EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF

LACTOBACILLUS FERMENTUM LfQi6

AS A FOOD INGREDIENT

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March, 2020

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1. PART I. SIGNED STATEMENT AND CERTIFICATION

1.1. Basis of Conclusion

This GRAS conclusion has been reached in accordance with requirements in 21 CFR 170.220.

1.2. Name and address of organization:

Quorum Innovations, LLC
2068 Hawthorne, Suite 102
Sarasota, FL 34239

1.3. Name of substance:

The common name of the substance of this GRAS assessment is *Lactobacillus fermentum* strain designated as LfQi6. It will be sold under trade-name BellaCell® as an ingredient to finished food formulation manufacturers.

1.4. Intended conditions of use:

The subject of this GRAS, *Lactobacillus fermentum* LfQi6, a standardized powder, is intended for use as a food ingredient for consumers in the following food categories: dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and functional/nutritional products. The intended uses of *L. fermentum* LfQi6 includes addition at levels up to 2 x 10^8 colony forming units (cfu)/serving (reference amounts customarily consumed, 21 CFR 101.12). The *L. fermentum* LfQi6 powder that is the subject of this GRAS assessment is not proposed for uses in foods that are intended for infants, such as infant formulas or in products that are regulated by USDA.

1.5. Statutory Basis for GRAS conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.6. Exemption from Premarket approval requirements:

Quorum Innovations, LLC (Quorum) has concluded that *L. fermentum* LfQi6 is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that *L. fermentum* LfQi6, meeting the specifications cited herein, and when used as a food ingredient, is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that *L. fermentum* LfQi6, when used as described in this dossier, is GRAS based on scientific procedures.

1.7. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to the FDA upon request by contacting Dr. Monsul at the below address. The data and information will be made available to the FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Dr. Nicholas Monsul
Quorum Innovations, LLC
2068 Hawthorne, Suite 102
Sarasota FL 34239

Phone: (941) 951-0126
Email: nickmonsulmd@quoruminnovations.com

1.8. Data exempt from Disclosure:

Parts II through Part VII of this GRAS does not contain any data or information that is exempt from disclosure under the Freedom of Information Act. There is no privileged or confidential information such as trade secrets and/or commercial or financial information in this document. Therefore, if needed, all of the information contained in this dossier can be made publicly available.

1.9. Certification:

Quorum certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by Quorum, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of *L. fermentum* LfQi6. Quorum accepts responsibility for the GRAS conclusion that has been made for *L. fermentum* LfQi6 as described in this dossier.

1.10. Name, position/title of responsible person who signs dossier and signature:

Dr. Nicholas Monsul
Quorum Innovations, LLC
2068 Hawthorne, Suite 102
Sarasota, FL 34239

Phone: (941) 951-0126
Email: nickmonsulmd@quoruminnovations.com

Signature: 

1.11. FSIS/USDA – Use in Meat and/or Poultry:

Quorum does not intend to add *L. fermentum* LfQi6 to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

2. PART II. IDENTITY AND TECHNICAL INFORMATION

2.1. Description of GRAS Organism

The subject of this GRAS assessment, *Lactobacillus fermentum* LfQi6 is a rod shaped, Gram-positive, non-spore forming, obligate heterofermentative bacterium derived from the human microbiome. It is manufactured as a free-flowing, cream to light beige, powder with a characteristic cultured, lactic malty odor. General descriptive parameters and properties of the *L. fermentum* LfQi6 preparations manufactured by Quorum are summarized in Table 1.

In general, the Lactobacilli are a highly heterogeneous taxonomic group, encompassing species with a wide range of genetic, biochemical and physiological properties. The genus *Lactobacillus* comprises the rod-shaped lactic acid bacteria. Several species of this genus are introduced in the food chain, in a range of food and feed fermentations products for humans and animals. These species are rod-shaped, non-motile and non-spore forming bacteria. Based on phylogenetic molecular taxonomy, 16S rRNA gene sequence, and comparative genomic analysis, the members of this genus and species are assigned to particular strains.

Table 1. General Descriptive Characteristics of *L. fermentum* LfQi6

Parameter	Description *
Organism	*Lactobacillus fermentum* LfQi6
Origin	Isolated from human microbiome
Physical characteristics	Dried powder
Odor	Cultured, lactic, malty
Shelf Life	12 months
Storage Condition	Refrigerated at 35-42ºF (2-5ºC)

*Based on information provided by Quorum

Lactobacillus fermentum is a member of the lactic acid bacteria (LAB) classification, a group related by the production of lactic acid as the major metabolic end product of carbohydrate metabolism and other physiological traits. These bacteria are not a defined taxonomic group; rather it is a functional grouping, and thus, the boundaries are controversial. Among the core genera classified as LAB are Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, and Streptococcus (Axelsson, 2004). Most LAB are considered to be non-pathogenic and have a long history of safe use in fermented and non-fermented foods (Axelsson, 2004; Douillard and De Vos, 2014). LAB are Gram-positive and generally non-spore forming, catalase negative, and devoid of cytochromes. LAB are of nonaerobic habit, but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative, forming lactic acid as the major end product of sugar fermentation (Holzapfel et al., 2001). The genus, *Lactobacillus*, the largest of the LAB genera, contains over 80 species. It may be categorized into three groups, obligate homo-fermentative, facultative hetero-fermentative, and obligate hetero-fermentative (Axelsson, 2004).

The name *L. fermentum* is given as it causes fermentation and is an obligatory heterofermentative species. It is Gram-positive, non-motile, rod-shaped (Figure 1), non-sporulating facultative anaerobic bacteria. Many *Lactobacillus* species have found applications in the food industry.

The hierarchical classification or taxonomic assignment of *L. fermentum* LfQi6 is presented in Table 2. The phenotypic and genotypic characteristics of *L. fermentum* LfQi6 have been established by Quorum.

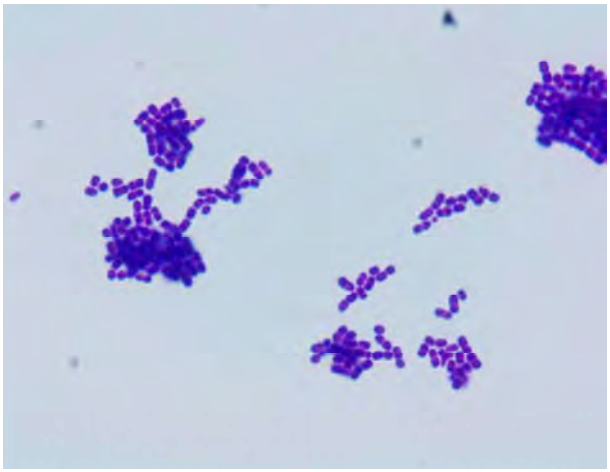

Figure 1. Typical Picture of *Lactobacillus fermentum* LfQi6 under Gram Stain (100x magnification)

Table 2. Taxonomical Lineage of *Lactobacillus fermentum* LfQi6

Taxonomy	Taxonomic Assignment
Kingdom	Bacteria
Phylum	Firmicutes
Class	Lactobacillales
Order	Lactobacillales
Family	Lactobacillaceas
Genus	*Lactobacillus*
Species	*Lactobacillus fermentum*
Strain	*Lactobacillus fermentum* LfQi6

2.1.1. Identification and Characteristics

A series of key characteristics of *L. fermentum* LfQi6 strain have been studied. This information demonstrates that *L. fermentum* LfQi6 is fully characterized. As indicated above the strain is a rod shaped, Gram-positive, aerobic organism appearing as individual rods or occasionally in short chains. *L. fermentum* LfQi6 strain has been deposited with the American Type Culture Collection (ATCC). The deposit has been assigned accession number ATCC No. PTA-122195 by the repository and was deposited on June 10, 2015.

2.1.1.2. Phenotypic Identification

The *L. fermentum* strain LfQi6 was initially identified at the species and strain level using whole genome sequencing as well as standard microbiological, biochemical and phenotypic techniques (Berkes et al., 2019). The phenotypic characteristics as evaluated by morphological, physical, biochemical and physiological characteristics for *L. fermentum* LfQi6 are presented in Table 3. In a starch hydrolysis test, *L. fermentum* LfQi6 demonstrated positive amylase activity showing starch degradation on and around the colony.

