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KEVIN O. GILLIES
CONSULTING SERVICES, LLC

1759 Grape St.
Denver, Colorado 80220
USA
Phone: +1 (816) 590 9836 | E-mail: kevin.o.gillies@gmail.com

June 13, 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 *Lactobacillus acidophilus* DDS®-1

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, *Lactobacillus acidophilus* DDS®-1 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 13, 2019

Sincerely,

Kevin O. Gillies

Enc.

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

KOG/Kg

Lactobacillus acidophilus DDS®-1

Generally Recognized as Safe Notice

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

Table of Contents

21 CFR 570. 225; Part 1: Generally Recognized as Safe (GRAS) Notice-Exemption Claim 4
1.1 Exemption Claim for *Lactobacillus acidophilus* DDS®-1 4
1.2 Information about Notifier 5
1.3 Basis for safety determination: 5
1.4 Intended use 5
1.5 Availability of Information 5
1.6 Certification Statement 6
1.8 Signature of Responsible Party or Agent 6
21CFR570.230; Part 2: Identity, Manufacturing, Specifications, Use 7
2.1 Identity of the Substance 7
2.1 Absence of Genetic Determinants Encoding Sequences of Concern for Human Health 11
2.1.1 Absence of Toxin and other virulence factors 11
2.1.2 Absence of Prophage and Extra-chromosomal elements 11
2.1.3 Assessment of Biogenic Amine Production 11
2.2 Absence of Acquired Antibiotic Resistance 12
2.3 Intended Use 13
2.4 Manufacturing; Production and Release Specifications 13
21CFR570.235; Part 3: Dietary Exposure 19
21CFR570.240; Part 4: Self-limiting levels of use 21
21CFR570.245; Part 5: Experience based on common use in food before 1958 22
21CFR570.250; Part 6: Safety Narrative 23
6.1 Background 23
6.2 Intended Use 25
6.3 Regulatory History 26
6.4 Clinical Trials 27
6.5 Strain Identity 28
6.6 Absence of virulence factors, acquired antibiotic resistance, biogenic amine production 29
6.7 Manufacturing 29
6.8 GRAS Conclusion 30
21CFR570.255; Part 7: List of supporting data and information 31
Part 8. Appendices 32
Appendix 1. DDS®-1 Safety Decision Tree[1] 33
Appendix 2. QPS Evaluation of *L. acidophilus* 36
Appendix 3. IDF List 42

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

Appendix 4. Genetic Identity Test.....55
Appendix 5. Biogenic Amine Production Assessment.....58
Part 9. Bibliography.....64
Bibliography.....65

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

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

1.1 Exemption Claim for *Lactobacillus acidophilus* DDS®-1

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 *Lactobacillus acidophilus* DDS®-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 *Lactobacillus acidophilus* DDS®-1 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 *Lactobacillus acidophilus* DDS®-1 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 *L. acidophilus,* in general, and the strain *L. acidophilus* DDS®-1, in particular, have a documented safe history of use in fermented food and other traditional uses and this claim extends the uses of strain *L. acidophilus* DDS®-1 that are considered GRAS.

In concluding that *L. acidophilus* DDS®-1 is GRAS, UASLabs has consulted the documented safe history of use, scientific literature, safety demonstrated 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:

1.2 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

1.3 Basis for safety determination:

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

1.4 Intended use

As an ingredient in conventional foods for the consumption by the general population, with the exception of USDA regulated meat and poultry products, 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 Certification Statement

UASLabs further certifies in accordance with 21CFR570.225(c)(9) that, to the best of our 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 *Lactobacillus acidophilus* DDS®-1.

1.8 Signature of Responsible Party or Agent

Kevin O. Gillies Consulting Services, LLC (member)
June 11, 2019,

UAS Laboratories, LLC
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: *Lactobacillus acidophilus* DDS®-1
- Chemical name: None
- Empirical formula: None
- Structural formula: None
- Quantitative formula: None

Lactobacillus acidophilus DDS®-1 is a single colony isolate of a group of similar microorganisms grouped under the taxa *Lactobacillus acidophilus*.

L. acidophilus is a member of the Phylum Firmicutes taxa (see Fig. 1 below) (Parte 2018). *L. acidophilus* is a well-characterized, non-pathogenic, non-toxigenic, homogeneous species grouping, believed to be first described by Dr. Ernst Moro in 1900.

There are no known pathogenic or toxigenic members of the *L. acidophilus* 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).

Fig. 1

Family: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Lactobacillales
Family: Lactobacillaceae
Genus: *Lactobacillus*
Species: *acidophilus*
Strain: DDS®-1

Members of the *Lactobacillus* genus, including *L. acidophilus*, are gram-positive, rod-shaped, non-spore forming and non-motile. Classically, the *Lactobacillus* genus is divided into three groups: Group 1, obligate homo-fermentative, Group 2, facultative hetero-fermentative and Group 3 obligate hetero-fermentative. They are catalase negative, aero-tolerant or anaerobic and

chemo-organotropic. This genus encompasses more than 100 different species with a large variety of phenotypic, biochemical and physiological properties. Of these, the *L. acidophilus* species belongs to the Group 3 obligate homo-fermentative group and represents significant constituents of the normal gut flora of humans and livestock.

L. acidophilus strains are also intentionally introduced in the food chain, being involved in a range of food and feed fermentations and widely in commerce globally as probiotics for humans and animals.

L. acidophilus is listed in the International Dairy Foundation list (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) of strains with a documented history of use in food (Bourdichon 2012).

The European Food Safety Authority (EFSA; Appendix 2) has published the results of its safety evaluation of the IDF listed organisms with some modifications in the Agency's Qualified Presumption of Safety (QPS) list. EFSA found that members of *L. acidophilus* species are safe for general use in foods without restriction and exempt, either food category or usage rate, from requirements for pre-market approval of use.

Prior sanctions were granted by the US FDA for the use of harmless lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (21CFR131.112), sour cream (21CFR131.160), cottage cheese (21CFR133.128), and yogurt (21CFR *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). FDA employed letters to manufacturers in pre-GRAS times and such prior sanction response is equivalent to a GRAS "no comment" letter today.

The International Food Additives Council has determined that the strains, including *L. acidophilus,* listed on the IDF list are GRAS for their traditional food uses by scientific procedures (Stevens 2009).

In accord with the safety decision tree of Pariza, et al. 2015 (Appendix 1) and EFSA recommendations (European Food Safety Authority n.d.), UASLabs has confirmed by state of the art methods that the strain *Lactobacillus acidophilus* DDS®-1 is (1) a member of the *Lactobacillus acidophilus* species; (2) does not contain gene sequences encoding known toxins; and (3) is free of known virulence genes.

Strain DDS®-1 has been confirmed as a member of the *L. acidophilus* species by ribotype 5s, 16s, and 23s rDNA 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.

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, strain DDS®-1 by extension 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.

Both EFSA (European Food Safety Authority) 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 could 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 the culture 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 DDS®-1 was judged

to be homogeneous and a pure culture and the five isolated colonies represented *L. acidophilus* DDS®-1. One of the five (5) colonies was picked for further sequence analysis.

The whole genome sequence of *L. acidophilus* DDS®-1 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.

A total of 28,311,550 paired-end sequence reads were generated using Illumina (HiSeq2500 system). FASTQ sequence files were generated using bcl2fastaq2 version 2.18 and processed for quality via Illumina Chastity filtering and FASTQC quality control. A total of 584,992 reads generated by PacBio (Sequel) were processed and filtered using the Single Molecule, Real Time (SMRT) Analysis software suite. Quality filtered Illumina FASTQ sequence reads were converted into contigs using ABySS version 1.51 and linked based on alignment of the PacBio CLR reads. This yielded 1 contig with a size of 1,987,143 bp.

