD or USP <2022> or AOAC
Salmonella	Negative by Test	TBD or USP <2022> or AOAC
Enterococcus	<100/g	M-SOPC-Q3 or CMMEF or SMEDP
Listeria	Negative in 25g	AOAC (VIDAS at Silliker)

Table 3. above lists the Quality Control specifications that must be satisfied for use of seed cultures and release of final products.

21CFR570.235; Part 3: Dietary Exposure

The strain *B. animalis ssp. lactis* UABla-12™ is envisioned for use as food ingredient in conventional foods, at levels of 10^9-10^{11} CFU per food serving. These levels are consistent with common and usual practice in the US marketplace and supported by citations in GRNs 445 ($2x10^9$ to $2x10^{11}$ CFU/250 gram serving) and 49 (10^6 to 10^{11} CFU /day fed to infants in clinical trials) (incorporated herein by reference) and in Section 6.3 below.

As the proposed usage of strain UABla-12™ is in keeping with current levels in the market and it is envisioned that the strain will be used in the same food categories as other *B. animalis ssp. lactis* strains already present in the market, the use is not expected to materially increase the dietary exposure of consumers to *B. animalis ssp. lactis* microbial food cultures.

21CFR570.240; Part 4: Self-limiting levels of use

The addition of *B. animalis ssp. lactis* UABla-12™ in foods is limited to those foods that will sustain the live culture through the shelf-life of the product. The inclusion rate of the strain is limited by the upper limit of fermentation and drying technology to produce cell concentrates.

21CFR570.245; Part 5: Experience based on common use in food before 1958

UASLabs is not aware of a common use of *B. animalis ssp. lactis* UABla-12™ in food before 1958. Therefore Part 5 does not apply to this GRAS Notice.

UASLabs
4375 Duraform Lane
Windsor, Wisconsin 53598, USA

21CFR570.250; Part 6: Safety Narrative

6.1 Background

UASLabs has undertaken a comprehensive safety determination for the use of *B. animalis* ssp. *lactis* UABla-12™ as a food ingredient for the US market. In this safety review, UASLabs has consulted articles in peer-reviewed scientific journals, governmental reviews and product approvals, in-house safety studies and the peer-reviewed Pariza, et al. (Pariza 2015) safety determination process to provide reasonable assurance that the ingredient does not present a significant or unreasonable risk (Arndt 2016) (Chen L. H. 2016) (Dobson A.E. 2007) (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP 2012) (Morton R. A. 2007) (Walker B. J. 2014) (Zhou 2011)U.S.C 342(f)(1)(B)). UASLabs has determined by scientific procedures that UABla-12™ is GRAS for use in conventional foods under the conditions of use described herein.

It is important to note at the outset that the human gut is essentially an open ecosystem containing a diverse microbial community and that in addition ingestion of microorganisms is the norm. The fully populated gut microbiome contains approximately 10^{14} organisms consisting of bacteria, fungi, and archea living in intimate contact with the host. This diverse population appears to play a key role in shaping human physiology and maintaining homeostasis of the gut and immune systems. Because the human gut is an open system, numerous opportunities are available for the introduction of microorganisms both intentional and accidental. *B. animalis* ssp. *lactis* is autochthonous resident of the human gut and can be considered to be part of the core microbial community in man.

There are numerous sources of microorganisms for inclusion in this community, including environmental and food. Quantitatively, fermented foods and increasingly probiotics perhaps are the most important source of organisms being introduced into the gut ecosystem (Derrien 2015). Fermented foods and beverages are estimated to make up approximately one-third of the human diet and are the major sources of those environmental bacteria that enter the GI tract. Lactic Acid Bacteria (primarily lactococci, lactobacilli, bifidobacteria, and propionibacteria make up a large proportion of ingested bacteria via fermented foods and probiotics. Such foods are estimaed to comprise approximately 30% of the human diet (Campbell-Platt 1997).

In addition to the common consumption of food fermentation microorganisms as part of traditional fermented foods, an additional 3.9 million adults and nearly 300,000 children (ages 4-17) in the US routinely and safely consume probiotic products. (http://nccih.nih.gov/research/statistics/NHIS/2012/natural-products/biotics).

The International Dairy Federation (IDF) has compiled a list organisms with a documented safe history of use in food (Morgensen 2002) (Bourdichon 2012) (Appendix 3) and the list is the only existing authoritative compendium documenting such uses. The source of these organisms in food can be from addition of starter cultures or from autochthonous organisms present on food raw materials. In either case, the organisms must be characterizing and not merely incidental components of the food microflora to be included in the IDF list. Thus, members of the IDF list qualify as "articles of food" for the purposes of regulation of dietary ingredients under the US Food, Drug and Cosmetic act. In addition to the widespread safe history of use in foods, clinical trials (see Section 6.3) have demonstrated the safety of dose from 10^9-10^{11} CFU per day.

In addition to a safe history of use in foods, including dietary supplements, regulatory agencies world-wide have evaluated the safety of LAB and other organisms in the food supply. The European Food Safety Authority has critically evaluated the components of the IDF list, including *B. animalis* ssp. *lactis* as well as other organisms and developed a list of organisms that have a Qualified Presumption of Safety. Uses of any of the organisms on the list as food ingredients in the EU do not require premarket approval from EFSA for use in food in the European Union. (EFSA; Appendix 2).

In addition to the documented safe history of use and EFSA evaluation, Lactobacilli and Bifidobacteria have been reviewed from scientific perspective and found to pose no additional risks that that posed by the commensal community into which they are introduced (Borriello SP 2003).

6.2 Regulatory History

There is virtually universal consensus that all members or strains of the *B. animalis* ssp. *lactis* species are safe and suitable for use in food and supplements.

B. animalis ssp*. lactis* is listed on both the IDF list and the EFSA QPS and is a prominent part of the food and supplement sources of ingested microorganisms. Further, *B. animalis* ssp*. lactis* is allowed in dairy products in the US under US FDA prior sanction, wherein the Agency approves all members of safe and suitable LAB species for use in the listed dairy products and is perhaps the most widely used "probiotic" in fermented milks in the US and Europe (Barangou 2009). FDA has also reviewed numerous GRAS Notices for uses of *B. animalis* ssp*. lactis* strains in various foods, supplements and antimicrobial preparations, incorporated herein by reference (GRN Nos: 445, 377, 49); https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices; last accessed April 10, 2018), wherein FDA has had "no comment" on the notifier's conclusion the organisms are GRAS under the conditions of use.

All members of the *B. animalis* ssp*. lactis* species, including those marketed as probiotics, are allowed for use in food in Canada, e.g. yogurt, without pre-market notification. In addition, where such probiotic cultures are contained in products that have a therapeutic use, Health Canada has approved numerous *B. animalis* ssp*. lactis* - containing therapeutic products as Licensed Natural Health Products and issued product licenses accordingly. Such products "have been assessed by Health Canada and found to be safe, effective and of high quality under their recommended conditions of use (https://health-products.canada.ca/lnhpd-bdpsnh/index-eng.jsp; last accessed February 10, 2018).