Table 3. Phenotypic Characteristics of *Lactobacillus fermentum* LfQi6*

Parameters	**Characteristics**
Microscopy	Bacillus (rod)
Gram-staining	Positive

Motility	Non-motile
Lactic acid production	Positive
Starch hydrolysis test	Positive
Sugar Fermentation Tests	
Glucose	Positive
Dextrose	Positive
Sucrose	Positive
FOS	Positive
Maltose	Positive

*Based on information provided by Quorum (2019)

2.1.1.2. Phylogenetic and Genotypic Identification

Phylogenetic multiple sequence alignments were performed using the 16S rRNA sequences of the indicated Lactobacillus strains on Clustal Omega (Larkin et al., 2007; Goujon et al., 2010). For *L. fermentum* LfQi6 isolate alignments, whole genome alignments were performed using the NCBI whole genome alignment tool (Dewey, 2012). A phylogenetic analysis performed by aligning the 16S rRNA gene sequences from the representative *Lactobacillus* species on PATRIC is depicted in Figure 2 (Wattam et al., 2017). *L. fermentum* LfQi6 clusters with other *L. fermentum* human microbiota species as well as *L. reuteri* which, until recently, was classified as a *L. fermentum* isolate (Reuter, 1965). Further whole genome sequencing alignment performed on NCBI (Altschul et al., 1990) shows LfQi6 phylogenetic placement among the available *L. fermentum* strains for which whole draft genome sequencing data is publicly available and its evolutionary distance from *L. fermentum* IFO 3956, used as the scaffold for *L. fermentum* LfQi6 contig generation. The results of phylogenetic analyses show *L. fermentum* LfQi6 evolutionary relatedness to reference *Lactobacillus* species (Figure 2A) and *L. fermentum* human microbiome isolates (Figure 2B).

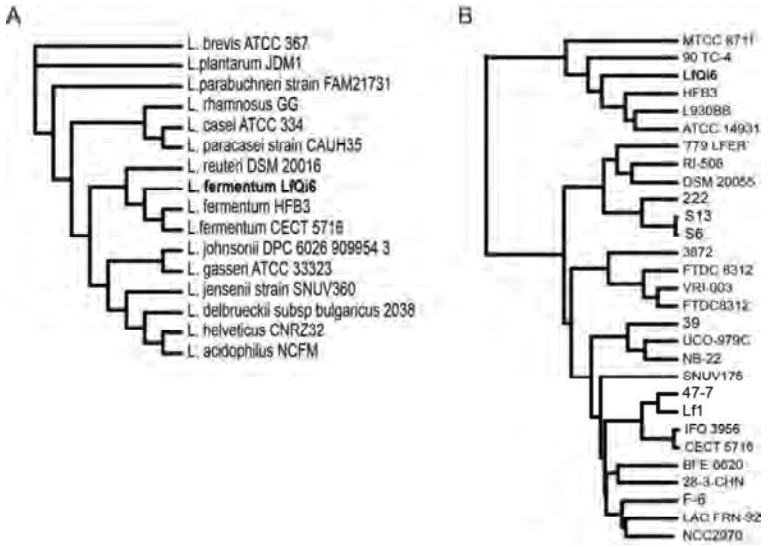

Figure 2. Phylogenetic Tree of *Lactobacillus fermentum* LfQi6 with Respect to (A) *Lactobacillus* Species and (B) *L. fermentum* Strains

Subhadra et al. (2015) published draft whole genome sequence of *L. fermentum* LfQi6. In this study, genomic DNA was used for the indexed Nextera XT sequencing library with 300- to 600-bp insert size and to prepare a Nextera mate-pair (4 to 10 kb) sequencing library. In this

study, pooled paired-end libraries were loaded onto MiSeq Flow Cell to generate 2 x 150-bp paired-end reads with average coverage of 250x per genome. Pooled libraries were loaded on the MiSeq platform and sequenced with 2 x 150-bp paired-end reads to obtain at least 50x coverage of the genome. Illumina sequence reads were demultiplexed, raw reads were converted into FASQT format, and low-quality and short reads were filtered out. MiSeq Reporter software filtered and demultiplexed the sequencing reads, giving 2,344,249 paired-end (2 x 150-bp) and 940,098 mate-pair reads (2 x 150-bp). The genome sequence of *L. fermentum* LfQi6 has been deposited in GenBank with accession number LAIK00000000.1.

The filtered reads were de novo assembled to generate contigs, combining paired-end and matepair read data. Sequences were trimmed using trim galore software to give 2,309,153 paired-end and 562,149 mate-pair reads and de novo assembled using Velvet and SPAdes. Assemblies with the largest N_{50} and/ or contig size were BLASTed against GenBank. BLAST results show that the sequenced sample has 99% identity with the three published *L. fermentum* genomes at the nucleotide level (Morita et al., 2008; Jimenez et al., 2010; Grover et al., 2013). Further BLAST searches suggest the possible presence of repeats and genomic rearrangements. The best contig assembly was scaffolded based on the *L. fermentum* IFO 3956 genome (AP008937) (Morita et al., 2008), using Scaffold_builder and a de novo approach using SSPACE.

The final assembly of *L. fermentum* LfQi6 is 2.21 Mbp with 37 scaffolds; the genome N50 is 252,403; the largest scaffold is 484,720 bp with a mean scaffold of 59,600 bp (Subhadra et al., 2015). The assembled genome was annotated with 2,012 coding sequences and 64 RNA genes. For the function-based comparison, 1,070 coding sequences were present in both *L. fermentum* IFO 3956 and the LfQi6 genome, with 64 coding sequences present only in *L. fermentum* IFO 3956 and 101 coding sequences present only in the *L. fermentum* LfQi6 genome. The investigators identified unique protein-coding sequences in the *L. fermentum* LfQi6 genome, including fibronectin-binding proteins, the cold shock protein CspB, the GTP-binding protein HflX, the Clp protease-like protein, murein hydrolases, and several para logs of DNA-repair proteins.

In addition to the above described published draft genome sequence, in an unpublished report (Appendix I), genome *L. fermentum* LfQi6 derived was of estimated size 1831981 base pairs, with GC content of 51%, an N50 of 223380 and an L50 of 3; all of which are indicating a very high-quality draft genome. Using RAST analysis of the closest genome to this was determined as *L. fermentum* IFO 3956 on RAST genome annotation server. This genome is consistent with the identity and classification as a *L. fermentum*, based upon genome similarity. Analysis of the genomes full 16s sequence using NCBI blast was performed. The top 100 hits were all identified as *L. fermentum* further indicating this DNA was derived from a *L. fermentum*.

2.2. Specifications

Food grade specifications of *L. fermentum* LfQi6 have been established by Quorum and are summarized in Table 4. Analytical results from three non-consecutive lots (Appendix II) demonstrate that the *L. fermentum* LfQi6 preparation is consistently manufactured to meet the standard food grade specifications. The purity of the bacterial culture is routinely checked by contamination testing based on generally recognized microbial limits. The mother cultures are

adequately maintained and the working cultures are derived from the mother culture. The genetic drift of *L. fermentum* LfQi6 will be monitored when the working cultures prepared.

Table 4. Specifications of *Lactobacillus fermentum* LfQi6 Powder

Parameters	Characteristics (Quorum, 2019*)	Test/Method
Description	Cream to light beige powder	Visual
Enumeration	Minimum $1x10^{11}$ (100 billion) cfu/g	SMEPD 17th Edition ISO 7889:2003
Moisture	6% maximum	AOAC #2008.06
Heavy Metals		
Arsenic	NMT 1.0 ppm	EPA 3050/6020 USP730
Lead	NMT 1.0 ppm	EPA 3050/6020 USP730
Cadmium	NMT 1.0 ppm	EPA 3050/6020 USP730
Mercury	NMT 1.0 ppm	EPA 3050/6020 USP730
Other Microbiological Assays		
Yeast & Mold count	NMT 100 cfu/g	BAM CH. 18
Coliforms	NMT 10 cfu/g	BAM CH. 4
Escherichia coli	None detected	BAM CH. 4
Salmonella	None detected	AOAC #999.08
Pseudomonas aeruginosa	None detected	Internal method
Staphylococcus (Coag Pos)	None detected	AOAC #975.55
Listeria	None detected	AOAC #996.14
Allergens		
β-Lactoglobulin	<0.1 ppm	ELISA systems
Casein	<0.28 ppm	ELISA systems
Soy	<2.5 ppm	ELISA systems

*Based on information provided by Quorum (2019). NMT = Not more than; cfu = colony forming units; ppm = parts per million

2.3. Manufacturing Process

The preparation containing *Lactobacillus fermentum* LfQi6 is manufactured at Jeneil Biotech, Inc. (400 North Dekora Woods Boulevard, Saukville, Wisconsin 53080), in accordance with current good manufacturing practices (cGMPs). The FDA food facility registration number is 149444840208. The manufacturing process is schematically presented in Figure 2.

The raw material ingredients for fermentation are selected, weighed, sterilized, cooled, and inoculated with *L. fermentum* LfQi6. The inoculum is incubated under constant temperature, pH, aeration, and agitation to achieve the fermentation endpoints. Once the endpoints have been reached, the bacterial cells are concentrated and recovered using centrifugation. The cells are then washed, stabilized, pelletized, dried, and milled. The milled dried concentrate is packaged and stored at refrigerated conditions until enumeration and all quality tests have been completed. The dried concentrate is then blended with food grade excipients for standardization to obtain a specified guaranteed cell concentration in the final product. The final product is stored under refrigerated conditions and tested periodically for stability.

The processing aids, fermentation medium and diluents used in the manufacturing of *L. fermentum* LfQi6 are either approved as food additives or are GRAS substances. The finished product is prepared from the approved concentrated product and food grade diluents, including but not limited to identity preserved maltodextrin, to comply with the finished product

specifications. These diluents are safe for the intended uses. The manufacturing facility is GMA-SAFE compliant (Assessment ID: 1625526).