The % GC content was 34.7%. Genome annotation was performed using a pipeline based on the Prokka Prokaryotic Genome Annotation System (Prokaryote gene prediction by Prodigal 2.6; rRNA prediction using barrnap 0.2; tRNA prediction by Aragorn 1.2.36).

Annotation statistics of *L. acidophilus* DDS-1 revealed a genome size of 1,987,143 bp, 1 contig (consensus), 1,862 coding sequences, a total of 30 hypothetical proteins, a total gene size of 1,767,163 bp and an average gene size of 920.4 bp (min = 70 bp, max = 12,980 bp). Genome alignment was carried out by progressive MAUVE with *L. acidophilus* NCFM (GenBank Acc No: NC_006814.3) as reference genome, revealing significant similarity overall.

The main differential region in the genome of *L. acidophilus* DDS®-1, as compared to *L. acidophilus* NCFM, is a triple repetition of homologous genes of the NCFM strain. Specifically, the gene pair constituted by parB and gIdB genes, involved in cell replication (Morton R. A. 2007) appeared to be repeated in *L. acidophilus* DDS®-1. The two strains share the presence of genes related with the production of at least one bacteriocin, which share sequence similarity with their homologous genes in *L. acidophilus* NCFM involved in the production of lactacin B (Dobson A.E. 2007).

2.1 Absence of Genetic Determinants Encoding Sequences of Concern for Human Health

2.1.1 Absence of Toxin and other virulence factors

The genome of *L. acidophilus* DDS®-1 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.

2.1.2 Absence of Prophage and Extra-chromosomal elements

The genome was found to not comprise any complete prophage, as an 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 Assessment of 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 *L. acidophilus* DDS®-1. 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 *L. acidophilus* DDS®-1 using BLAST. No relevant matches were detected within the *L. acidophilus* DDS®-1 genome for histidine decarboxylase, tyrosine decarboxylase, lysine decarboxylase or agmatine deiminase, which are involved in the generation of histamine, tyramine, cadaverine or N-carbamoyl putrescine, respectively.

Results obtained were confirmed using a biochemical assay according to (Bover-Cid 1999), using appropriate positive controls for production of histamine, tyramine, cadaverine or putrescine. The assay is based on a chromogenic change linked to the production of biogenic amines and allows for the detection of metabolic products regardless of the biosynthetic pathway. *L. acidophilus* DDS®-1 was confirmed to not yield a positive reaction for any of the tested biogenic amines. (Appendix 5).

2.2 Absence of Acquired Antibiotic Resistance

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 (FEEDAP 2012) recommend that microbial strains used in food applications must not harbor acquired antimicrobial resistance genes to clinically relevant antimicrobials. Analysis of the DDS®-1 antimicrobial susceptibility phenotype indicates that DDS®-1 does not express acquired antimicrobial resistance factors to known antibiotics of clinical significance.

In order to assess antibiotic resistance of *L. acidophilus* DDS®-1, 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. *L. acidophilus* DDS®-1 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 characteristic cut-off values (Table 1).

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	1	0.5	0.5	Sensitive
Vancomycin	2	0.5	0.5	Sensitive
Gentamicin	16	2	4	Sensitive
Kanamycin	64	64	64	Sensitive
Streptomycin	16	4	4	Sensitive
Erythromycin	1	0.125	0.063	Sensitive
Clindamycin	1	0.5	0.25	Sensitive
Chloramphenicol	4	4	4	Sensitive

As *L. acidophilus* DDS®-1 does not show any sign of acquired antibiotic resistance and all MIC values are below reported species characteristics (cut-off values); investigation at the genetic level to assess horizontal gene transfer is not necessary.

2.3 Intended Use

L. acidophilus DDS®-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 with the exception of infant formula at levels of 10^9 to 10^{11} per serving of food intended for the general population

2.4 Manufacturing; Production and Release Specifications

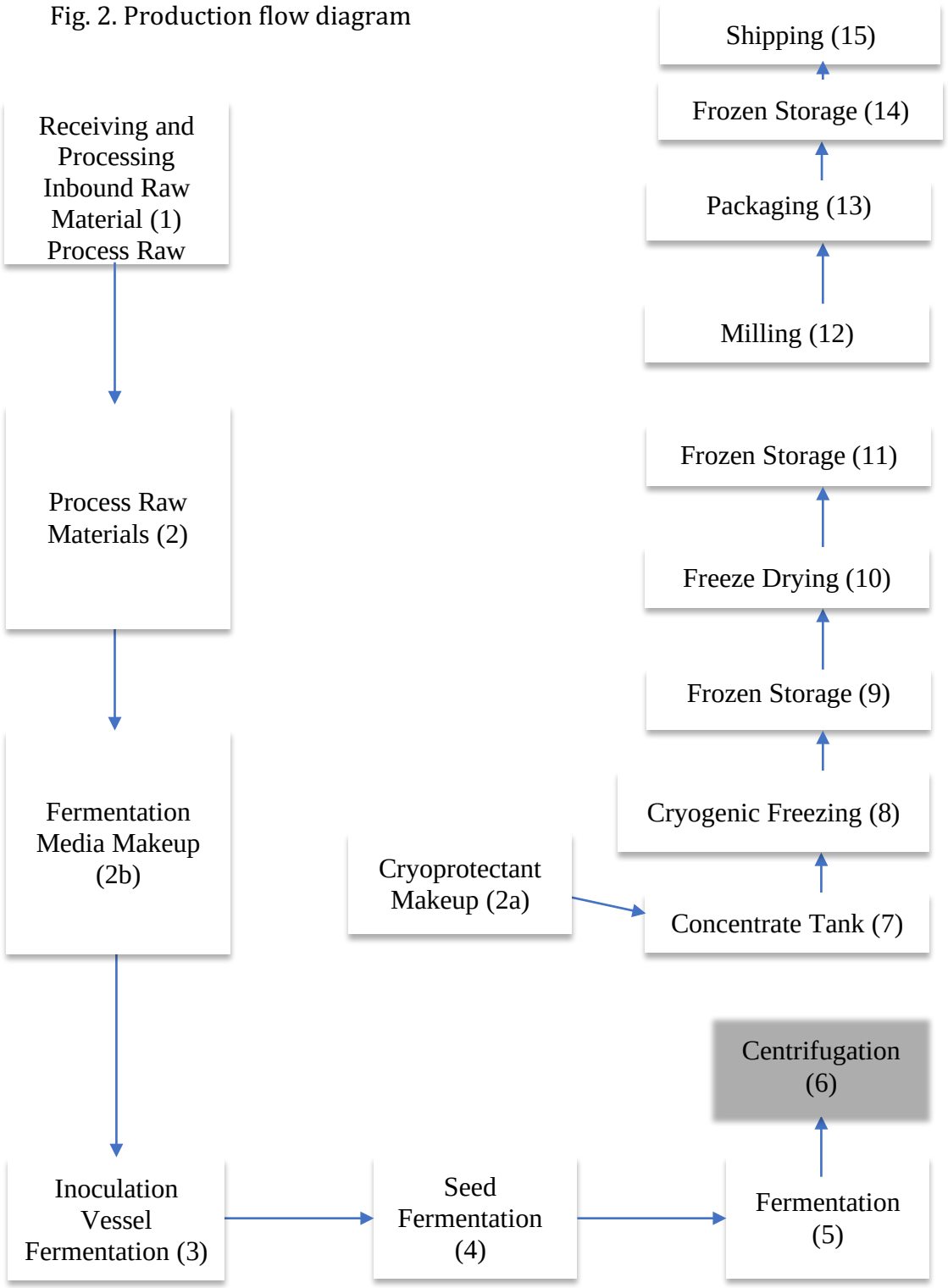
The UASLabs facility at 4375 Duraform Lane, Windsor, Wisconsin 53598 USA is a FDA-regulated and inspected purpose-built food microorganism production facility including seed preparation, fermentation, and post-fermentation processing capabilities. All raw materials used in the production of *L. acidophilus* DDS®-1 are safe and suitable GRAS ingredients 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.