In addition, all members of the *B. animalis* ssp*. lactis* species are considered traditional foods because of its "long history of use in yogurt and fermented milk products in Australia and New Zealand and are also used in complimentary medicines under the Australia Department of Health Therapeutic Goods Administration authority (http://www.foodstandards.gov.au/industry/novel/novelrecs/Documents/Novel%20Foods%20-%20Record%20of%20views%20Jan%202018%20Update.pdf; https://search.tga.gov.au/s/search.html?collection=tga-websites-web&query=lactobacillus+acidophilus&op=Search; accessed February 24, 2018;).

6.3 Clinical Trials

Results of clinical studies reinforce the overwhelming evidence of safety of the organisms on the IDF and QPS lists. The findings of safety in clinical trials involving premature infants for the prevention of necrotizing colitis demonstrate the safety in the most sensitive of at-risk populations (AlFaleh 2012). While, the use intended for B. *animalis* subsp. *lactis* UABla-12™ does not include such at-risk populations it is instructive that no product related adverse effects have been reported in clinical trials involving these populations (Sanders 2016).

Moreover, numerous *B. lactis* UABla-12™ human feeding trials in adults and children while not standard Phase I safety studies at dosages in the range of those proposed in Section 2.2 (above) have not noted any significant adverse effects on study participants (S. V. Gerasimov 2010) (S. V. Gerasimov 2016) (Naglaa 2011), which supports the level of use of 10^9 - 10^{11} CFU/dose.

1. Gerasimov 2010
    a. Dosage: 5 x 10^9 CFU (*B. lactis* UABla-12™ + *L. acidophilus* combined) twice daily (1 x 10^{10} CFU total)
    b. Demographic group: Children aged 1-3 years
2. Gerasimov 2016
    a. Dosage: 5 x 10^9 CFU (*B. lactis* UABla-12™ + *L. acidophilus* combined) once daily
    b. Demographic group: Children aged 3-12 years
3. Nagala 2011

a. Dosage: 1.2 x 10^{10} CFU (*B. lactis* UABla-12™ + 3 other strains combined) twice daily (2.4 x 10^{10} CFU total) followed by once daily (1.2 x 10^{10} CFU total)
b. Demographic group: Adults with symptoms of IBS aged 25-86 years

In addition to *B. lactis* UABla-12™™ strain specific studies, additional evidence is available that supports the probiotic safety of the *B. lactis* subspecies in various age groups, including infants, children and adults.

1. Ahmed et al. 2007
   a. Dosage: Three different doses [5 x 10^9 CFU/day (high), 1.0 x 10^9 CFU/day (medium) and 6.5 x 10^7 CFU/day (low)]
   b. Demographic group: Elderly human subjects (over 60 years old)

2. Bartosch et al. 2005.
   a. Dosage: 3.5 x 10^{10} CFU (B. lactis) + 3.5 x 10^{10} CFU (B. bifidum)
   b. Demographic group: Healthy elderly volunteers (over 62 years old)

3. Engelbrektson et al. 2009
   a. Dosage: 1 x 10^{10} CFU (B. lactis) + several other species at various doses
   b. Demographic group: Healthy adults, males and females, mean age of 37 yrs.

4. Gill et al. 2001
   a. Dosage: 5 x 10^9 CFU/day
   b. Demographic group: Elderly subjects

5. Gopal et al. 2003
   a. Dosage: 3 x 10^{10} CFU (*B. lactis*)
   b. Demographic group: Healthy adults, males and females, 20-60 years old

6. Kim et al. 2010
   a. Dosage: 1.6 x 10^9 CFU (*B. lactis*) + *B. bifidum* + *L. acidophilus*
   b. Demographic group: Pregnant women and infants aged 4-6 months

7. Langhendries et al. 1995
   a. Dosage: 10^6 CFU/g powder (Bifidobacteria)
   b. Demographic group: Full-term infants aged 0 – 2 months

8. Larsen et al. 2006
   a. Dosage: One of 4 doses: 10^8 – 10^{11} CFU (*B. lactis* and *L. paracasei* combined)

b. Demographic group: Healthy young adults (18 – 40 years)

9. Prescott et al. 2008)
   a. Dosage: 9 x 10^9 CFU (*B. lactis*)
   b. Demographic group: Pregnant and lactating women

10. Sazawal et al. 2004
   a. Dosage: 9.6 x 10^6 CFU (*B. lactis*)
   b. Demographic group: Children aged 1-3 years

11. Singh et al. 2013
   a. Dosage: 4 x 10^9 CFU (B. lactis)
   b. Demographic group: Adults aged 20-65 years with seasonal allergic rhinitis

12. Sullivan et al. 2003 (L. e. al. 1995) (L. et 1995)
   a. Dosage: 2.5 x 10^{10} CFU (B. lactis and 2 other strains combined)
   b. Demographic group: Adults aged 21-48.

6.4 Strain Identity

Exhaustive identification processes based upon PCR ribotyping and whole genome sequencing demonstrate that UABla-12™ is typical of the *B. animalis* ssp*. lactis* isolates currently in the global marketplace and conforms to the recommendations of the US FDA (Early Clinical Trials with Live Bio therapeutic Products: Chemistry, Manufacturing, and Control Information: Guidance for Industry June 2016; https://www.fda.gov/downloads/BiologicsBloodVaccines/Guidance-ComplianceRegulatoryInformation/Guidances/General/UCM292704.pdf; accessed February 24, 2018), EFSA Guidance (EFSA Journal 2012; https://www.efsa.europa.eu/en/corporate/pub/ejcompendium2012; accessed February 24, 2018) for probiotic strains development, and manufacturing, the Pariza et al. (Pariza 2015) decision tree and a probiotics industry recommendations (Sanders ME 2010) for the characterization of microbial food culture strains, including probiotics.

This confirmation of UABla-12™ as a strain of the *B. animalis* ssp*. lactis* species is important because the equivalence ties UABla-12™ directly to the safe history of use, QPS status, prior sanction and other competent authority approvals for *B. animalis* ssp*. lactis.*

6.5 Absence of virulence factors and acquired antibiotic resistance genes

Both EFSA and Pariza et al. recommend that new isolates of safe and suitable genus/species be tested for genes encoding sequences of concern for human health.

Based on whole genome sequence analysis, UABla-12™ is free of sequences that encode for known food toxins and virulence factors.

In addition, antimicrobial sensitivity phenotype analysis indicates that UABla-12™ is not resistant to clinically relevant antibiotics above established cut off levels except in the case of Tetracycline resistance. The identification of tetracycline resistance can be explained by the presence of tetW (COAIJIPA_00636, tetracycline resistance protein TetW). Tetracycline resistance is well conserved across a range of *Bifidobacterium* strains, including GRAS ingredient *B. animalis* subsp. *lactis* BB-12 (GRN 49) (Gueimonde et al. 2010) and has previously been shown to correlate directly with the presence of tetW (Delcour et al. 1999).