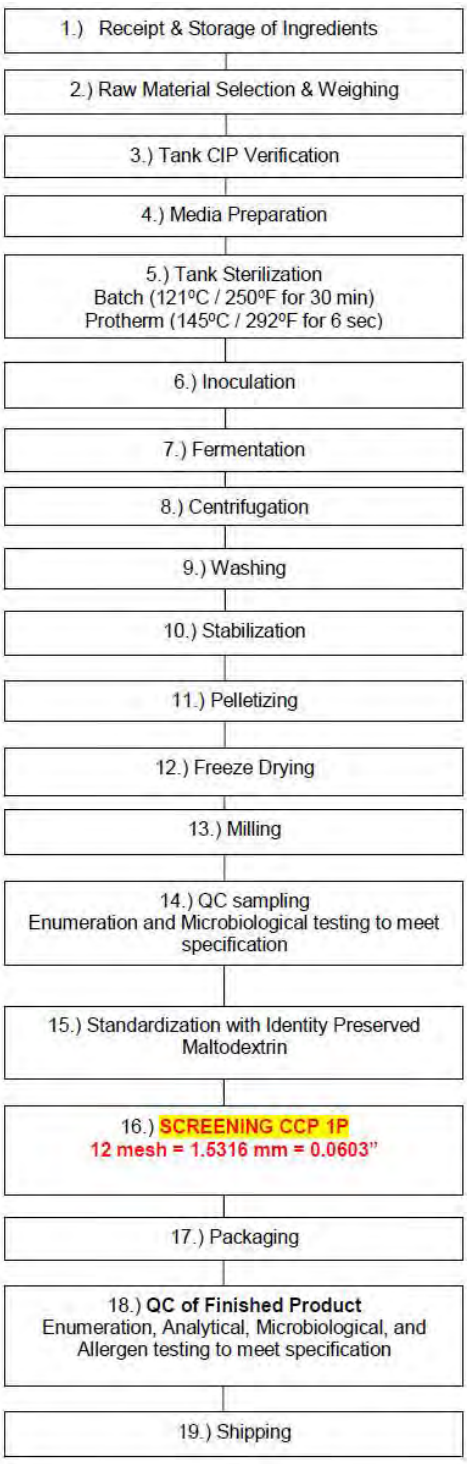

Figure 2. Manufacturing process of *Lactobacillus fermentum Lf*Qi6 preparation

3. PART III. DIETARY EXPOSURE

3.1.1. Intended Use Levels and Food Categories

Quorum intends to use *L. fermentum* LfQi6 as a food ingredient to conventional foods at concentrations consistent with cGMP needed to provide at least $2x10^8$ cfu/serving (reference amounts customarily consumed, 21 CFR 101.12) throughout the shelf life of the product. The addition level will usually be between $5x10^8$ and 10^{10} cfu/serving, which provides for the loss of viability of the bacteria added.

The foods to which *L. fermentum* LfQi6 is intended to be added are those foods that can sustain viable *L. fermentum* LfQi6 for the shelf life of the food, including but not limited to dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and functional/nutritional products, when not precluded by a standard of identity. Foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, and meat and poultry products that come under USDA jurisdiction are excluded from the list of intended food uses of *L. fermentum* LfQi6 preparation.

3.1.2. Estimated Daily Intake from the Intended Uses

In a recent GRAS notice (GRN 840) that received no question letter from FDA, Arla Foods Ingredients (Arla, 2018) proposed use of *L. paracasei* ssp. *paracasei* strain F-19 in the same food categories and at same levels as those mentioned above for *L. fermentum* LfQi6. Given the same uses and food categories, the intake estimates provided in GRN 840 are applicable to the present GRAS assessment for *L. fermentum* LfQi6. The intake estimate, as described and agreed upon by FDA, is based on the consideration that mean food consumption is about 20 food servings/day (Millen et al., 2006). This estimate allows for ten or more servings of foods or drinks containing the proposed food ingredient (i.e., *L. fermentum* LfQi6) and this is an extremely conservative estimate.

L. fermentum LfQi6 is expected to be present in a limited number of foods normally at levels $2x10^8$ cfu/serving (at the time of consumption) and to achieve this it may be added at levels between $5x10^8$ and 10^{10} cfu/serving. Based on these assumptions and considering that a person consumes maximum 10 servings of foods containing *L. fermentum* LfQi6, the resulting daily maximum intake will be $2x10^9$ cfu/day. *L. fermentum* LfQi6 will not proliferate in the foods and beverages to which it is added, but instead will decline over the shelf-life of the food. Its likely maximum ingestion is thus less than $2x10^9$ cfu/day. For safety assessment purposes, the high intake of $2x10^9$ cfu of *L. fermentum* LfQi6/person/day is considered. Based on the available scientific information, and, as discussed below, the estimated daily intake of *L. fermentum* LfQi6 preparation, if ingested daily over a lifetime, is considered as safe based on scientific procedures.

4. PART IV. SELF LIMITING LEVELS OF USE

There are no self-limiting intake levels but the use is restricted to foods that can sustain living *L. fermentum* for the shelf life of the food. Additionally, excessive amounts of Quorum's *Lactobacillus fermentum* LfQi6 product is unlikely to be added to food products because of the cost of the product.

5. PART V. EXPERIENCE BASED ON COMMON USE IN FOODS BEFORE 1958

The statutory basis for the conclusion of GRAS status of Quorum's *Lactobacillus fermentum* LfQi6 in this document is not based on common use in food before 1958. The GRAS conclusion is based on scientific procedures. As described below, *Lactobacillus fermentum* has been commonly present in food prior to 1958, providing support that *Lactobacillus fermentum* has been safely used in food products prior to 1958.

6. PART VI. NARRATIVE

6.1. Traditional and Current Safe Uses

The available information suggests that since historical times humans are regularly consuming live lactic acid bacteria (LAB) present in lactic acid fermented foods. The archaeological signs indicate that humans have historically consumed large numbers of live LAB. All through times up until the industrial revolution, lactic acid fermentation was applied as the simplest and often the safest way of preserving food. This suggest that the human gastrointestinal tract has evolved to adapt to a daily supply of live LAB. Since human started using fermented milk as food, *Lactobacilli* have been consumed on a daily basis (Salminen et al., 1998). As *Lactobacilli* are normal inhabitants of green plant material, it is almost certain that these bacteria are widely consumed even before that time. *Lactobacilli* have played a crucial role in the production of fermented products for millennia. In a review article, Bernardeau et al. (2006) reported that in healthy humans, "*lactobacilli* are normally present in the oral cavity (10^3-10^7 cfu/g), the ileum (10^3-10^7 cfu/g), and the colon (10^4-10^8 cfu/g) and they are the dominant microorganism in the vagina." Many strains of *Lactobacillus* have been used since ancient times in the manufacture of fermented foods. This would indicate a relationship between humans and *Lactobacillus* that stretches back as far as 8000 years.

The scientific rationale behind fermentation started with the identification of microorganisms in 1665 by Van Leeuwenhoek and Hooke. During the 1880s, Sir John Lister showed the role of a sole bacterium, "Bacterium" lactis (*Lactococcus lactis*), in fermented milk. Subsequently, Louis Pasteur defined fermentation, from the Latin word fervere, as "La vie sans l'air" (life without air). In 2002, an authoritative list of microorganisms (starter species) with a documented use in food was established by the International Dairy Federation (IDF) and the European Food and Feed Cultures Association (EFFCA)[1]. The genus *Lactobacillus* was already widely present in the initial inventory.

The available information suggests that for several decades, products containing *Lactobacillus* cultures have been used without safety concerns. The IDF-EFFCA list includes *Lactobacillus fermentum* reporting that it has been used in food (Bulletin of the International Dairy Federation). A recent IDF Bulletin provides an update to the earlier inventory of microbial species, taking a global perspective versus the original focus of European dairy products. The updated inventory lists 82 species of *Lactobacillus* (Bourdichon et al., 2012). In summary, *Lactobacillus* strains have a history of use in food production, with large levels of viable bacteria present in many foods, including yogurt, fermented milk, and cheeses. *L. fermentum* specifically is documented as used in ancient Egypt in fermented milk, and today as naturally present or as a starter culture in fermented milk products (Bernardeau et al., 2006).

In a 2001 report, Food and Agriculture Organization and World Health Organization expert consultation noted that lactobacilli have a long history of use without established risk to humans, and this remains the best proof of their safety and concluded that, "no pathogenic or

[1] Available at: http://www.effca.org/sites/effca.drupalgardens.com/files/2002-Inventory-of-Microorganisms-with-a-documented-history-of-use-in-food.pdf

virulence properties have been found for lactobacilli" (FAO/WHO, 2001). Cabana et al. (2006) reported that the optimal dose of lactobacilli remains an area of active investigation and no specific pediatric dose has been established in general. However, there are no known reports of toxicity associated with exceeding a specific dose in either adults or children. *Lactobacilli* and other similar bacteria become undetectable a few days after stopping the administration, thus indicating that these bacteria do not colonize the gastro-intestinal tract (Vandenplas et al., 2007). This also suggests the absence of any risk for long-term side effects. Several studies have demonstrated that lactobacilli are not recovered from feces by 1-2 weeks after administration ceases.