The following flow chart (Fig. 2) and accompanying description in Table 2. provide a summary of the production process. Included are references to critical control points identified by UASLabs.

Fig. 2. Production flow diagram



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

Table 2. 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
Seed Can Fermentation	3	Inoculation of the bacteria into a canister that contains nutritional media using a thawed, frozen culture. This process takes place in the controlled seed room located within the production laboratory.
Fermentation	4	The product is moved through the closed system into a larger vessel that contains a new nutritional media
Fermentation	5	The product is moved through the closed system into a larger 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 used is *L. acidophilus* DDS®-1. Stock seed cultures are produced and maintained in the secure culture bank of UASLabs as frozen 1 ml. vials at -80^{0}C under the control of the Quality function. UASLabs verifies the identity of the seed by 16s rDNA 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 inoculated with DDS®-1. The food grade ingredients are used in the production are used for the sole purpose as substrates for growth of DDS®-1 or stabilization of the final product; and no chemical alteration of the resulting fermented product is intended or expected.

Table 3. UASLabs *L. acidophilus* DDS®-1 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	*Lactobacillus acidophilus*	SOP-J61 or 16s rRNA Gene Sequence
Purity Testing		
Non Lactics	< 5,000 CFU/g	M-SOP-Q13 or ISO 13559
Potency/Strength		
Total Viable Cell Count	NLT 200 Billion CFU/g	M-SOP-Q23 or ISO 7889/IDF 117
Microbial Testing		
Escherichia coli	Negative by Test	M-SOP-Q29 or USP <2022> or AOAC
Staphylococcus aureus	Negative by Test	M-SOP-Q27or USP <2022> or AOAC
Salmonella	Negative by Test	M-SOP-Q28 or USP <2022> or AOAC
Enterococcus	<100/g	M-SOP-Q3 or CMMEF or SMEDP
Listeria	Negative in 25g	AOAC (VIDAS at Silliker)

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

Table 3. (above) lists the Quality Control specifications that must be satisfied for release of final products.

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

21CFR570.235; Part 3: Dietary Exposure

The strain *L. acidophilus* DDS®-1 is envisioned for use as food ingredient at levels of 10^9-10^{11} CFU per food serving. These levels are consistent with common and usual practice in the US and as described in GRNs 357 and 502 (incorporated herein by reference). Further, fermented milks, such as yogurt, may have concentrations of 10^8-10^9 CFU of Lactic Acid Bacteria including *L. acidophilus* per gram of the finished product at the start of shelf-life. A 250-gram serving would contain 2.5 x 10^{10} to 2.5x10^{11} per serving (for example see White Mountain yogurt advertises 9x10^{10} CFU of per serving of yogurt; https://www.whitemountainfoods.com/yogurt.html; last accessed 13 November 2018).

As the proposed usage of strain DDS®-1 is in keeping with current levels in the market and it is envisioned that the strain will be used in the same foods as other *L. acidophilus* strains already present in the market, the replacement use is not expected to materially increase the dietary exposure of consumers to *L. acidophilus* microbial food cultures.

Similarly, *L. acidophilus* DDS®-1 is used both as a food ingredient and as a dietary ingredient globally and the proposed use in conventional foods corresponds to the usage norms of *L. acidophilus* products in supplement products. *L. acidophilus* strains are commonly used in the 10^9-10^{10} CFU per dose range in supplement form. For example:

1. Nature Made Acidophilus 10^9 CFU/per tablet; https://www.walgreens.com/store/c/nature-made-acidophilus-dietary-supplement-tablets/ID=prod6109087-product?ext=msnKBM_PLA_-_Vitamins&kpid=sku6096591&sst=b6ebf408-1e64-460b-ba6a-dfb630ffa7a3&msclkid=ee46e4cea3a4158075a2bf24427b9748; last accessed 11 November 2018)
2. Pure Encapsulations Lactobacillus acidophilus - 1.5 x 10^9/capsule; 1-3 times per day; http://www.clinicalnutritioncenters.com/lactobacillus-acidophilus-60-capsules-by-pure-encapsulations/?keyword=SearchAd&matchtype=e&query=lactobacillus%20acidophilus%20capsules&pla=ShoppingAd&device=c&msclkid=5

79712a3c31416a1c52f49492256df0d&utm_source=bing&utm_medium=cpc&utm_campaign=TPA®%20-%20Shopping&utm_term=4574655566554592&utm_content=All%20Products; last accessed 11 November 2018)

Again, DDS®-1 is envisioned to replace other *L. acidophilus* strains already present in foods in the US, and is not expected to materially increase the dietary exposure of consumers from the intended uses.

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

The addition of *L. acidophilus* DDS®-1 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 dry microbial food culture cell concentrates.

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

UASLabs is not aware of a common use of *L. acidophilus* DDS®-1 in food before 1958. Therefore, Part 5 does not apply to this GRAS Notice.

UAS Laboratories, LLC
4375 Duraform Lane
Windsor, Wisconsin 53598
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21CFR570.250; Part 6: Safety Narrative

6.1 Background

UASLabs has undertaken a comprehensive safety determination for the use of *L. acidophilus* DDS®-1 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. process to provide reasonable certainty that the ingredient does not present a significant or unreasonable risk of harm (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)) and that *L. acidophilus* DDS®-1 is GRAS for the uses described herein.

The following is a summary of the UASLabs finding that *L. acidophilus* DDS®-1 is safe and suitable for its intended uses.

It is important to note at the outset that the human gut is essentially an open ecosystem containing a diverse microbial community and that 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.

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. Safe and suitable Lactic Acid Bacteria (primarily lactococci and lactobacilli, bifidobacteria, and propionibacteria) make up a large proportion of ingested bacteria via fermented foods and probiotics and such fermented products are estimated to comprise upto 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).

L. acidophilus is a non-pathogenic and non-toxigenic microorganism (Appendix 2) with a safe history of use as an article of food in the global food supply. The International Dairy Federation (IDF) has compiled a list of organisms with a documented safe history of use in food (Morgensen 2002) (Bourdichon 2012) (Appendix 3) including Lactic Acid Bacteria and the list is the only existing 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. As the IDF list documents species with a safe history or use, all isolates of *Lactobacillus acidophilus* including *L. acidophilus* DDS®-1 are considered to have a documented safe history of use in the IDF list and are considered GRAS for their traditional uses (Stevens 2009).

In addition to a safe history of use in foods, including dietary supplements, regulatory agencies world-wide have evaluated the safety of Lactic Acid Bacteria including *Lactobacillus acidophilus* and other organisms in the food supply. There is virtual consensus globally that *L. acidophilus* is safe and suitable for use in food (see section 6.2 below). The European Food Safety Authority (EFSA; Appendix 2) has critically evaluated the components of the IDF list, including *L. acidophilus* 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 and feed ingredients in the EU does not require premarket approval from EFSA. (European Food Safety Authority n.d.). In addition to the documented safe history of use and EFCA evaluation, Lactobacilli and Bifidobacteria have been reviewed from scientific perspective and found to pose no additional risks than that posed by the commensal community into which they are introduced (Borriello SP 2003).