All *B. animalis* ssp. *lactis* strains that have been the subject of GRAS Notices to date are resistant to Tetracycline at levels similar to UABla-12™ and the lack of prophage and extra-chromosomal elements in its genome and the location of tetW in a highly conserved region of the UABla-12™ genome indicate the low risk of the strain participating in horizontal transmission of such factors. Thus UABla-12™ is shown to be free of identified risk factors to human health.

6.6 Manufacturing

Finally, *B. animalis* ssp. *lactis* UABla-12™ is produced in a purpose-built, state-of-the-art microbial food culture production facility in accordance with the appropriate food and dietary supplement cGMPs. All raw materials used in the fermentation and production processes are GRAS or approved food additives.

The risk of product contamination is minimized at each step of the process. Seed preparations are prepared in clean rooms under HEPA filtered air. The fermentation process is completely closed in a CIP-prepared process of inoculation, fermentation, harvest. All post-harvest steps are done in clean rooms under HEPA filtered air, positive pressure and limited access.

6.7 Conclusion of the Pariza et al. Decision Tree

In summary, UASLabs has followed rigorously the Pariza et al. safety decision tree and employs a safe and suitable production organism, cGMP-compliant processes and food safety systems to insure the safety of the final *B. animalis* ssp. *lactis* product. The safety determination process is summarized in Appendix 1 including the final decision tree answers leading to the conclusion that *B. animalis* ssp. *lactis* UABla-12™ is safe and suitable for use in food and dietary supplements.

In addition, UASLabs has determined, by scientific procedures and based upon the publically available evidence reviewed in detail above that *B. animalis* ssp. *lactis* UABla-12™ is Generally Recognized as Safe (GRAS) in accordance with 21 CFR 170.30 and is

exempt thereby from pre-market approval requirements of the Food, Drug and Cosmetic Act.

21CFR570.255; Part 7: List of supporting data and information

7.1 Regulations, competent authority guidance and evaluations
All regulatory history information is publicly available and references are provided.

7.2. Scientific literature
All scientific journal articles are publicly available and references are provided

7.3 History of safe use
The safe history of use is documented in publicly available information and references are provided.

7.4 Clinical Data
All clinical data used in making a conclusion of GRAS status have been published and references are provided.

Appendix 1. Strain Safety Decision Tree[1]

Decision Tree Question	Response
1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? [ii] (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding).	YES
2. Has the strain genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.) [iii]	YES
3. Is the strain genome free of genetic elements [iv] encoding virulence factors [v] and/or toxins [v] associated with pathogenicity? [vi] (If YES, go to 4. If NO, go to 15.)	YES
4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? [vii] (If YES, go to 5. If NO, go to 15.)	YES
5. Does the strain produce antimicrobial substances? [viii] (If NO, go to 6. If YES, go to 15.)	NO
6. Has the strain been genetically modified using rDNA techniques? (If YES, go to 7a or 7b. If NO, go to 8a or 8b.)	NO
7a. For strains to be used in human food [ix]: Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in food? (If YES, go to 8a. If NO, the expressed product(s) must be shown to be safe before proceeding to 8a.)[s]	N/A
7b. For strains to be used in animal feed [ix]: Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in feed for the target animal species? (If YES, go to 8b. If NO, the expressed product(s) must be shown to be safe for the target animal species before proceeding to 8b.) [x]	N/A
8a. For strains to be used in human food: 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 [xi] and characterizing [xii] component (not simply an 'incidental isolate')? (If YES, go to 9a. If NO, go to 13a.) [xiii]	YES
8b. For strains to be used in animal feeds: Was the strain isolated from a feed (for example, silage) that has a history of safe consumption by target animals, for which the species, to which the strain belongs, is a substantial [xi] and characterizing [xii] component (not simply an 'incidental isolate')? (If YES, go to 9b. If NO, go to 13b.) [xiv]	N/A
9a. For strains to be used in human food: Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food use by an authoritative group of qualified scientific experts? [xv] (If YES, go to	YES

10a. If NO, go to 13a.)	
9b. For strains to be used in animal feeds: Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for feed use by an authoritative group of qualified scientific experts? [xvi] (If YES, go to 10b. If NO, go to 13b.)	N/A
10a. For strains to be used in human food: Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9a continue to support the conclusion that the species, to which the strain belongs, is safe for use in food? (If YES, go to 11a. If NO, go to 13a.)	YES
10b. For strains to be used in animal feeds: Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9b continue to support the conclusion that the species, to which the strain belongs, is safe for use in feed? (If YES, go to 11b. If NO, go to 13b.)	N/A
11a. For strains to be used in human food: Will the intended use of the strain expand exposure to the species beyond the group(s) that typically consume the species in "traditional" food(s) in which it is typically found (for example, will a strain that was isolated from a fermented food typically consumed by healthy adults be used in food intended for an 'at risk' group)? (If NO, go to 12a. If YES, go to 13a.)	NO
11b. For strains to be used in animal feeds: Will the intended use of the strain expand exposure to the species beyond the target animals that typically consume the species in "traditional" feed(s) in which it is typically found (for example, will a strain that was isolated from silage be used in swine feed)? (If NO, go to 12b. If YES, go to 13b.)	N/A
12a. For strains to be used in human food: Will the intended use of the strain expand intake of the species (for example, increasing the number of foods beyond the traditional foods in which the species typically found, or using the strain as a probiotic rather than as a fermented food starter culture, which may significantly increase the single dose and/or chronic exposure)? (If NO, go to 14a. If YES, go to 13a.)	NO
12b. For strains to be used in animal feeds: Will the intended use of the strain expand intake of the species (for example, increasing the number of feeds beyond the traditional feeds in which the species is typically found, or using the strain as a probiotic rather than as a silage starter culture)? (If NO, go to 14b. If YES, go to 13b.)	N/A
13a. For strains to be used in human food: Does the strain induce undesirable physiological effects in appropriately designed safety	NO

evaluation studies? [xvii] If yes, go to 15. If no, go to 14a.)	
13b. For strains to be used in animal feeds: Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? [xviii] If yes, go to 15. If no, go to 14b.)	N/A
14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.	YES
14b. The strain is deemed to be safe for use in the manufacture of feeds, probiotics, and dietary supplements for animal consumption.	
15. The strain is NOT APPROPRIATE for human or animal consumption [xix].	

[1] Pariza, M.W., Gillies, K. O., Krack-Ripple, S., Leyer, G., and Smith, A.B. "Determining the safety of microbial cultures for consumption by humans and animals." *Regulatory Toxicology and Pharmacology* 73 (2015): 164-171.