In the US, as dietary supplements[2], *Lactobacillus fermentum* has been in use before 1994. As a supplement, *L. fermentum* and its preparations are marketed under the Dietary Supplement Health and Education Act (DSHEA, 1994). The available information from the National Institute of Health reveals: two ingredient name(s) which contain "*Lactobacillus fermentum*"; and 52 products which contain "*Lactobacillus fermentum*" anywhere on the label (ODS/NLM, 2019). Common conditions of use for such products containing a single strain of *L. fermentum* include: dose range of 6 x 10^9 to 8 x 10^9 cfu/serving, one serving/day, intent for chronic use, an oral route of administration, a capsule delivery format, and is recommended to be consumed with meals or on an empty stomach. *L. fermentum* species are also used as a dietary ingredient product that contain multi-species blends. In a New Dietary Ingredient Notification (NDIN), Danisco USA Inc. proposed the use of *L. fermentum* SBS-1 at use levels up to 2 x 10^9 cfu/serving/day. Following its review, the FDA filed this NDIN on March 3, 2018 under the number 1061. These uses indicate that *L. fermentum* is unlikely to cause adverse effects.

In addition to above described uses, *L. fermentum* species has been isolated from various traditionally made fermented foods whose origins date back to ancient times from different countries, including Indian 'Dosa', Vietnamese 'Dua muoi', Tanzanian 'Togwa', batter (Soni et al. 1986; Mugula et al., 2003; Nguyen et al. 2013). In a recent review article, Nagmouch et al. (2019) described an account on the application of *L. fermentum* strains in the biomedical and food preservation fields. The available information suggests that several products are made from utilization of *L. fermentum* that have been consumed by humans for thousands of years to present times. This also suggest that *L. fermentum* species have been safely used by humans through the production and consumption of a variety of fermented foods from dairy, grain and plant materials (Swain et al., 2014; Ray et al., 2016).

6.2. Safety and Toxicity Studies

In several *in vitro* and *in vivo* studies, including human trials, the safety of a number of strains of *L. fermentum* has been demonstrated.

6.2.1. Specific Studies with *Lactobacillus fermentum* LfQi6

Evaluation of *L. fermentum* LfQi6 according to guidelines adopted by the European Food Safety Authority (EFSA 2007, 2012, 2015) and by FAO/WHO (2001) demonstrates strain safety. A standard safety evaluation of microorganisms, via antimicrobial resistance pattern

[2] See: https://www.dsld.nlm.nih.gov/dsld/rptQSearch.jsp?item=Lactobacillus+fermentum&db=adsld

determination and assessment for potentially harmful metabolic activities, such as biogenic amine production, mucin degradation, various enzymatic activities and pathogenic hemolytic activity, was undertaken. None of these experiments reveal any safety concerns (Berkes et al., 2019). Some of these *in vitro* studies are further described below. These *in vitro* studies support the safety-in-use of *L. fermentum* LfQi6.

6.2.1.1. Specific Antibiotic Susceptibility Studies

In an *in vitro* study, antibiotic susceptibility of *L. fermentum* LfQi6 was investigated using BD BBL™ Sensi-Disc™ antimicrobial susceptibility test discs, according to the manufacturer's instructions (Berkes et al., 2019). The assay was performed as per the disc manufacturer instructions and an in-house established standard operation procedure (SOP). The antibiotics tested were ampicillin (10 µg), amoxicillin with clavulanic acid (Amoxi-Clav; 20/10 µg), cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin [2 µg], daptomycin [30 µg] erythromycin [15 µg], fosfomycin [200 µg], gentamycin (10 µg), imipenem (10 µg), linezolid (30 µg), meropenem (10 µg), oxacillin (1 µg), penicillin G (10 U), rifampin (5 µg), tetracycline (30 µg), SMZTMP (5 µg) and vancomycin (30 µg), in accordance with EFSA 2012 recommendations (Bover-Cid and Holzap, 1999; FEEDAP, 2012).

The findings of antibiotic susceptibility tests with minimum inhibitory concentration (MIC) values are summarized in Table 5. The MIC is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic. *L. fermentum* LfQi6 displays antibiotic susceptibility typical of a generally considered safe LAB strain, with only the intrinsic resistance pattern expected for lactobacilli observed, with resistance to cefoxitin, fosfomycin, gentamycin, sulfamethoxazole and trimethoprim (SMZ-TMP) and vancomycin (Berkes et al., 2019).

Table 5. Antibiotic Susceptibility Testing for *Lactobacillus fermentum* LfQi6

Antibiotics	Dose (µg)	Planktonic Sensitivity	Basis of Resistance	Biofilm Sensitivity
Amoxi-Clav	20/10	S		
Ampicillin	10	S		S
Cefoxitin	30	R	Intrinsic	
Chloramphenicol	30	S		
Ciprofloxacin	5	S		
Clindamycin	2	S		
Daptomycin	30	S		
Erythromycin	15	S		S
Fosfomycin	200	R	Intrinsic	
Gentamicin	10	R	Intrinsic	
Imipenem	10	S		
Linezolid	30	S		
Meropenem	10	S		S
Oxacillin	1	S		
Penicillin (Units)	10	S		
Rifamopin	5	S		
Tetracycline	30	S		
SMZ-TMP	5	R	Intrinsic	
Vancomycin	30	R	Intrinsic	

S = sensitive; R = resistant. Doses in micrograms except where noted

6.2.1.2. Specific Hemolysis Assay

Hemolytic activity, if any, of *L. fermentum* LfQi6 was evaluated using sheep's blood agar plates (5% defibrinated sheep's blood), and incubated at 30°C for 48 hours (Berkes et al., 2019). Recorded characteristics of hemolysis on blood agar were β-hemolysis (clear zones around colonies), α-hemolysis (green zone around colonies), and γ-hemolysis (no halo around colonies) (Noriega et al., 2006). The assay was performed in duplicate. The findings from this study show that *L. fermentum* LfQi6 is non-hemolytic (or gamma-hemolysis) (Berkes et al., 2019).

6.2.1.3. Specific Biogenic Amine Production Assay

Biogenic amine production of tyramine, histamine and putrescine was assessed using the decarboxylase agar method (Bover-Cid and Holzapfel, 1999). Precursor amino acids (tyrosine, histidine and ornithine, respectively) were purchased from Sigma, MO. *L. fermentum* LfQi6 was inoculated onto decarboxylase plates and incubated for four days at 37°C under aerobic and anaerobic conditions. A positive result was defined as a color change of the medium from yellow to purple due to pH shift based on production of alkaline biogenic amines from the amino acids present in the medium. The assay was conducted in duplicate. The findings of biogenic amines were compared with *Lactobacillus rhamnosus GG* (LGG) strain.

Like LGG, *L. fermentum* LfQi6 does not possess amino acid decarboxylase activity capable of generating potentially harmful biogenic amines such as histamine and tyramine. These results are in agreement with previous *L. fermentum* GRAS assessment as described in GRN 531 for *L. fermentum* CECT5716 (Biosearch, 2014) and evaluated by FDA (2015) without any question.

6.2.1.4. Specific Mucin Degradation Assay

Mucin degradation was studied using 0.3% mucin supplemented agarose medium with or without glucose as described in a publication by Zhou et al. (2001). In brief, cells were grown overnight in MRS broth at 37^0C under aerobic conditions and spotted on Medium B plates. The pH of medium was adjusted to 7.0 with NaOH. Mucin degradation activity was evaluated by the diameter of the halo observed after plate staining with amido black in glacial acetic acid and washing with glacial acetic acid. A stool sample collected from a two-month old infant was used as a positive control. The findings of this study revealed that *L. fermentum* LfQi6 was non-mucinolytic.

6.2.2. Safety Based on Genome Sequencing and Bioinformatics

As mentioned earlier, in an unpublished report, a bioinformatics safety assessment of *L. fermentum* LfQi6 was carried out based on the whole genome sequence (Appendix II). The findings from this analysis revealed: (1) positive identification of the strain; (2) 16S rRNA confirmation and sequence provided; (3) annotation is provided using RAST; (4) no virulence genes, transposable elements, nor pathogenicity islands found; (5) no significant, unexpected antibiotic resistance genes were found; and (6) subsystems screening in SEED revealed nothing remarkable from a safety point of view.

6.2.2.1. Virulence Factors and Transposable Elements

As mentioned earlier, in an unpublished report, a bioinformatics safety assessment of *L. fermentum* LfQi6 was carried out based on the whole genome sequence. In an attempt to screen for virulence factors in *L. fermentum* LfQi6, the contigs were annotated using RAST (Rapid Annotation using Subsystem Technology), which includes an up-to-date collection of virulence factors. RAST is an annotation tool for bacterial and archaeal genomes and provides a high-quality annotation.