6.2 Intended Use

L. acidophilus DDS®-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.

In addition to the consensus view that *L. acidophilus* strains are safe for use in food, strain DDS®-1 has a safe history of use in in fermented milks since at least 1980 (Ayebo 1980). Usage rates envisioned herein for the uses are common rates for the addition of *L. acidophilus* strains to food, including supplements and as part of Lactic Acid Starter cultures in fermented foods.

For example, GRN 357 (incorporated herein by reference) envisions addition of *L. acidophilus* to foods that can sustain living *Lactobacillus acidophilus*, including dairy products, functional beverages, nutrition powders, juices, bars, RTE breakfast cereals, chewing gum and confections, at levels that will achieve 5 x 10^{10} CFU / day dose at the beginning of shelf-life.

GRN 502 (incorporated herein by reference) envisions addition of *L. acidophilus* La-14 up to 5 x 10^{11} CFU per 250 g serving.

Further, fermented milks, such as yogurt, may have concentrations of 10^{6}-10^{8}CFU of Lactic Acid Bacteria per gram of the finished product at the start of shelf-life. A 250-gram serving would contain 10^{8} to 10^{10} per serving (White Mountain yogurt advertises 9x10^{10} CFU of viable microorganisms per serving of yogurt; https://www.whitemountainfoods.com/yogurt.html; last accessed 13 November 2018).

As supporting information on the safety of the intended usage rates, supplement use of *L. acidophilus* strains consists commonly of 10^{9} CFU per dose. For example:

1. Nature Made Acidophilus 10^{9} CFU/per tablet; https://www.walgreens.com/store/c/nature-made-acidophilus-dietary-supplement-tablets/ID=prod6109087-product?ext=msnKBM_PLA_-_Vitamins&kpid=sku6096591&sst=b6ebf408-1e64-460b-ba6a-dfb630ffa7a3&msclkid=ee46e4cea3a4158075a2bf24427b9748; last accessed 11 November 2018)
2. Pure Encapsulations Lactobacillus acidophilus - 1.5 x 10^{9}; 1-3 times per day

http://www.clinicalnutritioncenters.com/lactobacillus-acidophilus-60-capsules-by-pure-encapsulations/?keyword=SearchAd&matchtype=e&query=lactobacillus%20acidophilus%20capsules&pla=ShoppingAd&device=c&msclkid=579712a3c31416a1c52f49492256df0d&utm_source=bing&utm_medium=cpc&utm_campaign=TPA®%20-%20Shopping&utm_term=4574655566554592&utm_content=All%20Products; last accessed 11 November 2018)

As demonstrated in the examples above, the intended use envisioned for *L. acidophilus* DDS®-1 in food are within the range of common usage for *L. acidophilus* strains in food, including supplements, and have been demonstrated to be safe in use.

6.3 Regulatory History

Lactobacillus acidophilus 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, *L. acidophilus* is allowed in dairy products under US FDA prior sanction. This finding by FDA is the equivalent of a GRAS finding. These prior sanction reviews were made prior to the start of the GRAS process. FDA has also reviewed numerous GRAS Notices for uses of *L. acidophilus* strains in various foods, supplements and anti-microbial preparations, incorporated herein by reference (GRN Nos: 502, 463, 378, 367,171; https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices; last accessed February 4, 2018) and had no objections to the conclusions that the organisms were safe for the intended uses.

L. acidophilus and other safe and suitable microorganisms, including those marketed as probiotics, are allowed for use in food in Canada, e.g. yogurt, without pre-market approval. Again, *L. acidophilus* is approved as a species. Therefore, all isolates of *L. acidophilus* are approved. In addition, where such probiotic cultures are contained in products that have a therapeutic use, Health Canada includes *L. acidophilus* in the Probiotic Monograph of strains allowed in Natural Health Products and has approved numerous *L. acidophilus*-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).

L. acidophilus is considered a traditional food because of its "long history of use in yogurt and fermented milk products in Australia and New Zealand and is 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;).

6.4 Clinical Trials

Results of clinical studies reinforce the overwhelming evidence of safety of the organisms on the IDF and QPS lists; the dose rates in the studies support the usage rates envisioned, herein, for DDS®-1 use in conventional food.

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 the DDS®-1 does not include such at-risk populations, it is instructive that no product related adverse effects have been reported in clinical trials involving these at-risk populations (Sanders 2016). Moreover, numerous *L. acidophilus* DDS®-1 human feeding trials in adults and children, while not standard Phase I safety studies, have not noted any significant adverse effects on study participants (Ayebo 1980) (Frese 2012) (Frese 2012) (Gerasimov 2004) (S. V. Gerasimov 2010) (S. V. Gerasimov 2016) (Greany 2004) (Nagala 2011) (Pakdaman 2016).

The patient groups and dosage rates are summarized below.

Ayebo 1980
Patient group: healthy adults
Dose: 1x 10^9 CFU / day; administered as *L. acidophilus* DDS®-1 fermented milk

S. V. Gerasimov 2004
Patient group: single 6 year old
Dose: 2 x 10^9 CFU / dose; administered twice daily for one (1) month then once daily for long term

S. V. Gerasimov 2010
Patient group: Children age 12-36 months diagnosed with Atopic Dermatitis

Dose: $1x10^{10}$ CFU/day mixture of strain DDS®-1 and *Bifidobacterium lactis* UABLa-12™

S. V. Gerasimov 2016
Patient group: health 3-12 year old children
Dose: $5x10^{9}$CFU/day mixture of strain DDS®-1 and *Bifidobacterium lactis* UABLa-12™

Greany 2004
Patient group: Adult women 40-70 years of age: two (2) groups were studied, recruited patients were split into two groups; one with a history of breast cancer but not treated with chemotherapy and the other no history of breast cancer.
Dose: 1×10^{9} CFU/day: combination of strain DDS®-1 and *Bifidobacterium longum*

Nagala 2011
Patient group: 25 clinically diagnosed IBS patients ages 25-86 years
Dose: $2.4x10^{10}$ CFU/day followed by $1.2x10^{10}$ CFU/day; combination product strain DDS®-1, *B. longum*, *B. lactis* and *B. bifidum*

Pakdaman 2016
Patient group: healthy adults with symptoms of lactose intolerance ages 18-75 years
Dose: $1x10^{10}$/day

Again, while the studies above did not address safety as a primary study endpoint, no significant adverse effects were observed or noted in the trials. Further, the dosage range in the studies is consistent with the intended usage rates as described in Section 6.2 above.

6.5 Strain Identity

An exhaustive identification processes based upon ribotyping and whole genome sequencing demonstrates that DDS®-1 is typical of the *L. acidophilus* isolates currently in the global marketplace and conform to the recommendations of the US FDA (Early Clinical Trials with Live Biotherapeutic 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 strain development and manufacturing, the Pariza et al. (Pariza 2015) decision tree and probiotic industry recommendations (Sanders ME 2010) for the characterization of microbial food culture strains, including probiotics.

This confirmation of DDS®-1 as a strain of the *L. acidophilus* species is important because the equivalence ties DDS®-1 directly to the safe history of use, QPS status, prior sanction and other competent authority approvals for *L. acidophilus* and all isolates thereof, and is, thereby, approved for use in all jurisdictions where the species is allowed.

6.6 Absence of virulence factors, acquired antibiotic resistance, biogenic amine production

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, DDS®-1 is free of sequences that encode for known food toxins and virulence factors and lacks the capacity to produce biogenic amines.

In addition, antimicrobial sensitivity phenotype analysis indicates that DDS®-1 is not resistant to clinically relevant antibiotics and that the antimicrobial resistance is innate and shared with other members of the species and, therefore, it is unlikely that the strain has acquired antibiotic resistance. The lack of prophage and extra-chromosomal elements in its genome indicate the low risk of the strain participating in horizontal transmission of such factors.