Appendix 2. QPS Evaluation of B. animalis ssp. lactis

The EFSA Journal (2007) 587, Qualified Presumption of Safety
http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2007.587
/epdf

APPENDIX A. Scientific report on the Assessment of Gram-Positive Non-Sporulating Bacteria

Many of the species included in this broad grouping are common constituents of the normal gut flora of humans and livestock although their occurance and numbers are host dependent. Additionally, species of Gram-positive bacteria constitute common components of the microbial community of food and, for their relevant role in food fermentation; these microorganisms have been deliberately introduced into food as starter cultures. In addition, several bacterial strains belonging to this group have a long history of apparent safe use as food starter cultures, feed additives (*e.g.* animal probiotics and silage inoculants) and source of additives (*e.g.* enzymes and amino acids). Based on their habitat and their extensive application in the food and feed sector, many species were judged potentially suitable for their safety to be assessed by the "Qualified Presumption of Safety" (QPS) methods, according to (EFSA 2005). The following genera, all belonging to the phylum *Firmicutes*, have been considered: *Bifidobacterium, Corynebacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Propionibacterium* and *Streptococcus.*

1 *Bifidobacterium*

Bifidobacteria are part of the normal gut microbiota of adults and are also one of the first genera to colonise the gut of infants. In addition, they are normal inhabitants of the gut of animals. A limited number of *Bifidobacterium* species have a history of use in dairy products, especially sour milk products like yoghurts and more recently yoghurt and fermented milk drinks.

1.1 Taxonomic unit defined

Bifidobacteria belong to the *Actinomycetes* branch of phylum Firmicutes. They are non-motile, non-sporeforming rods of variable appearance, usually curved and clubbed, and are often branched including Y and V forms. They are normally strictly anaerobic, although some species and strains tolerate oxygen. The type species is *Bifidobacterium bifidum*. Bifidobacteria are saccharolytic organisms and they have the ability to ferment glucose, galactose and fructose. Glucose is fermented via the fructose-6-phosphate shunt to acetic and lactic acid. Differences occur between species in their ability to ferment other carbohydrates and alcohols.

The genus consists currently of following species: *Bifidobacterium adolescentis, B. angulatum*, *B. animalis* subsp. *Animalis, B. animalis* subsp. *lactis*, *B. asteroides*, *B. bifidum*, *B. boum, B. breve, B. catenulatum, B. choerinum, B. coryneforme, B. cuniculi*, *B. dentium*, *B. gallicum*, *B. gallinarum*, *B. indicum*, *B. longum*, *B. magnum*, *B. merycicum*, *B. minimum, B. pseudocatenulatum*, *B. pseudolongum* subsp. *globosum*, *B. pseudolongum* subsp. **Appendix A - Assessment of gram-positive non-sporulating bacteria** *The EFSA Journal* (2007) 587, Qualified Presumption of Safety *B. pseudolongum*, *B. psychraerophilum*, *B. pullorum*, *B. ruminantium*, *B. saeculare*, *B. scardovii*, *B. subtile*, *B. thermacidophilum* subsp. *porcinum*, *B. thermacidophilum* subsp. *thermacidophilum*, *B. thermophilum* .

1.2 Is the body of knowledge sufficient?

The characteristics and habitat of the species of the genus *Bifidobacterium* are well known. The number of established or proposed species has increased only slightly during recent years.

Only a few species have a long history of use in industrial applications. Bifidobacteria are mainly exploited in dairy products like yogurts or yogurt drinks, but also a whole range of sour milk and other milk based products. Occasionally they are also used in feed in combination with other genera. In Europe only a few species are used (*B. animalis, B. longum, B. breve*, *B. bifidum* and *B. adolescentis,*) and often applied in combination with lactic acid bacteria (Reuter 1990; Reuter 1997; Klein, Pack *et al*. 1998; Reuter 2002). The genome sequences of *B. longum* (Schell, Karmirantzou *et al*. 2002) and *B. breve* have been determined, while the genome sequencing project of *B. adolescentis* is ongoing.

1.3 Are there safety concerns?

Humans. Safety concerns are so far related mainly only to one species, *B. dentium*, which has been associated with dental caries. It has also been isolated from a case of peritonsillar abscess together with other anaerobes (Civen, Vaisanen *et al*. 1993) and, under its previous designation "*Actinomyces eriksonii",* from pulmonary and subcutaneous abscesses (Slack 1974). Occasionally, other species have been reported to be isolated from human clinical cases, but none of them was the primary cause of disease. Only immunocompromised hosts were infected (Crociani, Biavati *et al*. 1996). These species are not used as food or feed supplements. None of the bifidobacteria used for industrial purposes have been associated with human clinical disease.

Although there are few studies on the antibiotic resistance of bifidobacteria strains, the presence of the acquired tetracycline resistance gene *tet*(W) has been reported in *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium bifidum* (Kastner, Perreten e*t al*. 2006; Masco, Van Hoorde *et al*. 2006).

Livestock. No report can be found on safety concerns related to *Bifidobacteria* in animals.

1.4 Can the safety concerns be excluded?

There are apparently no specific safety concerns regarding the genus *Bifidobacterium* (especially concerning *B. animalis; B. longum, B. breve, B. adolescentis,* and *B. bifidum*) with the exception of the species associated with dental caries, *B. dentium*. Susceptibility to antibiotics should be assessed as defined by the EFSA opinion (EFSA 2005) for each strain.

1.5 Units proposed for QPS status

Due to the long history of safe use of *B. adolescentis, B. animalis; B. longum, B. breve* and *B. bifidum*, these species are proposed for QPS status. Other species could be included subsequent to their industrial application with the exception of the species associated with dental caries (*B. dentium*).