Based upon the RAST annotation there were no identified virulence factors found within the genome sequence. No transposable elements were found, no pathogenicity islands identified, and standard prophage elements were identified as follows: Phage tail proteins (3); Phage replication (5); Phage packaging machinery (7); Phage tail proteins 2 (5); and Phage capsid proteins (6). In summary, based on RAST analysis no virulence or transposable elements were identified that not also present in similar form in other known *L. fermentum* strains.

6.2.2.2. Antibiotic Resistance Gene

One important consideration to determine the safety of *L. fermentum* is transferable resistant genes. For *L. fermentum* to be considered for its potential uses in food for humans, it must not contain any transferable resistant genes. If a resistance gene is transferable, it could lessen the effect of the use of antibiotics. Out of ten common antibiotic genes that were tested (gentamicin, cefazolin, penicillin, trimethoprim/sulfmethoxazole, ampicillin, carbenicillin, erythromycin, amikacin, chloramphenicol, and norfloxacin), *L. fermentum* was found to only be resistant to amikacin and norfloxacin. Other studies have reported that most LABs (lactic acid bacteria) are also resistant to these antibiotics, which led to the conclusion that it was a common characteristic of LABs. The resistance to these antibiotics can be considered natural or intrinsic. So far, no observed *L. fermentum* strains have been observed to have transferable resistance or acquired resistance genes.

6.2.3. Safety Related Studies of Other Strains of *L. fermentum*

In addition to the above described specific studies with *L. fermentum* LfQi6, in several studies (including *in vitro*, animal and human studies), safety and efficacy of *L. fermentum* has been investigated. Some of the relevant (safety related) studies with *L. fermentum* are described below.

6.2.3.1. Studies with *L. fermentum* CECT 5716

In multiple studies, the safety of *L. fermentum* CECT 5716, isolated from human milk of healthy mothers, has been extensively investigated. Some of the relevant studies with this strain are described below. As mentioned earlier, and also described below, this strain has been the subject of a GRAS notification (GRN 531) for its use in term infant formula. The available studies with this strain suggest that intake of *L. fermentum* species at doses identical to, or near to, the proposed dose for *L. fermentum* strain LfQi6 are safe and well tolerated in humans.

In a human study, Olivares et al. (2007) investigated the safety of *L. fermentum* CECT 5716. In this, randomized, double-blinded, placebo-controlled study, 50 volunteers (31 male and

19 female) participated to address the immunologic effects of an intramuscular anti-influenza vaccine in adults (33±7.7-year-old). Fifty percent of volunteers received an oral daily dose of methylcellulose (placebo) or *L. fermentum* CECT 5716 ($1x10^{10}$ cfu/day) for two weeks before vaccination and two weeks after vaccination. The investigators noted that the intake of this strain was safe and well tolerated over the study period. This was evident by a lack of adverse events reported that were associated with consumption of *L. fermentum* CECT 5716 and the fact that no participants had to be removed or dropped from the study. Further, compliance was demonstrated to be good for both groups and was confirmed by fecal detection of the *L. fermentum* CECT 5716 strain which was shown to be present in 92% of participants in the supplement group compared to only 12% of participants in the placebo group. This study indicates that intake of the *L. fermentum* species is safe and well tolerated in adult humans.

Maldonado et al. (2012) investigated safety of follow-on formula containing *L. fermentum* CECT 5716 ($1x10^{7}$ cfu/g) taken daily at an average dose of $2x10^{8}$ cfu/day by healthy 6-12 months old formula-fed infants for six months. In this randomized double-blinded controlled study, 188 infants that completed the study were assigned to either follow-on formula supplemented with *L. fermentum* plus galactooligosaccharide (GOS), or the same formula supplemented with only GOS (control group). The main outcome was the incidence of infections for the 6-month duration of the study. The consumption of the formula did not cause adverse events and no between-group differences were noted in growth and development (weight, length, and head circumference), indicating that both study formulas were safe. There were no dropouts or withdrawals from the study, indicating that both study formulas were well tolerated.

In another study, similar to the one described above (Maldonado et al., 2012), Gil-Campos et al. (2012) investigated the safety and tolerance of infant formula supplemented with *L. fermentum* $1x10^{7}$ cfu/g consumed by healthy formula-fed 1-6-month-old infants for five months. In this randomized double blinded controlled study, 121 healthy one-month old infants received a prebiotic infant formula supplemented with *L. fermentum* or the same formula without the microorganism. No significant differences in weight gain were observed between groups at four months of age (29.0±7.8 vs. 28.9±5.7 g/day) nor at six months (25.1±6.1 vs. 24.7±5.2 g/day). There were no statistically significant differences in the consumption of the formulas or symptoms related to the tolerance of the formula. The findings from this study indicate that the consumption of study formula containing *L. fermentum* strain (average dose $2x10^{8}$ cfu/day) was safe and well tolerated.

In a 3-year follow-up study, Maldonado-Lobón et al. (2015) aimed at evaluating the long-term effects produced by the early consumption of an infant formula supplemented with *L. fermentum* CECT5716 compared with a control formula without the microorganism (control group). The infants included in this follow-up study had previously completed a 5-month randomized double-blind controlled trial (from 1 to 6 months of age), where the safety and tolerance of the formula containing *L. fermentum* strain was investigated. The main outcome of the follow-up study was the growth of the children. At three years, the mean values of weight, length and head circumference were similar in children receiving the formula containing *L. fermentum* as compared with the control group. No differences were observed in the incidence of infectious and non-infectious diseases or disorders related with intestinal function. The pattern of fecal microbiota was also similar between both groups. In conclusion, this 3-year study shows

that the early administration of *L. fermentum* CECT5716 in an infant formula is safe and it does not produce measurable differences in children compared with a control formula.

In a recent randomized, double blind, controlled, parallel group study in healthy, formula-fed infants, Maldonado et al. (2019) investigated safety, tolerance and efficacy of 1-year consumption of infant formula supplemented with *L. fermentum* CECT5716 Lc40 or *B. breve* CECT7263. In this study, 236 one-month-old infants were selected and randomly divided into three study groups. Infants in the control group received a standard powdered infant formula until 12 months of age. Infants in the treatment groups received the same infant formula but supplemented with *L. fermentum* or *B. breve*. The daily consumption of the formula corresponded to an average dosage of the bacteria at levels of $1x10^9$ cfu/day up to six months and $7\text{-}8x10^8$ cfu/day between 6 and 12 months. Main outcome was weight-gain of infants as safety marker. Of the selected infants, 189 completed the 11 months of intervention (61 in control group, 65 in *L. fermentum* group and 63 in *B. breve* group). The growth of infants in the three groups was consistent with standards. No significant differences were observed in the main outcome, weight-gain. The three milk formulae were well tolerated, and no adverse effects were related to the consumption of any of the formula. The investigators concluded that the addition of *L. fermentum* or *B. breve*, two strains naturally found in breast milk, to infant formulae is safe.

Lara-Villoslada et al. (2009) evaluated potential toxicity and translocation ability of *L. fermentum* CECT5716 following oral administration to mice. In this study, 40 Balb/C mice were divided in two groups (n=20/group). One group of mice was treated orally with $1x10^{10}$ cfu/mouse/day of *L. fermentum* for 28 days. The other group only received the excipient and was used as control. Food intake, body weight, bacterial translocation and different biochemical and hematological parameters were analyzed. Oral administration of *L. fermentum* to mice had no adverse effects on mice. There were no significant differences in body weight or food intake between control and treated mice. No bacteremia was observed and there was no treatment-associated bacterial translocation to liver or spleen. The results of this study suggest that *L. fermentum* CECT 5716 is non-pathogenic for mice even in doses 10,000 times higher than those normally consumed by humans.

In another study Cardenas et al. (2015), characterized *L. fermentum* CECT 5716 and reported that the strain does not contain plasmids, nor biogenic amine, or bacteriocin biosynthesis capability. These investigators also reported that no prophages could be induced and the strain was found to be sensitive to 16 antibiotics tested with MIC values at or below the microbiological breakpoints stated by EFSA. Further, genomic analysis showed no transmissible genes with potential for antibiotic resistance and that CECT 5716 consists of a circular chromosome with 2,100,449 base pair and a GC content of 51.49% (Cardenas et al., 2015).

6.2.3.2. Studies with *L. fermentum* ME-3

In a number of human trials, safety of a unique strain of *Lactobacillus* species, *L. fermentum* ME-3 DSM-14241 has been reported. In these studies, a variety of different formats (capsules, cheese, fermented milk) up to 3×10^{11} cfu/day have clearly demonstrated the safety of this strain (Songisepp et al., 2005; Kaur et al., 2008; Mikelsaar and Zilmer, 2009).

As reported in a review article by Mikelsaar and Zilmer (2009), *L. fermentum* ME-3 colonization and safety, along with other effects, have been tested in several open placebo-controlled and randomized double-blind placebo-controlled trials using capsules with ME-3, goat milk fermented with ME-3, commercial foodstuffs (kefir, cheese) and synbiotics enriched with ME-3. A large spectrum of indices measured in healthy adult volunteers showed that the use of strain ME-3 was safe regarding the physiological values of blood cytokines (including IL-6), inflammatory markers (WBCs, hsCRP), principal markers of carbohydrates and lipids or lipid-like compounds (glycose, triglycerides, cholesterol, LDL, HDL), several metabolites (homocysteine, creatinine, bilirubin) and several other biochemical indices such as blood calcium and iron, and endothelial functionality and arterial stiffness.