Thus, DDS®-1 is shown to be free of identified risk factors to human health.

6.7 Manufacturing

Finally, *L. acidophilus* DDS®-1 is produced in a purpose-built, state-of-the-art facility in accordance with the appropriate food and dietary supplement cGMPs. All raw materials used in the process are GRAS ingredients or approved food additives.

The risk of product contamination is minimized at each step of the process. Seed preparations are done 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.

6.8 GRAS Conclusion

In summary, UASLabs has followed rigorously the Pariza et al. (Pariza 2015) safety decision tree to conclude by scientific procedures that *L. acidophilus* DDS®-1 is GRAS for the intended uses described herein.

In addition to the GRAS status of the organism, UASLabs employs a safe and suitable system to preserve the quality of the production organism, cGMP-compliant production processes and food safety systems to insure the safety of the final *L. acidophilus* DDS®-1 product.

The outcome of the safety decision tree as proposed by Pariza et al. 2015 is summarized in Appendix 1 including the final decision tree answers leading to the conclusion by scientific procedures that *L. acidophilus* DDS®-1 is Generally Recognized as Safe (GRAS) under the conditions of use in accordance with 21 CFR 170.30 and is exempt thereby from pre-market approval requirements of the Food, Drug and Cosmetic Act.

Finally, UASLabs has reviewed the available data and information and is not aware of any data and information that are, or may appear to be, inconsistent with the conclusion of GRAS status of *L. acidophilus* DDS®-1 under the conditions of use as described herein.

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.

Part 8. Appendices

Appendix 1. DDS®-1 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

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

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 10a. If NO, go to 13a.)	YES
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	NO

may significantly increase the single dose and/or chronic exposure)? (If NO, go to 14a. If YES, go to 13a.)	
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 evaluation studies? [xvii] If yes, go to 15. If no, go to 14a.)	NO
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.	YES
15. The strain is NOT APPROPRIATE for human or animal consumption [xix].	NO

[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 *L. acidophilus*

Appendix A - Assessment of gram-positive nonsporulating bacteria (The EFSA Journal (2007) 587, Qualified Presumption of Safety http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2007.587/epdf *Lactobacillus).*

The genus *Lactobacillus* is a wide and heterogeneous taxonomic unit, comprising the rod-shaped lactic acid bacteria. This genus encompasses more than 100 different species with a large variety of phenotypic, biochemical and physiological properties. Many of the species are significant constituents of the normal gut flora of humans and livestock although their occurrence and numbers are host dependent. Several species of the genus are intentionally introduced in the food chains, being involved in a range of food and feed fermentations and applied as probiotics for humans and animals.

Taxonomic unit defined

As for other lactic acid bacteria, lactobacilli belong to the phylum *Firmicutes*. They are rod shaped, non-motile and non-sporeformers. Classically, the *Lactobacillus* genus is divided into three groups: group 1, obligate homofermentative, group 2, facultative heterofermentrative and group 3 obligate heterofermentrative (for a review, see Axelsson 2004). The application of phylogenetic molecular taxonomy and 16S rRNA gene sequence analysis resulted in several changes within the taxonomy of this genus, with an increase in the number of species. At present 112 species belong to the genus *Lactobacillus*. Several molecular methods are available for the identification of lactobacilli to species level.

Is the body of knowledge sufficient?

The characteristics and habitat of most of *Lactobacillus* species are well known. Some of the species of this genus have a long history of apparent safe use in industrial and agricultural applications. Lactobacilli are used as starter cultures in a variety of food fermentation, such as dairy products, fermented and cured meats, fermented vegetables, sourdough and silage. Moreover, they are among the dominant populations in microbial communities of traditional fermented foods, being part of the natural starter cultures. Increased information on this genus is being derived from the sequence analysis of several genomes of *Lactobacillus* species.

Are there safety concerns?

Members of the *Lactobacillus* genus are daily consumed in large quantities in a variety of fermented foods by people of all ages, ethnic groups and health status with apparently no ill effects. Apart from their possible involvement in the development of dental caries, lactobacilli have generally been considered to be non-pathogenic. However, there has been an increasing number of reports that these organisms might occasionally be involved in human disease (Sharpe, Hill *et al*. 1973; Gasser 1994; Salminen, Rautelin *et al*. 2006). A variety of different *Lactobacillus* species has been recovered from human clinical specimens. These include *L. rhamnosus, L. fermentum, L. plantarum, L. casei, L. jensenii, L. salivarius, L. gasseri, L. salivarius,* and *L. acidophilus.* Clinical conditions from which these species were derived were chiefly subacute endocarditis and bacteremia or systemic septicemia, but also included abscesses, chorioamnionitis, and urosepsis (Lorenz, Appelbaum *et al*. 1982; Dickgiesser, Weiss *et al*. 1984; Salminen, Tynkkynen *et al*. 2002; Salminen, Rautelin *et al*. 2004; Salminen, Rautelin *et al*. 2006).

Even the strain *L. rhamnosus* ATCC 53103, used as human probiotic, has occasionally been encountered in clinical specimens such as blood or pus samples (Rautio, Jousimies-Somer *et al*. 1999; Salminen, Tynkkynen *et al*. 2002; Salminen, Rautelin *et al*. 2004; De Groote, Frank *et al*. 2005; Salminen, Rautelin *et al*. 2006). However, Salminen and co-workers (Salminen, Rautelin *et al*. 2006) demonstrated that increased probiotic use of *L. rhamnosus ATCC* 53103 had not led to an increase in *Lactobacillus* bacteraemia. Furthermore, it has been demonstrated that strains isolated from clinical samples, show phenotypic, differences from probiotic *L. rhamnosus* strains (Klein,Hack *et al*. 1995; Ouwehand, Saxelin *et al*. 2004). Many of the patients with apparent *Lactobacillus* infection were immunocompromised or had other severe underlying illnesses. As far as endocarditis due to lactobacilli is concerned, this infection usually develops on the basis of preceding anatomical alterations of the heart valves. There are indications, however, that good adhesion properties of lactobacilli and, thus, of probiotic strains, might be a potential risk for bacteremia (Apostolou, Kirjavainen *et al*. 2001). In conclusion, most of the *Lactobacillus* species described to date can rightly be considered to be non-pathogenic to humans (Bernardeau, Guguen *et al*. 2006). Only certain strains of *L. rhamnosus* may be considered to be potential human opportunistic pathogens because they not only affect severely immunocompromised, but also immunologically healthy individuals with a history of rheumatic endocarditis or heart valve replacement. Several examples of antibiotic resistant lactobacilli isolated from food or from the gut of animals exist. Acquired genes for antibiotic resistance have been detected in *Lactobacillus* species: *tet*(M) has been found in *L.*

plantarum, L. brevis, L. sakei and *L. curvatus* (Danielsen 2002; Gevers, Danielsen *et al*. 2003) and *tet*(S) in *L. plantarum* (Huys, D'Haene *et al*. 2006). Erythromycin resistance determinants *erm* (B) has been found in *L. plantarum*, *L. salivarius, L. animalis, L. fermentum, L. reuteri* (Axelsson, Ahrne *et al*. 1988; Fons, Hege *et al*. 1997; Gevers, Danielsen *et al*. 2003; Martel, Meulenaere *et al*. 2003). Moreover, the gene coding for the bifunctional aminoglycoside-modifying enzyme AAC(6')-APH(2") was detected in *L. salivarius* and *L. acidophilus* (Tenorio, Zarazaga *et al*. 2001) and chloramphenicol resistance gene *cat* was identified in *L. reuteri* (Lin, Fung *et al*. 1996). Obligate and facultative heterofermentative lactobacilli, and *L. salivarius,* are intrinsically resistant to vancomycin and other glycopeptide antibiotics. Several genetic determinants for antibiotic resistance in *Lactobacillus* are harboured by extrachromosomal elements (Lin, Fung *et al*. 1996; Danielsen 2002; Gevers, Danielsen *et al*. 2003; Gfeller, Roth *et al*. 2003; Huys, D'Haene *et al*. 2006). However, transferable elements encoding resistances of clinical relevance, such as to the glycopetides have been excluded for some probiotic *L. reuteri* and *L. rhamnosus* strains (Klein, Hallmann *et al*. 2000).