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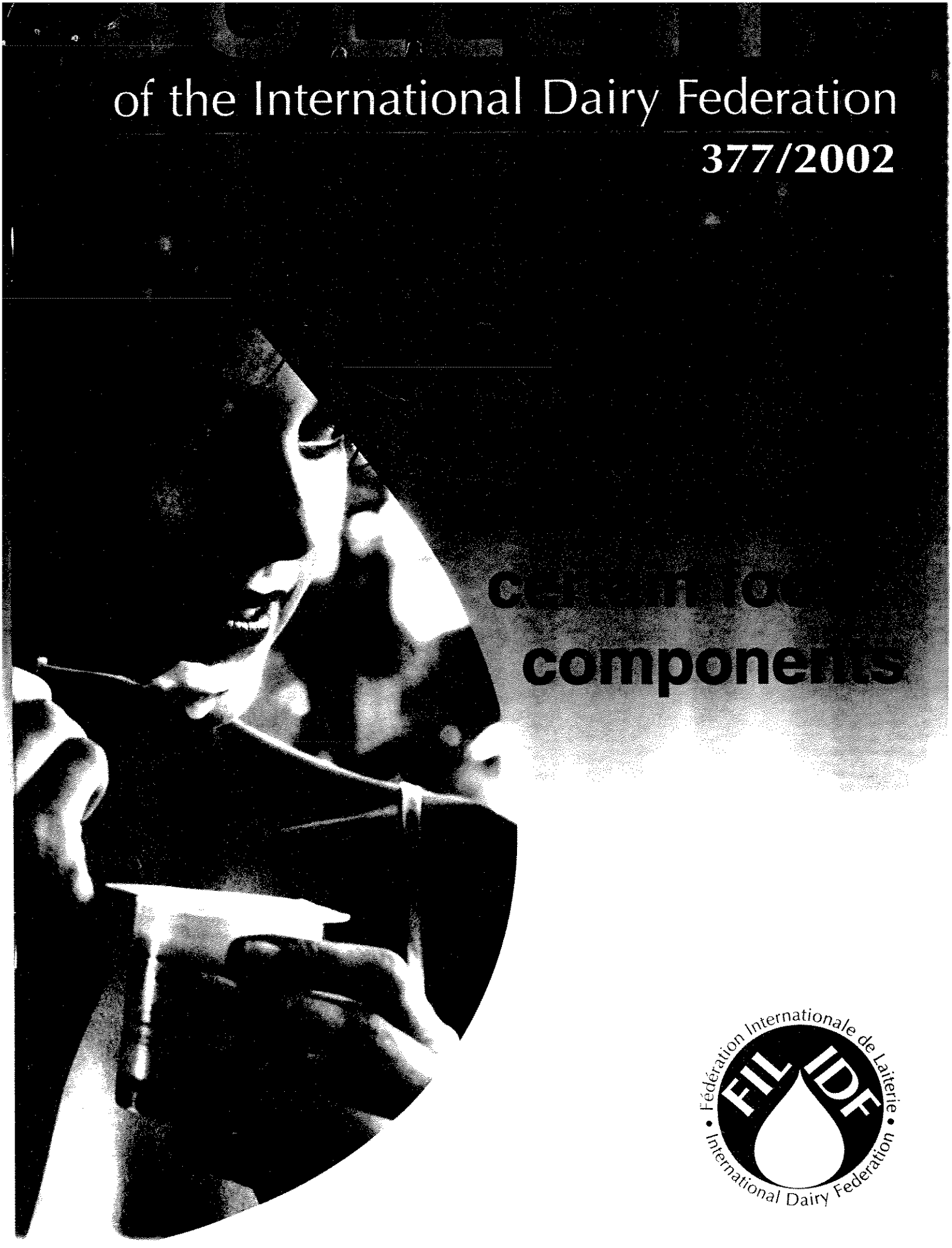
Appendix 3. IDF List

of the International Dairy Federation

377/2002



certain food components

Fédération Internationale de Laiterie
FIL IDF
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CONTENTS

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377/2002

Ten pages have been removed in accordance with copyright laws. The removed reference is:

Morgensen, G., Salminen, S., O'Brien, J., Ouwehand, A., Holzapfel, W., Shorit, C., Fonden, R., Miller, G.D., Donohue, D., Playne, M., Critterenden, R., Bianchi Salvadori, B., and Zink, R. 2002. "Inventory of Microorgnaisms with a Documented History of Use in Food." *Bulletin of the International Dairy Federation* 377.

Appendix 4. Genetic Identity test

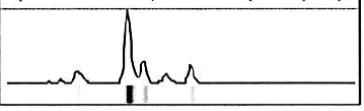
Genetic Identity Test - *B. animalis ssp. lactis*

UAS Labs incorporates genetic identity testing for each lot of bacterial raw material produced. Three primary technologies may be utilized. Descriptions of those technologies in relation to *Bifidobacterium lactis* UABla-12™ are below.

RiboPrinter® Microbial Characterization System

The RiboPrinter® system is an automated southern blotting (ribotyping) platform for microbial identification and characterization. It automates restriction fragment length polymorphism (RFLP) analysis and targets the RNA-coding region of the bacterial genome. Restriction enzymes, such as *Eco*RI or *Pvu*II, cut bacterial DNA into fragments that are then separated via gel electrophoresis, and hybridized with labeled DNA probes derived from regions encoding the 5S, 16S and 23S sequences, as well as the spacer regions and flanking genes on either side processed to form a characteristic banding pattern or "fingerprint." The system captures an image of the banding pattern and digitizes it as a RiboPrint™ pattern. This pattern can then be compared to a reference database of over 1,700 microbial species patterns from historic samples. This rich depth of information is what allows highly precise differentiation among strains of the same species, even those with the same 16S sequence.

Riboprint pattern from master seed vial of *Bifidobacterium lactis* UABla-12™

	RiboGroup	Number	Label	Custom ID Label	DuPont ID Label	RiboPrint™ Pattern
						1 kbp 5 10 15 50
1	ECORI 434-412-S-4	434-637-S-5	B. lactis Master Seed	Bifidobacterium lactis	Bifidobacterium animalis	

16s rDNA Sequencing

16S ribosomal RNA (rRNA) sequencing is a common and well-established amplicon sequencing method used to identify bacteria using a highly-conserved region at the species level. The output of the 500 basepair 16S rRNA gene sequencing is an alignment report, whereby genetic relationships are expressed as the percentage of positions that differ when two sequences are aligned and compared against validated libraries hosted at a third-party contract laboratory (Sherlock DNA software – MIDI Labs).

16s sequence analysis from master seed vial of *Bifidobacterium lactis* UABla-12™

MIDI LABS

Alignment Report - 500BP Identification

Customer: Allen , Shara
Company: UAS Labs
Address: 555 72nd Ave, Wausau, WI 54401 USA

125 SANDY DRIVE▪NEWARK, DE 19713▪PH 302-737-4297▪FX 302-737-7781▪WWW.MIDILABS.COM

D16M3 Library Revision: 3.12

Created: 2/14/2018 9:36:08 AM
Sample ID: C1802091244-B. lactis 180131-seed

16S DNA: 520 base pairs

D16M3 DNA Match Report

Match	%Diff	Length	Library Entry Name
1	0.00	520	Bifidobacterium-animalis-lactis
2	1.72	523	Bifidobacterium-animalis-animalis
3	4.04	520	Bifidobacterium-pseudolongum-pseudolongum-
4	4.22	521	Bifidobacterium-pseudolongum-globosum-T
5	4.58	521	Bifidobacterium-choerinum
6	5.93	521	Bifidobacterium-gallicum-T
7	7.85	510	Bifidobacterium-boum
8	8.32	511	Bifidobacterium-breve
9	8.62	509	Bifidobacterium-thermophilum-R1425
10	8.80	511	Bifidobacterium-thermacidophilum-thermacid

Exact match with Bifidobacterium-animalis-lactis

PCR Amplicon Analysis

The polymerase chain reaction (PCR) process is an *in vitro* technique that amplifies a targeted segment of the DNA template. A typical reaction requires DNA template, two specific oligonucleotide primers, and a reaction mixture that includes deoxynucleotide triphosphates (dNTPs), a thermostable DNA polymerase, a reaction buffer, and a fluorescent nucleotide stain. The reaction is placed in a thermocycler that cycles through a series of timed temperature set points that allow amplification of the targeted sequence (amplicon). The reaction is separated via gel electrophoresis and visualized with UV illumination, allowing determination of amplicon size. The specificity of the reaction is based on the specificity of the oligonucleotide primers. These can be potentially strain-specific based on genome knowledge and the uniqueness of the region being amplified. The absence of an amplicon of the proper size is confirmed with proper controls. The specificity of the PCR reaction lies within the primer design and optimization of the temperatures and times of the PCR run.