In a study by Kullisaar et al. (2016), the safety of consuming a supplement containing $6x10^9$ cfu/serving *L. fermentum* ME-3 for four weeks in 45 healthy adults was investigated. The parameters measured were total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, oxLDL, hsCRP, IL-6 and glycosylated hemoglobin (HbA1c%). In this study, consumption of *L. fermentum* ME-3 was well tolerated with no major safety concerns reported during the course of the trial.

In an open label, randomized, double blind, placebo-controlled study, Songisepp et al. (2005), investigated the safety of *L. fermentum* ME-3. For these investigations, two 3-week trials were performed in healthy volunteer. The participants (n=24) received either capsules with *L. fermentum* ME-3 (daily of dose $1x10^{9.2}$ cfu/day) or placebo capsules. The fecal lactoflora composition, fecal ME-3 recovery, effect of the consumption on intestinal lactoflora, and oxidative stress markers of blood was measured. In this study, *L. fermentum* ME-3 administered in fermented goats' milk ($1x10^{11.8}$ cfu/day) or as a capsule ($1.0x10^{9.2}$ cfu/day) did not result in adverse side effects or study product related dropouts over the course of the trial. The findings from this study suggest that *L. fermentum* ME-3 doses equal to or higher than the proposed daily intake for *L. fermentum* LfQi6 are safe and well tolerated in humans.

Sepp et al. (2018) evaluated the effects of *L. fermentum* ME-3 on composition of gut microbiota and urine polyamines level. In this study, healthy volunteers were divided into two groups: treatment group received kefir with *L. fermentum* ME-3 (n=71) and control group (n=66) regular kefir both for eight weeks. The *L. fermentum* ME-3 content of the kefir was $4x10^7$ cfu per ml and the individuals in treatment group received $8x10^9$ cfu/day. In the treatment group there was an increase in the richness, diversity and counts of lactoflora. After consumption of kefir containing *L. fermentum* ME-3, the level of urine putrescine had negative correlation with counts of lactobacilli and bifidobacteria. The investigators reported that at week 8, measurements were conducted with 71 participants: 42 in the treatment group and 29 in the control group. Fifteen participants withdrew due to infections, intestinal complaints, and loss of contact; in addition, five healthy subjects did not bring their biological material (both urine and feces samples) and 46 lost the motivation to complete the trial for four weeks more.

6.2.3.3. Studies with *L. fermentum* MTCC 5898

In a study in aging mice, Sharma et al. (2014) investigated the effects of the consumption of milk that is fermented with *L. fermentum* MTCC 5898. In this 2-month study, 16-month-old male Swiss mice were kept on three experimental diets: basal diet (BD), BD supplemented with

skim milk, and BD supplemented with *L. fermentum* fermented milk. A concurrent analysis of several parameters such as neutrophil functions, interleukins profile, inflammation, and antibody responses in the intestine as well as analysis of antioxidant enzymes in the liver and red blood cells was performed. No adverse effects were reported.

In a recent study, Pradhan et al. (2019) evaluated the safety and toxicity of *L. fermentum* MTCC 5689 using both *in vitro* and *in vivo* approaches. The *in vitro* assays included mucin degradation, hemolytic activity, biogenic amine production and platelet aggregation assay. The safety was also assessed using acute, subacute and subchronic assays, bacterial translocation studies, intravenous and intravenous administration and genotoxicity assay in murine model. The outcome of this toxicological safety assessment indicated that *L. fermentum* strain lacked any harmful metabolic activity or any genotoxic effects. Furthermore, the results of oral toxicity studies in mice revealed that short term administration of high cell mass concentration of 1 x 10^{12} cfu/animal as well as long term feeding of *L. fermentum* did not alter any hematological, general health parameters or cause any organ specific disorder. The investigators suggested that based upon these scientific assessments and supported by long history of safe use, *L. fermentum* may be considered to be safe for human consumption.

In a 28-day repeat dose toxicity study, Samtiya et al. (2019) studied the safety of *L. fermentum* MTCC-5898 at doses of $1x10^7$, $1x10^9$, and $1x10^{11}$ cfu/day/animal in Swiss albino mouse (weanling) using oral route. Health status of animals was monitored by physical assessment of body weight, organ indices, and histological appearances of liver and intestine along with measurement of hematological parameters (Hb, WBC, RBC count, MCHC, MCV, MCH), biochemical analyses in blood involving glucose, serum enzymes (ALT, AST and LDH), urea, creatinine, and lipid profile (total cholesterol, triglycerides, HDL, VLDL, LDL, and atherogenic index). *L. fermentum* treatment showed no adverse effects on above parameters of general health status after continuous consumption for the experimental period. Further, safety of *L. fermentum* was also confirmed by insignificant changes in release of FITC-dextran (4 kDa) in blood on its consumption than control group where only saline was given orally. The investigators concluded that *L. fermentum* MTCC 5898 is safe and non-toxic to weanling mice.

6.2.3.4. Additional Studies with Other Strains of *L. fermentum*

Simons et al. (2006) assessed the effects of *L. fermentum* (PCC®) in human subjects. In this single center, double blind, placebo-controlled, parallel design trial in volunteers having total cholesterol > or =4 mmol/L, subjects (n=46) were randomized to receive either *L. fermentum* [2 capsules twice daily (each capsule containing $2x10^9$ cfu)] or matching placebo for a period of 10 weeks. Main outcome measures were percentage changes in LDL cholesterol and other lipids, changes in liver enzymes and other safety tests. Two subjects withdrew early in the study, one for personal reasons and one because of bowel discomfort. Three other subjects experienced some bowel discomfort but still completed the study. There were no significant changes over time or between treatments noted in total cholesterol, high density lipoprotein cholesterol or triglycerides. There were no significant changes in liver enzymes or other safety parameters with time or between treatments. The investigators concluded that *L. fermentum* did not appear to produce a major change in serum lipid fractions.

In a double-blind, randomized, controlled trial, competitive cyclists (64 males and 35 females; age 35 ± 9 and 36 ± 9 y, VO2max 56 ± 6 and 52 ± 6 ml.kg-1.min-1, mean ± SD) were randomized to receive minimum $1x10^9$ *L. fermentum* (PCC®/day) or placebo treatment for 11 weeks (West et al., 2011). *Lactobacillus* numbers increased 7.7-fold more in males receiving *L. fermentum*, while there was an unclear 2.2-fold (0.2- to 18-fold) increase in females receiving *L. fermentum*. The number and duration of mild gastrointestinal symptoms were ~2-fold greater in the treatment group. The investigators concluded that *L. fermentum* may be a useful nutritional adjunct for healthy exercising males. However, uncertainty in the effects of supplementation on URTI and on symptoms in females needs to be resolved. No adverse effects were reported.

In a randomized, double-blind, and controlled trial, Akbari et al. (2016) investigated the effects of milk containing *L. acidophilus, L. casei, B. bifidum*, and *L. fermentum* in 60 Alzheimer's disease patients. The patients were randomly divided into two groups (*n*=30 in each group) treating with either milk (control group) or milk containing microorganisms. The supplemented group ingested 200 ml/day milk containing *L. acidophilus, L. casei, B. bifidum*, and *L. fermentum* ($2x10^9$ cfu/g for each bacterium; apparently $4x10^{11}$/person/day) for 12 weeks. Mini-mental state examination (MMSE) score was recorded in all subjects before and after the treatment. Pre- and post-treatment fasting blood samples were obtained to determine the related markers. Intake of milk containing microorganisms had no considerable effect on other biomarkers of oxidative stress and inflammation, fasting plasma glucose, and other lipid profiles. No adverse effects were reported.

In another randomized, double-blind, placebo-controlled trial, Badadi et al. (2019) investigated the effects of a mixture of common known microorganisms such as *L. acidophilus, L. casei, B. bifidum, L. fermentum* in 48 patients with gestational diabetes mellitus (GDM). Participants were randomly divided into two groups to intake either capsule containing mixture of ($2x10^9$ cfu/g each bacterium; apparently $4x10^{11}$/person/day) (n = 24) or placebo (n = 24) for 6 weeks. No adverse effects were reported.

Huang et al. (2018) investigated the effects of *L. paracasei*, *L. fermentum* GM-090 (BCRC 910259, CCTCC M204055), and their combination in children with asthma. In this double-blind, prospective, randomized, placebo-controlled trial, 160 children with asthma, aged 6-18 years, received *L. paracasei*, *L. fermentum*, combination or a placebo for three months. The *L. fermentum* group was treated at a dose of $2x10^9$ cfu/day for three months. The investigators concluded that *L. paracasei*, *L. fermentum* and their combination were well tolerated with fair compliance and without adverse effects reported.