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

Can the safety concerns be excluded?

There are apparently no specific safety concerns regarding a number of *Lactobacillus* species which have a long history of apparent safe use in the food chain. Susceptibility to antibiotics should be assessed as defined by the EFSA opinion for each strain (EFSA 2005).

Units proposed for QPS status

Due to the long history of safe use the following species are proposed for QPS status:
L. acidophilus, L. amylolyticus, L. amylovorus, L. alimentarius, L. aviaries, L. brevis, L. buchneri, L. casei, L. crispatus, L. curvatus, L. delbrueckii, L. farciminis, L. fermentum, L. gallinarum, L. gasseri, L. helveticus, L. hilgardii, L. johnsonii, L. kefiranofaciens, L. kefiri, L. mucosae, L. panis, L. paracasei, L. paraplantarum, L. pentosus, L. plantarum, L. pontis, L. reuteri, L. rhamnosus, L. sakei, L. salivarius, L. sanfranciscensis and *L. zeae.*

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Appendix 3. IDF List

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Windsor, Wisconsin 53598
USA

of the International Dairy Federation

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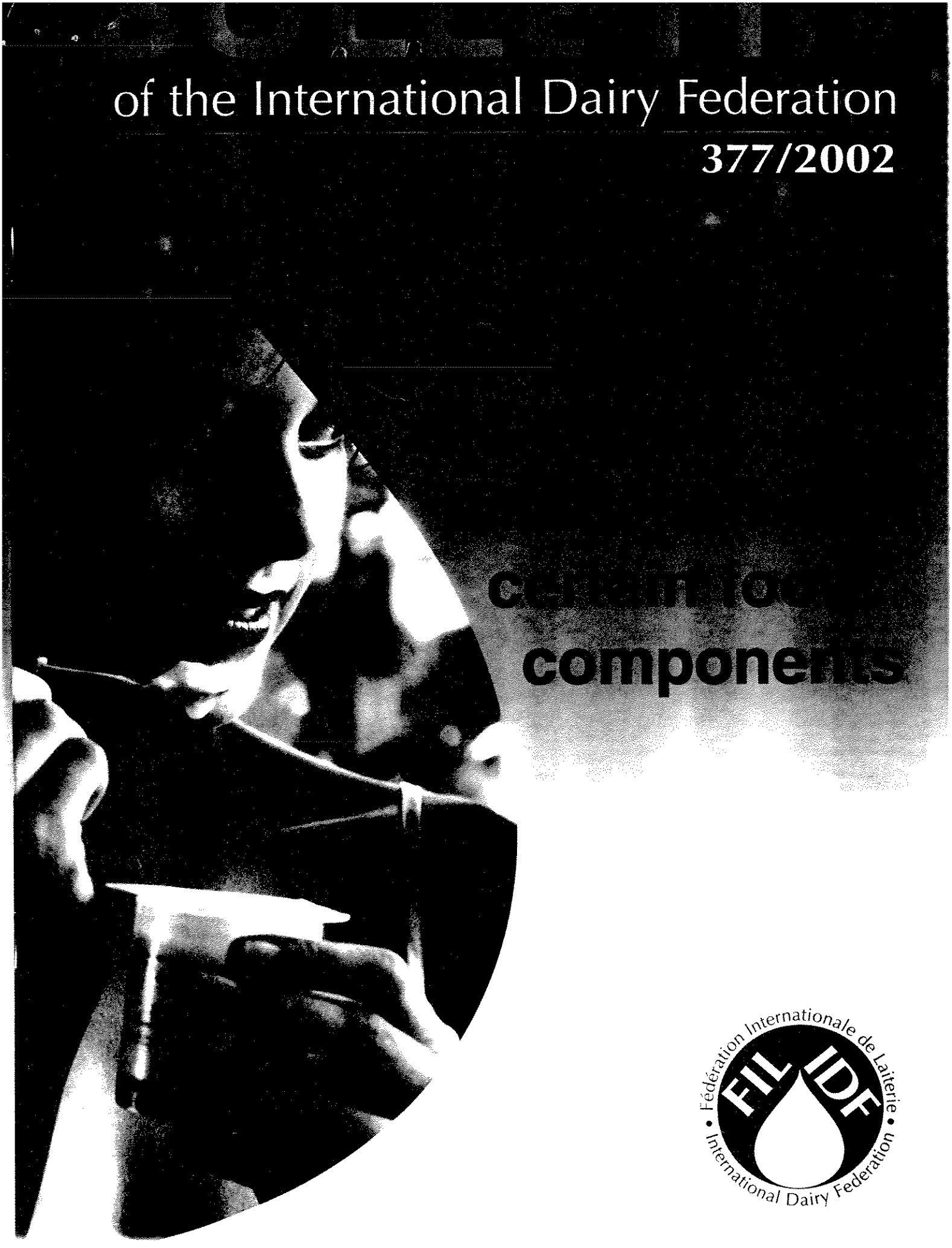

certain food components

Fédération Internationale de Laiterie
FIL IDF
International Dairy Federation

CONTENTS

IDF news

Schedule of future IDF events 2

Health benefits and safety evaluation of certain food components

Foreword 3

1 Food Microorganisms - Health Benefits, Safety Evaluation and Strains with Documented History of Use in Foods

G. Mogensen, S. Salminen, J. O'Brien, A. Ouwehand, W. Holzapfel, C. Shortt, R. Fondén, G.D. Miller, D. Donohue, M. Playne, R. Crittenden, B. Bianchi Salvadori & R. Zink

1 Introduction 4
2 Clinical studies on health benefits involving LAB 5
3 Safety evaluation of LAB 6
4 Clinical cases involving LAB 6
5 IDF/EFFCA inventory of microorganisms with a history of use in foods 7
6 Conclusion 8
References 8

2 Inventory of Microorganisms with a Documented History of Use in Food

G. Mogensen, S. Salminen, J. O'Brien, A. Ouwehand, W. Holzapfel, C. Shortt, R. Fondén, G.D. Miller, D. Donohue, M. Playne, R. Crittenden, B. Bianchi Salvadori & R. Zink

1 Introduction 10
2 Microorganisms with a documented history of use in foods 11
References 15

3 Trans Fatty Acids

Y. Soustre, B. Laurent, J. Schrezenmeir, M. Pfeuffer, G. Miller & P. Parodi

1 Introduction 20
2 Trans fatty acids in food 21
2.1 Milk and dairy fats 21
2.2 Vegetable fats 23
2.3 Meat and meat products 24
3 Trans fatty acids and health 25
3.1 Between CLA and CLA: the beneficial effects of CLA 25
3.2 Between TFA and TFA: the disparate biological effects of *trans* fatty acid isomers 25
References 27

4 Milk Lipids in Diet and Health - Medium Chain Fatty Acids (MCFA)

M. Pfeuffer & J. Schrezenmeir

1 Introduction 32
2 Digestion and lymphatic transport 32
3 Intermediary metabolism 33
4 Effect on plasma cholesterol 35
5 Effect on postprandial triglyceride response 35
6 Effect on plasma fasting triglyceride levels 36
7 Effect on weight control 36
8 Effect on diabetes risk 37
9 Effect on hypertension 37
10 Effect on exercise performance 37
11 Effect on immune response 38
12 Perspectives 38
References 38

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Diamant Building, Boulevard Auguste Reyers, 80 - 1030 Brussels, Belgique / Belgium
Tel: +32 2 733 98 88 • Fax: +32 2 733 04 13
e-mail: Info@fil-idf.org • Web site: http://www.fil-idf.org ISSN 0250-5118

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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. "fInventory of Microorgnaisms with a Documented History of Use in Food." *Bulletin of the International Dairy Federation* 377.