PCR gel from master seed vial *of Bifidobacterium lactis* UABla-12™

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Appendix 5. Biogenic Amine Analysis

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microbion

OPEN INNOVATION IN AGRI-FOOD MICROBIOLOGY

in-silico analysis for detection of Biogenic Amines biosynthesis related genes and multi-copper oxidase coding gene

P18117UL

Scope of the project

AIM

The aim of the project is detect the genetic determinant for the synthesis of Biogenic Amine (BA) screening the genomes data obtained during former projects.

This *in-silico* analysis ought to replace and improve the PCR based methods (Li *et al.*, 2018).

UASLabs strains subject of this analysis

Strain	Description
MB34	P17025.UL_01 (DDS®-1)
MB35	P17025.UL_02 (UALp-05)
MB36	P17025.UL_03 (NCIMB 30242)
MB37	P17025.UL_04 (ATCC 53103)
MB38	P17025.UL_05 (UABla-12)
MB39	P17145.UL_01 (BRN17)
MB40	P17145.UL_02 (M5-26-17 - UALC-03)
MB41	P17145.UL_03 (Lpc06 M 2-16-17 - UALpc-04)
MB42	P17145.UL_04 (BL077 - UABL-14)
MB43	P17145.UL_05 (1_6-16-17)
MB48	P18042UL_02

Li L, Wen X, Wen Z, Chen S, Wang L, Wei X. (2018). Evaluation of the biogenic amines formation and degradation abilities of *Lactobacillus curvatus* from chinese bacon. Front Microbiol. 2018 May 15;9:1015.

Summary of results

The «query» sequences to be used for BLAST analysis

Gene		Source	Length (nt)	Gene Bank Acc. N°	Prot. Length (aa)
Histidine Dec.	***hdcA***	*Lactobacillus buchneri*	951	AJ749838.1	317
Tyrosine Dec.	***tyrDC***	*Lactobacillus curvatus*	1866	MF537630.1	622
Lysine Dec.	***ldc***	*Lactobacillus saerimneri* 30A	2181	ANAG01000014.1	727
Ornithine Dec.	***odc***	*Lactobacillus acidophilus*	2091	AY542890.1	697
Agmatine Deim.	***aguA***	*Lactobacillus brevis*	1095	AF446085.5	365
	aguD	*Lactobacillus brevis*	1389	AF446085.5	463
	sufI	*Lactobacillus paracasei* strain CB9CT	1530	KU962939.1	510

Biogenic Amines: summary of results

This table present summary of results obtained screening the genomes to identify gene with relevant homology with genes sequences detected in biogenic amine producing *Lactobacillus* species (sequence query listed in the previous slide).

We used BlastX that compare translated nucleotide sequences from the genomes with aminoacidic sequences of the probe genes. We considered relevant only above 30% of identity.

We did not detect relevant matches for Histidine decarboxylase, Tyrosine decarboxylase nor Lysine decarboxylase. Ornithine decarboxylase instead yield relevant pairing within the following genomes: MB34, MB37, MB39, MB40, MB41 and MB48.

Results of BlastX Analysis

Strain ID	Description	Histidine Dec.	Tyrosine Dec.	Lysine Dec.	Ornithine Dec.		Agmatine Deim.
						Identity	
MB34	P17025.UL_01 (DDS®-1)	bt	bt	bt	relevant	100% (697/697)	bt
MB35	P17025.UL_02 (UALp-05)	bt	bt	bt	bt	bt	bt
MB36	P17025.UL_03 (NCIMB 30242)	bt	bt	bt	bt	bt	bt
MB37	P17025.UL_04 (ATCC 53103)	bt	bt	bt	relevant	44.33% (301/679)	bt
MB38	P17025.UL_05 (UABla-12)	bt	bt	bt	bt	bt	bt
MB39	P17145.UL_01 (BRN17)	bt	bt	bt	relevant	73.07% (510/698)	bt
MB40	P17145.UL_02 (M5-26-17 - UALC-03)	bt	bt	bt	relevant	45.44% (284/625)	bt
MB41	P17145.UL_03 (Lpc06 M 2-16-17 - UALpc-04)	bt	bt	bt	relevant	45.92% (276/601)	bt
MB42	P17145.UL_04 (BL077 - UABL-14)	bt	bt	bt	bt	bt	bt
MB43	P17145.UL_05 (1_6-16-17)	bt	bt	bt	bt	bt	bt
MB48	P18042UL_02	bt	bt	bt	relevant	46.09% (277/601)	bt

bt: below threshold

Multi-copper oxidase: summary of results

This table present summary of results obtained screening the genomes to identify gene with relevant homology with genes sequences detected multi-copper oxidase (query listed in the previous slide).

We used BlastX that compare translated nucleotide sequences from the genomes with aminoacidic sequences of the probe genes. We considered relevant only above 30% of identity.

Multi-copper oxidase yields relevant pairing within the following genomes:
MB35, MB37, MB40, MB41 and MB48.

Results of BlastX Analysis

Strain ID	Description	Multi copper Oxidase (*sufI*)	
			Identity
MB34	P17025.UL_01 (DDS®-1)	bt	bt
MB35	P17025.UL_02 (UALp-05)	relevant	68.34% (341/499)
MB36	P17025.UL_03 (NCIMB 30242)	bt	bt
MB37	P17025.UL_04 (ATCC 53103)	relevant	94.70% (482/509)
MB38	P17025.UL_05 (UABla-12)	bt	bt
MB39	P17145.UL_01 (BRN17)	bt	bt
MB40	P17145.UL_02 (M5-26-17 - UALC-03)	relevant	92.53% (471/509)
MB41	P17145.UL_03 (Lpc06 M 2-16-17 - UALpc-04)	relevant	99.80% (508/509)
MB42	P17145.UL_04 (BL077 - UABL-14)	bt	bt
MB43	P17145.UL_05 (1_6-16-17)	bt	bt
MB48	P18042UL_02	relevant	99.21% (502/505)

bt: below threshold

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•END OF DOCUMENT•

Bonnette, Richard

Subject: FW: Filing status of submissions to the GRAS notification program

From: Kevin Gillies <kevin.o.gillies@gmail.com>
Sent: Friday, July 26, 2019 9:57 AM
To: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Subject: Re: Filing status of submissions to the GRAS notification program

Hi Richard,

Thanks for the note. You are correct, USDA regulated uses are out of the scope of the Notices. We will go to USDA/FSIS directly for suitability determination if needed in future.