The safety of *L. fermentum* SJRP30, isolated from water buffalo mozzarella cheese, has been investigated by Casarotti et al. (2017) by employing *in-vitro* and *in-silico* experiments. This strain did not demonstrate hemolytic or mucin degradation capabilities and tested negative for a number of genes related to endocarditis, antigen, collagen adhesion, tyrosine decarboxylase, ornithine decarboxylase, tetracycline resistance, and erythromycin resistance. Further, *L. fermentum* SJRP30 was sensitive to a majority of antibiotics tested (Ampicillin, Tetracycline, Chloramphenicol, Erythromycin, Clindamycin) but was resistant to vancomycin, kanamycin, and gentamicin. This pattern of antibiotic resistance was considered to be an intrinsic factor of *L. fermentum* SJRP30 as it was chromosomally encoded and thus did not present a risk for

transferring acquired antibiotic resistance to pathogens, leading researchers to conclude that *L. fermentum* strain SJRP30 is safe (Casarotti et al., 2017).

Shokryazdan et al. (2016) investigated the safety of *L. fermentum* HM3 from human milk using acute and subacute oral toxicity tests in Sprague-Dawley rats. In addition, its effects on cecal microflora and harmful bacterial enzymes (β-glucuronidase and β-glucosidase) of the tested animals were also studied. The findings from this study showed that *L. fermentum* HM3 was safe up to a level of $1x10^{10}$ cfu/kg bw/day in a 14-day or 28-day treatment period. The strain was well tolerated and there were no observed adverse effects on growth, feed consumption, cellular blood components and vital organs of the treated animals. These results suggest that *L. fermentum* HM3 is safe.

In summary, the effects of different strains of *L. fermentum* have been investigated in several human (double blind, placebo controlled) studies as well as in *in vitro, in-silico* and animal studies. The findings from these studies indicate that, as such, *L. fermentum* species is unlikely to cause adverse effects.

6.2.4. Comparison with Known Safe Strains

As described above, *L. fermentum* CECT 5716 is a well-characterized strain isolated from human milk. The safety of this strain for human exposure has been demonstrated and it is currently used in commercial infant formulas. This strain has been identified as a safe strain for human and showed a strong adherence to intestinal cells. Hence, the subject of this present GRAS assessment, *L. fermentum* LfQi6, was compared for genetic similarity and differences with *L. fermentum* CECT 5716 from a safety point of view. To enhance genetic comparison, both organisms were annotated using the same methodology. Sequence based using gene classifications and function based. *L. fermentum* LfQi6 contained 1859 genetic features, 235 subsystems and 1785 coding sequences with 74 RNAs while *L. fermentum* CECT 5716 possessed 2191 features, 320 subsystems and 2119 coding sequences with 72 RNAs, suggesting similar sized genomes. Sequence based comparison of 1616 genes that were shared by each organism showed 95.6% identity average across all these features. Using SEED both organisms had closest global identity to *L. fermentum* IFO 3956 another well characterized *Lactobacillus* strain.

Analyzing unique subsystems in SEED revealed similarity of genome segments assigned to the functions: amino acid and derivatives, cell division and cell cycle, cell wall and capsule, clustering-based subsystems, cofactors, vitamins, prosthetic groups, pigments, dormancy and sporulation, fatty acids, lipids and isoprenoids, iron acquisition and metabolism, membrane transport, metabolism of aromatic compounds, miscellaneous, nitrogen metabolism, nucleosides and nucleotides, phosphorus metabolism, potassium metabolism, protein metabolism, regulation and cell signaling, regulons, respiration, RNA metabolism, stress response, sulfur metabolism, and virulence, disease and defense, indicating a high level of homogeneity in many genes between these two strains. The *L. fermentum* LfQi6 has three subsystems that are not present in *L. fermentum* CECT 5716 which related to cyclic AMP signaling, citrate metabolism, mercuric resistance (resistance to mercury), and RelB/StbD replicon stabilization protein which is involved in transcription regulation. *L. fermentum* CECT 5716 has a wide range of subsystems

not found in the smaller genome of the *L. fermentum* LfQi6 including arsenic resistance and Tetracycline resistance.

In summary, the genome of *L. fermentum* LfQi6 showed high similarity to that of *L. fermentum* CECT 5716, with most genetic differences occurring in subsystems pertaining to carbohydrate utilization, DNA metabolism, and phages, prophages, transposable elements and plasmids. There were no differences detected in the genomes related to virulence, disease, and defense and as such the safety of these strains was determined to be similar. Moreover, the safety of *L. fermentum* strain CECT 5716 has been documented in human studies at doses up to $1x10^{10}$ cfu/day in adult humans. Taken together, these results suggest that *L. fermentum* LfQi6 may be reasonably expected to be safe under the proposed conditions of use.

6.2.5. Regulatory Agency Assessments

6.2.5.1. FDA Evaluation of GRAS Notice

In 2014, the FDA received a GRAS notice on *L. fermentum* CECT 5716 from Biosearch Life S.A. (Biosearch, 2014). This GRAS notice is designated as GRN 531. Following review of this GRAS notice, the FDA issued a "no question" letter (FDA, 2015). In this GRAS notice, Biosearch (2014) described *L. fermentum* CECT 5716 as a white-yellowish powder. L. *fermentum* CECT 5716 was described as a Gram-positive, rod shaped, lactic acid-producing bacterium that is facultatively anaerobic and heterofermentative. The strain was isolated from human milk and deposited in the Spanish Type Culture Collection. *L. fermentum* CECT5716 was reported to be manufactured using standard fermentation techniques and under conditions that are suitable for producing human food. In this GRAS notice, Biosearch proposed the use of *L. fermentum* in term infant formula at a maximum level of 10^7 cfu/g of powdered non-exempt milk-based infant formula, resulting in an estimated daily intake of $2x10^8$ cfu.

In this GRAS notice, Biosearch described published studies conducted using animals, published human studies conducted in adults and infants, and an unpublished *in vitro* study supporting the safety of *L. fermentum* strain CECT5716. In a published study in which mice received 10^{10} cfu of *L. fermentum* strain CECT5716/mouse/day by oral gavage for 28 days. The results of this study did not reveal any adverse effects on body weight or food intake, nor were there any changes in biochemical or hematological parameters in the treated group compared to the control group. The results of the study also showed an absence of bacteremia and treatment-associated bacterial translocation in the livers and spleens of animals in the treatment group. This study suggests the nonpathogenicity and nontoxicity of *L. fermentum* strain CECT5716.

Additionally, Biosearch discussed three published studies in which *L. fermentum* strain CECT5716 was orally administered to human adults or infants. In one study, healthy adults received $1x10^{10}$ cfu of *L. fermentum* strain CECT5716/person/day via capsules for one month, followed by observation for five months. In another study, one-month old healthy infants received $8.4x10^8$ cfu/infant/day in formula for five months. In a third study, six-month-old healthy infants received $2x10^8$ cfu/infant/day in follow-on formula for five months. No treatment-related adverse effects were observed in these studies. Biosearch noted that there is one published case report describing adverse effects caused by *L. fermentum*, and this case occurred in an immunocompromised individual. In addition to the published studies described

above, Biosearch discussed the results of an unpublished *in vitro* study showing that *L. fermentum* strain CECT5716 is susceptible to clinically relevant antibiotics. Biosearch further stated that the strain does not contain transmissible genes encoding resistance to clinically relevant antibiotics, nor does the strain contain plasmids. The FDA reviewed the notice and responded to the notifier that, based on the information provided in the notification, as well as other information available to the FDA, the agency has no questions at this time regarding the conclusion that *L. fermentum* CECT5716 is GRAS under the intended conditions of use.

6.2.5.2. EFSA Evaluation

In European countries, the Scientific Committee recommended to the European Food Safety Authority (EFSA) a generic approach to assess the safety of microorganisms used in food or feed and the production of food/feed additives (EFSA, 2007). This system is somewhat similar to GRAS. The European approach is modified to account for the regulatory practices in Europe. The system is referred to as Qualified Presumption of Safety (QPS). The Scientific Committee recommended policies and practices for the routine assessment of microorganisms based on taxonomy, familiarity, pathogenicity, and end use. If a microorganism is approved as QPS, it would not require further regulatory review prior to introduction into the food supply. Lactic acid bacteria (including *Lactobacillus* species) were among the microorganisms recommended to be reviewed in this initial document.

The available information suggest that *Lactobacillus* species were reviewed under the QPS system multiple times. *L. fermentum* was among the taxonomic units included in the initial QPS review of lactobacilli. In the initial review, the Scientific Committee concluded that the weight of evidence available for these species was sufficient and provided as least the same degree of confidence as a case-by-case assessment (EFSA, 2007). The Scientific Committee reviewed the available evidence regarding the involvement of lactobacilli in human disease. Reviewing and summarizing the occasional reports of *Lactobacillus* bacteremia, the committee concluded lactobacillemia occurred primarily in immunocompromised or those suffering from severe underlying illness and that the *Lactobacillus* species described herein can be considered non-pathogenic to humans. The committee emphasized the long history of safe use in the food chain and reported no safety concerns. The subsequent yearly QPS reviews evaluated the totality of the scientific information each year and reaffirmed the QPS status of *L. fermentum*. Given the EFSA classification of *L. fermentum* as an organism having a QPS and thus being freed from the need for further safety assessment. This conclusion has been maintained through all annual reappraisals to date.