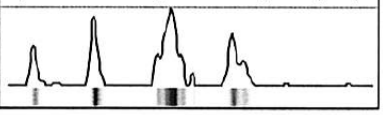
Appendix 4. Genetic Identity Test

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 Lactobacillus acidophilus DDS®-1 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.

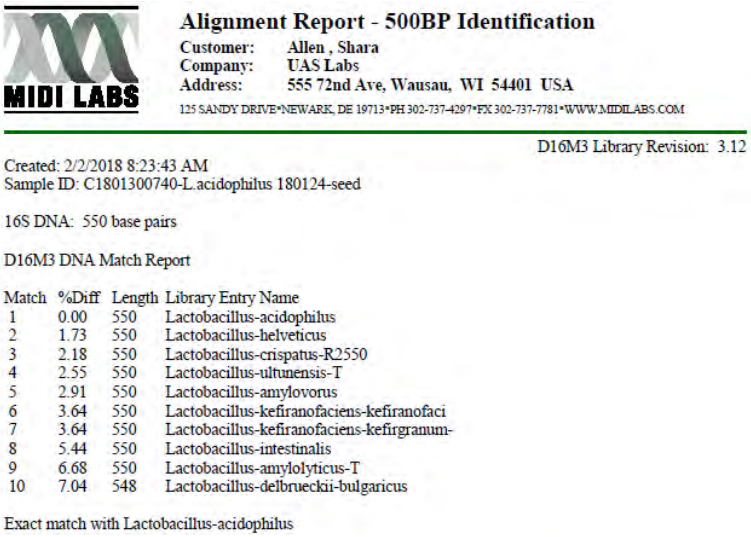
Riboprint pattern from master seed vial of *L acidophilus* DDS®-1

	RiboGroup	Number	Label	Custom ID Label	DuPont ID Label	RiboPrint™ Pattern
						1 kbp 5 10 15 50
1	ECORI 434-409-S-7	434-633-S-4	DDS-1 Master Seed	Lactobacillus acidophilus DDS-1	Lactobacillus acidophilus	

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 base pair 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 rDNA sequence analysis from master seed vial of *L acidophilus* DDS®-1



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: 2/2/2018 8:23:43 AM
Sample ID: C1801300740-L.acidophilus 180124-seed

16S DNA: 550 base pairs

D16M3 DNA Match Report

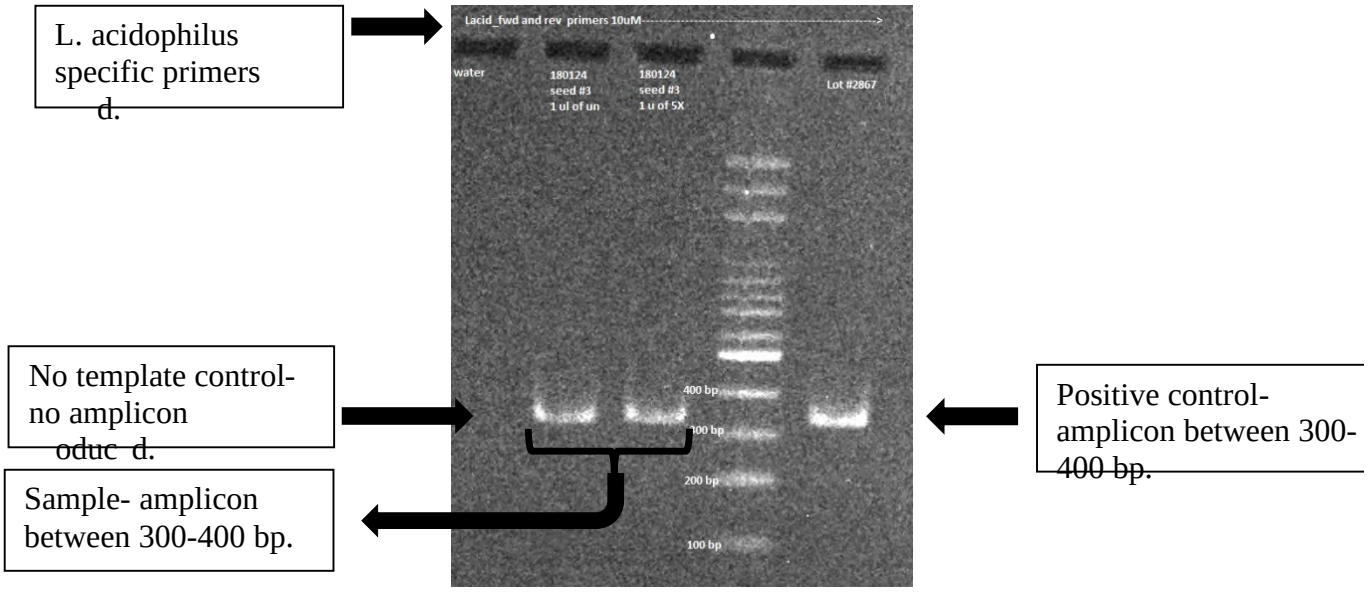
Match	%Diff	Length	Library Entry Name
1	0.00	550	Lactobacillus-acidophilus
2	1.73	550	Lactobacillus-helveticus
3	2.18	550	Lactobacillus-crispatus-R2550
4	2.55	550	Lactobacillus-ultunensis-T
5	2.91	550	Lactobacillus-amylovorus
6	3.64	550	Lactobacillus-kefiranofaciens-kefiranofaci
7	3.64	550	Lactobacillus-kefiranofaciens-kefirgranum-
8	5.44	550	Lactobacillus-intestinalis
9	6.68	550	Lactobacillus-amylolyticus-T
10	7.04	548	Lactobacillus-delbrueckii-bulgaricus

Exact match with Lactobacillus-acidophilus

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 - Master Seed Vial - *L. acidophilus* DDS®-1



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Appendix 5. Biogenic Amine Production Assessment

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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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

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

KEVIN O. GILLIES
CONSULTING SERVICES, LLC

1759 Grape St.
Denver, Colorado 80220
USA
Phone: +1 (816) 590 9836 | E-mail: kevin.o.gillies@gmail.com

September 25, 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 000871

Dear Dr. Hice:

Thank you for your email of September 23, 2019 asking for UAS Laboratories, LLC (UASLabs) input on questions that have arisen during FDA's review of GRN 000871. 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 000871:

1. **Please state whether *Lactobacillus acidophilus* strain "DDS-1" 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:

L. acidophilus DDS®-1 is deposited with the National Collection of Industrial, Food and Marine Bacteria, Bucksburn Aberdeen, Scotland UK (NCIMB; https://www.ncimb.com) under designation "NCIMB 30333".

2. **Please provide a list of food categories associated with the intended use. The GRAS notice includes dietary supplements in Part 3: Dietary Exposure (p.19-20) as well as in subsection 6.1: Intended Use of Part 6: Safety Narrative (p. 25-26). FDA**

does not consider GRNs for the use of dietary ingredients in dietary supplements. Please clarify the intended use.