Best,
Kevin

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Tel: +1 816 590 9836

> On Jul 26, 2019, at 7:50 AM, Bonnette, Richard <Richard.Bonnette@fda.hhs.gov> wrote:
>
> Hello Kevin,
> Regarding these two microbial submissions from June, we note that they are intended for use in foods generally (excluding infant formula) where appropriate. Can you also confirm that foods under USDA's authority (meat and poultry products, primarily) would also be outside the scope of the intended uses described in these submissions? I presume that they likely are, but just wanted to confirm with you. If your clients do intend meat and poultry uses, there are separate and specific data needs that USDA will require as part of their review that these submissions lack.
> Thanks,
> Richard

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November 14, 2019

Stephanie Hice, PhD
Staff Fellow (Biologist)
Division of Food Ingredients
CFSAN/OFAS
US Food and Drug Administration

Re: Response to FDA Inquiries related to GRN 000872

Dear Dr. Hice:

Thank you for your email of November 6, 2019 asking for UAS Laboratories, LLC (UASLabs) input on questions that have arisen during FDA's review of GRN 000872. On behalf of my client, we are providing answers to the Agency's questions below. Please note that UASLabs' responses do not contain confidential information.

Questions/Comments Regarding GRN 000872:

1. Please state whether *Bifidobacterium animalis* subsp. *lactis* strain "UABla-12" has been deposited in a recognized culture collection and provide the non-trade name designation. If the strain is not deposited, describe how the source was verified and identified.

UASLabs Response:

B. animalis subsp. *lactis* UABla-12™ is deposited with the National Collection of Industrial, Food and Marine Bacteria, Bucksburn Aberdeen, Scotland UK (NCIMB; https://www.ncimb.com) under designation "NCIMB 30334". We note, specifically, that "*B. animalis* subsp. *lactis* UABla-12" is the common and usual name for the strain and is the designation used by UASLabs for internal documentation and trade purposes, as well as, the name that that was submitted to NCIMB. The NCIMB number is a designation that only is used as a NCIMB repository bookkeeping designation. Should FDA prefer to reference the NCIMB number, an appropriate synonym, in our opinion, would be either "*B. animalis* subsp. *lactis* UABla-12™ (NCIMB 30334)" or "*B. animalis* subsp. *lactis* NCIMB 30334" with a reference to the common and usual name as discussed with you and Dr. Highbarger during our telephone conference of November 13, 2019.

2. Please state whether any of the raw materials used in the fermentation media and during production of *B. animalis* "UABla-12" are major allergens or derived from major allergens. Please state whether the final ingredient contains any major allergens.

UASLabs Response:

UAS Labs does not use major allergens or substances derived from major allergens as raw materials in the fermentation media for the production of *B. animalis* subsp. *lactis* UABla-12™. Further, the final formulation of *B. animalis* subsp. *lactis* UABla-12™ does not contain any major allergens. To ensure that major allergens are not present, no major allergens are utilized in the production of any UASLabs products.

3. Please specify whether the manufacturing process is monitored for contamination, and if so, how often this is performed.

UASLabs Response:

Yes, the manufacturing process is monitored for contamination at three (3) process control points. Because the fermentation process for the production of *B. animalis* subsp. *lactis* UABla-12™ is entirely enclosed starting from the inoculation of the production fermentation vessel through the frozen pellet stage, there is limited opportunity in the process for contamination monitoring other than the initial seed vial, the frozen pellet stage and the final milled powder finished product. Each batch of seed vials is tested and must meet product specifications listed in Table 3 (p.14). Each batch of production frozen pellets are tested for coliforms, *Enterobacteriaceae*, enterococci and non-lactic contaminants and must meet the release criteria for those organisms listed in Table 3 (p.14) in order to continue in the process to the lyophilization step. The final milled powder, finished product is then tested for the product release specifications listed in Table 3 (p.14).

4. Please indicate if the analytical methods used to analyze the batches for conformance with the stated specifications are validated for that particular purpose.

UASLabs Response:

The analytical methods used to analyze the batches for conformance to the stated specifications are validated compendial methods that have been verified to be fit for purpose. The one exception is the potency method for total cell count, which is not a compendial method but has been validated internally for that particular purpose.

5. Please specify the sample size for analysis of *E. coli*, *S. aureus* and *Salmonella* as listed in Table 3 (p.14).

UASLabs Response:

The sample size for analysis of E. coli, S. aureus, and Salmonella as listed in Table 3 is 10 grams.

6. Please specify whether *Listeria* refers to *Listeria monocytogenes* on p. 14.

UASLabs Response:

Listeria listed on Table 3 (p.14) refers to *Listeria* species in general. If the initial test is positive for *Listeria* species, the sample is then tested for *Listeria monocytogenes*.

7. Please provide results of three non-consecutive batch analyses to demonstrate that the manufacturing can meet the provided specifications

UASLabs Response:

Please see attached below, three (3) Certificate of Analysis documents for non-consecutive batches of *B. animalis* subsp. *lactis* UABla-12™, demonstrating that the manufacturing process described in GRN 000872 can meet the specifications provided therein.

UAS Laboratories, LLC
Certificate of Analysis
Milled Powder, Finished Product,
Bla-12, 500B
20 kg

Revision: 6 | **Pages:** 1 of 1

Item Number: 60008 Storage: 24 months when stored at 4°C or below

Production Lot#: 64700
Manufacture Date: 2/27/2019 Best By Date: 2/27/2021

UAS Labs Testing Requirements			
Parameter	**Standard Test Procedure**	**Specification**	**Final Results**
Physical Testing			
Color	Visual	Off-White – Cream	PASS
Appearance	Visual	Powder	PASS
Active Water (Aw)	M-SOP-Q11	< 0.1	0.111
Identification			
Identification	16s rRNA Gene Sequence	*Bifidobacterium lactis*	*Bifidobacterium lactis*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	1,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 500 Billion CFU/g	730 Billion CFU/g
Microbial Testing			
Escherichia coli	M-SOP-Q29	Negative in 10g	NEGATIVE in 10g
Staphylococcus aureus	M-SOP-Q27	Negative in 10g	NEGATIVE in 10g
Salmonella	M-SOP-Q28	Negative in 10g	NEGATIVE in 10g
Enterococcus	M-SOP-Q3	< 100 CFU/g	< 100 CFU/g
Listeria	AOAC 2004.06 (VIDAS at Silliker)	Negative in 25g	NEGATIVE in 25g
Enterobacteriaceae Count via Petrifilm	M-SOP-Q2	< 100 CFU/g	< 100 CFU/g
Coliform Count via Petrifilm	M-SOP-Q24	< 100 CFU/g	< 100 CFU/g
Heavy Metal Testing			
Arsenic	EPA 3050/6020 USP <730>	NMT 1 mg/kg (ppm)	0.02 mg/kg
Cadmium	EPA 3050/6020 USP <730>	NMT 0.30 mg/kg (ppm)	0.020 mg/kg
Mercury	EPA 3050/6020 USP <730>	NMT 0.05 mg/kg (ppm)	<0.005 mg/kg
Lead	EPA 3050/6020 USP <730>	NMT 1.0 mg/kg (ppm)	< 0.01 mg/kg
Other Testing			
Gluten	M-SOP-Q54	Negative	NEGATIVE