UASLabs Response:

The intended use of *Lactobacillus acidophilus* DDS®-1 is as an ingredient in conventional foods for consumption by the general population, with the exception of USDA regulated meat and poultry products, that are compatible with the addition of live, safe and suitable food microbial cultures in accordance with cGMP levels of 10^9-10^{11} colony forming units(CFU) per serving. The uses include, but are not limited to, the following:

- Wet, chilled and ambient products such as fermented and non-fermented milks, fruit drinks, and plant-based food products
- Dry, chilled food products
- Dry and shelf-stable food products stored at ambient temperatures such as cereals, candy, bars, cookies, gums, and confectionary

GRN 000871 states in Part 3: Dietary Exposure (p. 19-20) the intended food ingredient use of DDS®-1 in relation to all current uses of *L. acidophilus* strains in the US for the purpose of discussing the total current dietary exposure to the strain and to *L. acidophilus*. UASLabs uses this comparison to demonstrate that the proposed usage rates for strain DDS®-1 in conventional foods are consistent with current market usage conditions. In addition, the usage rates and product descriptions for use as a dietary ingredient are intended as supporting documentation for the safe history of use of the ingredient at the indicated usage rates.

We are aware that FDA does not review GRAS notices for dietary ingredients, and the intended uses, outlined in GRN 000871 for FDA review, are explicitly limited to use as an ingredient in conventional foods. However, we feel that in assessing the safety of the intended usage, including the dietary exposure to an ingredient, it is important to acknowledge all of the potential exposure routes that are currently known for the ingredient or related strains including uses that are not the subject of the notice. In doing so, UASLabs explains that the proposed usage of DDS®-1 as an ingredient in conventional foods is consistent with current usage rates in the US for *L. acidophilus* strains in conventional food, as well as in supplements and, thereby, there is a safe history of use of the ingredient at the intended usage rates. In addition, such uses of DDS®-1 are envisioned to be direct replacements for other *L. acidophilus* strains currently in the marketplace, and will in all likelihood not result in an increase in overall dietary exposure to the strain, in particular, or to the species *L. acidophilus*, in general.

Similarly, in subsection 6.1: Intended Use of Part 6: Safety Narrative (p. 25-26), UASLabs discusses dietary ingredient use of *L. acidophilus* strains and DDS®-1 as supporting evidence for the safety of DDS®-1 for the intended use in conventional foods in the context of total dietary exposure to the ingredient class and safe history of use in food. Again, the information is provided as supporting evidence of safety of the use of DDS®-1 in conventional foods and not for the purpose of FDA review of the safety of the dietary ingredient use.

The combination of the safety of the species *L. acidophilus,* including the universal agreement that the species is safe for its traditional uses and safe history of use of DDS®-1 in fermented milks and supplements, the safe history of use of the species, in all forms of food including supplements, at the usage rates intended for DDS®-1 in conventional foods, the absence of genetic determinants for known microbial-related hazards, and the likelihood of no material increase in the total dietary exposure to *L. acidophilus*, in general, constitutes the evidence for the General Recognized as Safe for the intended use in conventional food conclusion documented in GRN 000871.

3. **Please state whether any of the raw materials used in the fermentation media and during production of *L. acidophilus* "DDS-1" are major allergens or derived from major allergens. Please state whether the final ingredient contains any major allergens.**

UASLabs Response:

UASLabs does not use major allergens or substances derived from major allergens as raw materials in the fermentation media for the production of *Lactobacillus acidophilus* DDS®-1. Further, the final formulation of *L. acidophilus* DDS®-1 does not contain any major allergens.

4. **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 *Lactobacillus acidophilus* DDS®-1 is entirely enclosed starting from the inoculation of the production fermentation vessel through the frozen pellet stage, the opportunity in the process for contamination monitoring is limited to the initial seed vial, the frozen pellet stage and the final milled powder finished product.

Each lot of seed vials is tested and must exceed product specifications listed in Table 3 (p.17). The release criteria for seed vials is absence of contaminants for all tests,

for example the *Enterococcus* specification is listed as <100/g, whereas seed vials must meet the more stringent specification of negative/g.

Each lot of production frozen pellets is tested for coliforms, *Enterobacteriaceae*, enterococci and non-lactic contaminants and must meet the release criteria for those organisms listed in Table 3 (p.17) in order to continue in the process to the lyophilization step.

Similarly, each lot of final milled powder, finished product is then tested for the product release specifications listed in Table 3 (p.17).

5. **Please specify the sample size for analysis of *E. coli*, *S. aureus*, and *Salmonella* as listed in Table 3 (p.17).**

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. 17.**

UASLabs Response:

Listeria listed on Table 3 (p.17) refers to *Listeria* species. If the test is positive in 25g for *Listeria* species, the lot is rejected. The rejected lot sample is then tested for *L. monocytogenes* per the UASLabs Food Safety Plan for cGMP purposes.

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 *Lactobacillus acidophilus* DDS®-1, demonstrating that the manufacturing process described in GRN 000871 can meet the specifications provided therein.

UAS Labs The Probiotic Company	UAS Laboratories, LLC **Certificate of Analysis** **Milled Powder, Finished Product, DDS®-1, 200B** **20 kg**	
Revision: 7	**Pages:** 1 of 1	

Item Number: 60004 Storage: 24 months when stored at 4°C or below

Production Lot#: [redacted]
Manufacture Date: 3/25/2019 Best By Date: 3/25/2021

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	0.067
Identification			
Identification	SOP-J61	*Lactobacillus acidophilus*	*Lactobacillus acidophilus*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	< 1,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 200 Billion CFU/g	286 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)	0.009 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] 04/09/19

Quality Signature / Date

UAS Laboratories, LLC
Certificate of Analysis
Milled Powder, Finished Product, DDS®-1, 200B
20 kg

Revision: 7 | **Pages:** 1 of 1

Item Number: 60004 Storage: 24 months when stored at 4°C or below

Production Lot#: [redacted]
Manufacture Date: 4/17/2019 Best By Date: 4/17/2021

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	PASS
Identification			
Identification	SOP-J61 AND 16s rRNA Gene Sequence	*Lactobacillus acidophilus*	*Lactobacillus acidophilus*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	<1,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 200 Billion CFU/g	233 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)	0.012 mg/kg
Mercury	EPA 3050/6020 USP <730>	NMT 0.05 mg/kg (ppm)	<0.01 mg/kg
Lead	EPA 3050/6020 USP <730>	NMT 1.0 mg/kg (ppm)	<0.005 mg/kg
Other Testing			
Gluten	M-SOP-Q54	Negative	Negative

[redacted] 6-13-19
Quality Signature / Date

UAS Laboratories, LLC
Certificate of Analysis
Milled Powder, Finished Product, DDS®-1, 200B
20 kg

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

Item Number: 60004 Storage: 24 months when stored at 4°C or below

Production Lot#: [redacted]
Manufacture Date: 2/20/2019 Best By Date: 2/20/2021

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%	0.013
Identification			
Identification	16s rRNA Gene Sequence	*Lactobacillus acidophilus*	*Lactobacillus acidophilus*
Purity Testing			
Non Lactics	M-SOP-Q13	< 5,000 CFU/g	< 1,000 CFU/g
Potency/Strength			
Total Viable Cell Count	M-SOP-Q23	≥ 200 Billion CFU/g	346 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)	0.013 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] 02/28/19

Quality Signature / Date

Please note, in the COAs, the addition of specifications for *Enterobacteriaceae* and coliforms that are not listed in the specification in Table 3 (p. 17). These specifications were added after the submission of GRN 000871.

Again, thank you for the opportunity to provide additional information regarding GRN 000871. Should you have further comments or questions, please feel free to contact me.

Sincerely,

Kevin O. Gillies