[redacted] 3/7/19
Quality Signature / Date

UAS Laboratories, LLC
Certificate of Analysis
Milled Powder, Finished Product,
Bla-12, 500B
1 kg

Revision: 6 | **Pages:** 1 of 1

Item Number: 60007 Storage: 24 months when stored at 4°C or below

Production Lot#: 87400
Manufacture Date: 2/25/2019 Best By Date: 2/25/2021

UAS Labs Testing Requirements			
Parameter	**Standard Test Procedure**	**Specification**	**Final Results**
Physical Testing			
Color	Visual	Off-White – Cream	PASS
Appearance	Visual	Powder	PASS
Active Water (Aw)	M-SOP-Q11	< 0.1	0.022
Identification			
Identification	SOP-J61 & 16s rRNA Gene Sequence	*Bifidobacterium lactis*	*Bifidobacterium lactis*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	2,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 500 Billion CFU/g	635 Billion CFU/g
Microbial Testing			
Escherichia coli	M-SOP-Q29	Negative in 10g	NEGATIVE in 10g
Staphylococcus aureus	M-SOP-Q27	Negative in 10g	NEGATIVE in 10g
Salmonella	M-SOP-Q28	Negative in 10g	NEGATIVE in 10g
Enterococcus	M-SOP-Q3	< 100 CFU/g	< 100 CFU/g
Listeria	AOAC 2004.06 (VIDAS at Silliker)	Negative in 25g	NEGATIVE in 25g
Enterobacteriaceae Count via Petrifilm	M-SOP-Q2	< 100 CFU/g	< 100 CFU/g
Coliform Count via Petrifilm	M-SOP-Q24	< 100 CFU/g	< 100 CFU/g
Heavy Metal Testing			
Arsenic	EPA 3050/6020 USP <730>	NMT 1 mg/kg (ppm)	0.02 mg/kg
Cadmium	EPA 3050/6020 USP <730>	NMT 0.30 mg/kg (ppm)	0.016 mg/kg
Mercury	EPA 3050/6020 USP <730>	NMT 0.05 mg/kg (ppm)	<0.005 mg/kg
Lead	EPA 3050/6020 USP <730>	NMT 1.0 mg/kg (ppm)	<0.01 mg/kg
Other Testing			
Gluten	M-SOP-Q54	Negative	NEGATIVE

[redacted] 05/10/19

Quality Signature / Date

UAS Laboratories, LLC
Certificate of Analysis
Milled Powder, Finished Product,
Bla-12, 500B
20 kg

Revision: 6 | **Pages:** 1 of 1

Item Number: 60008 Storage: 24 months when stored at 4°C or below

Production Lot#: 64100
Manufacture Date: 2/25/2019 Best By Date: 2/25/2021

UAS Labs Testing Requirements			
Parameter	**Standard Test Procedure**	**Specification**	**Final Results**
Physical Testing			
Color	Visual	Off-White – Cream	PASS
Appearance	Visual	Powder	PASS
Active Water (Aw)	M-SOP-Q11	< 0.1	0.020
Identification			
Identification	SOP-J61	*Bifidobacterium lactis*	*Bifidobacterium lactis*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	1,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 500 Billion CFU/g	560 Billion CFU/g
Microbial Testing			
Escherichia coli	M-SOP-Q29	Negative in 10g	NEGATIVE in 10g
Staphylococcus aureus	M-SOP-Q27	Negative in 10g	NEGATIVE in 10g
Salmonella	M-SOP-Q28	Negative in 10g	NEGATIVE in 10g
Enterococcus	M-SOP-Q3	< 100 CFU/g	< 100 CFU/g
Listeria	AOAC 2004.06 (VIDAS at Silliker)	Negative in 25g	NEGATIVE in 25g
Enterobacteriaceae Count via Petrifilm	M-SOP-Q2	< 100 CFU/g	< 100 CFU/g
Coliform Count via Petrifilm	M-SOP-Q24	< 100 CFU/g	< 100 CFU/g
Heavy Metal Testing			
Arsenic	EPA 3050/6020 USP <730>	NMT 1 mg/kg (ppm)	0.03 mg/kg
Cadmium	EPA 3050/6020 USP <730>	NMT 0.30 mg/kg (ppm)	0.028 mg/kg
Mercury	EPA 3050/6020 USP <730>	NMT 0.05 mg/kg (ppm)	< 0.005 mg/kg
Lead	EPA 3050/6020 USP <730>	NMT 1.0 mg/kg (ppm)	<0.01 mg/kg
Other Testing			
Gluten	M-SOP-Q54	Negative	NEGATIVE

[redacted] 3/6/19
Quality Signature / Date

Please note, in the COAs above, the addition of specifications for *Enterobacteriaceae* and coliforms that are not listed in the specification in Table 3 (p. 14). These specifications were added after the submission of GRN 000872.

8. Please provide the names of the methods intended to be used to analyze the product for conformance with the stated specifications. Several of the methods in Table 3 (p. 14) are stated as "TBD" or only AOAC without indicating the specific AOAC method.

UASLabs Response:

The names of the methods used to analyze the product for conformance are as follows:

- Non-lactics: ISO 13559 / IDF 153 "Butter, fermented milks and fresh cheese – Enumeration of contaminating microorganisms – Colony-count technique at 30ºC".
- *E. coli, Salmonella, Staph*: U.S. Pharmacopoeia. (2010). <2022> Microbiological Procedures for the Absence of Specified Microorganisms – Nutritional and Dietary Supplements. U.S Pharmacopoeia 34 National Formulary 29 (Vol. 1). The United States Pharmacopeial Convention: Rockville, MD.
- Enterococci: Compendium of Methods for the Microbiological Examination of Foods, 4th Edition. Chapter 10 (section 10.51).
- Listeria: AOAC Official Method 2004.06. Listeria in select foods. Modified VIDAS LIS Assay Screening Method.

Again, thank you for the opportunity to provide additional information regarding UASLabs' GRN 000872. Should you have further comments or questions, please feel free to contact me.

Sincerely,

[redacted]

Kevin O. Gillies

Cc: G. Leyer PhD