6.3. GRAS Panel Evaluation, Summary and Discussion

At the request of Quorum Innovations, LLC (Quorum), an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[3], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to evaluate the Generally Recognized As Safe (GRAS) status of a *Lactobacillus fermentum* LfQi6 for use as a food ingredient in selected food products such as

[3] Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

bakery products (biscuits, pastries, cookies, brownies, crackers), chocolate (also includes gummies-candy), yogurt and flavored dairy beverages at use levels up to $2x10^8$ cfu/serving (reference amounts customarily consumed, 21 CFR 101.12). *L. fermentum* LfQi6 is intended for its use in food products as a food ingredient. It will not proliferate in the foods and beverages to which it is added but instead will decline over the shelf-life of the food. A comprehensive search of the scientific literature for safety and toxicity information on *L. fermentum* LfQi6 and other strains was conducted through December 2020, and made available to the Expert Panel. The Expert Panel, independently and critically, evaluated materials submitted by Quorum and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred, and unanimously agreed to the decision described herein.

Quorum ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel were not contingent upon the outcome of their deliberations. The *L. fermentum* LfQi6, subject of this present GRAS assessment, isolated from human microbiome is well characterized. It is deposited with the American Type Culture Collection (ATCC) and has been assigned the accession number ATCC No. PTA-122195. It is a rod shaped, Gram-positive, non-spore forming, obligate hetero-fermentative bacterium. The identity of *L. fermentum* LfQi6 has been fully investigated and confirmed by phenotypic and genotypic analysis. *L. fermentum* LfQi6 is manufactured using standard, well-documented fermentation techniques as per current Good Manufacturing Practices (cGMP) conditions using approved food grade materials. The strain is manufactured consistently to meet standard food grade specifications. Based on conservative considerations, the intended uses of *L. fermentum* LfQi6 in the above mentioned conventional foods will results in the total estimated consumption of $2x10^9$ cfu/person/day.

In general, *Lactobacillus* is a non-pathogenic genus consisting of over a hundred species with a large variety of phenotypic, biochemical, and physiological properties. Lactobacilli have played a crucial role in the production of fermented products for millennia. In healthy humans, Lactobacilli are normally present in the oral cavity, the ileum, and the colon and they are the dominant microorganism in the vagina. *Lactobacillus* strains have a history of use in food production, with large levels of viable bacteria present in many foods, including yogurt, fermented milk, and cheeses. *L. fermentum* is documented to be used in ancient Egypt in fermented milk, and today as naturally present or as a starter culture in fermented milk products. Given its presence in different foods, it has long been ingested during normal food-consumption activities with no apparent adverse effects.

In a series of studies that included standard microorganism safety evaluations for human exposure, *L. fermentum* LfQi6 was evaluated for antimicrobial resistance pattern determination and assessment for potentially harmful metabolic activities, such as biogenic amine production, mucin degradation, various enzymatic activities and pathogenic hemolytic activity. None of these investigations reveal any safety concerns. Additionally, the safety of *L. fermentum* LfQi6 has been investigated by employing whole genome sequencing analysis and bioinformatics. This analysis confirmed that *L. fermentum* LfQi6 is indeed a strain of *Lactobacillus fermentum*. Based

on RAST analysis no virulence or transposable elements were identified that not also present in similar form in other known *L. fermentum* strains. Based on genetic comparison of *L. fermentum* LfQi6 to a well-known organism *L. fermentum* CECT 5716 used for exposure to humans, the subject of present GRAS *L. fermentum* LfQi6 has no identifiable unique subsystems that are associated with known safety issues.

In addition to the specific studies, the safety of *L. fermentum* LfQi6 is further corroborated by multiple *in vitro,* animal and human studies with other strains of *L. fermentum* that are substantially similar to the subject strain of this present GRAS assessment. In multiple studies, the safety of different strains of *L. fermentum* has been extensively investigated. Most prominently, the safety of *L. fermentum* CECT 5716 has been established, as this strain did not possess acquired antibiotic resistance, was not toxic when given at a high dose to Balb/C mice, was sensitive to all antibiotics tested, did not contain plasmids, biogenic amine, or bacteriocin biosynthesis capability, and as such did not pose a risk to human health. Human studies with this strain shows that it is safe and well tolerated at doses of $2x10^8$ cfu/day and $1.0x10^7$ cfu/g when administered as part of infant formula for five and six months in healthy infants. Moreover, this strain was shown to be safe and well tolerated in adult humans. This strain has been the subject of a GRAS notification (GRN 531) for its use in term infant formula and received no question letter from the FDA.

There is sufficient qualitative and quantitative scientific, as well as history of use, evidence to determine the safety-in-use of standardized *L. fermentum* LfQi6. The safety evaluation of *L. fermentum* LfQi6 is based on the totality of available evidence, including phenotypic and genotypic characterization, and animal and human studies. The historical and current uses of products containing *L. fermentum* strains further corroborate the safety in use of *L. fermentum* LfQi6.

The evidence of *L. fermentum* LfQi6 safety is supported by:

- Traditional and current safe use of products containing *Lactobacillus* species.
- Full identity characterization of *L. fermentum* LfQi6 by phenotypic and genotypic means.
- *L. fermentum* LfQi6 is not able to produce biogenic amine and does not carry any transferrable gene coding for antibiotic resistance in line with no phenotypic antibiotic resistance
- Findings from phenotypic tests showed that *L. fermentum* LfQi6 did not cause cytotoxic activity and the strain is non-hemolytic.
- Corroboration of safety from multiple human and animal studies with substantially equivalent/similar strains.

In summary, on the basis of scientific procedures[4] including knowledge from a history of exposure and endogenous presence of the *Lactobacillus fermentum* in the gastrointestinal tract, the consumption of *L. fermentum* LfQi6 as an added food ingredient is considered safe at levels up to $2x10^9$ cfu//person/day. The intended uses are compatible with current regulations, *i.e., L. fermentum* LfQi6 in selected food products such as bakery products (biscuits, pastries, cookies, brownies, crackers), chocolate (also includes gummies-candy), yogurt and flavored dairy beverages at use levels up to $2x10^8$ cfu/serving (reference amounts customarily consumed, 21CFR 101.12) and is produced according to current good manufacturing practices (cGMP).

[4]21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

6.4. GRAS Panel Conclusion

Based on a critical evaluation of the publicly available data, summarized above, the Expert Panel members whose signatures appear below, have individually and collectively concluded that *Lactobacillus fermentum* LfQi6 preparation, meeting the specifications cited above, when used at $2x10^8$ cfu/serving in food products such as bakery products (biscuits, pastries, cookies, brownies, crackers), chocolate (also includes gummies-candy), yogurt and flavored dairy beverages (yogurt, and other dairy products, soy products, beverages, chewing gum, confectionary snacks and other foods), as described in this monograph and resulting in the total estimated likely consumption of $2x10^9$ cfu of *L. fermentum* LfQi6/person/day is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that *L. fermentum* LfQi6, when used as described, is Generally Recognized As Safe (GRAS).

Signatures

[redacted]
Robert L. Martin, Ph.D.

March 24, 2020
Date

[redacted]
Douglas L. Archer, Ph.D.

March 30, 2020
Date

[redacted]
Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.
Advisor to Expert Panel

April 2, 2020
Date

7. PART VI- SUPPORTING DATA AND INFORMATION- REFERENCES

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8. APPENDIX I

Whole Genome Sequencing and Bioinformatics Analysis Report
(Included separately- Pages 1-9)

9. APPENDIX II

Analytical results from three non-consecutive lots
PDF file attached separately

APPENDIX I

Molecular Research LP
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Genetic Evaluation of L. fermentum LfQi6 and SEED-Based Comparison Against L. fermentum CECT 5716: Project No. 062719EBLferm

Prepared by

Molecular Research LP (dba MR DNA)
503 Clovis Rd.
Shallowater, Texas 79363
Director: Dr. Scot E Dowd Ph.D.

Genome Methods for Project No. 062719EBLferm

A Sample of extracted DNA was provided to Molecular Research LP (MR DNA), 503 Clovis Rd.. Shallowater, TX. 79363. This DNA was provided by Quorum Innovations, Sarasota Florida on 6-27-19 and reported as L. fermentum LfQi6.

Molecular Research was commissioned to sequence the genome of this organism and provide a basic report and opinion letter regarding the ability of this organism whose DNA was reported as L. fermentum LfQi6 to be considered GRAS (Generally Recognized as Safe) as determined by analysis of the sample of extracted DNA provided to Molecular Research LP.

Disclaimer: Molecular Research is a research support and service provider specializing in next generation sequencing and bioinformatics. We have no specific ability to determine if an organism is Generally Recognized as Safe. Opinions provided here are based on our analysis of the DNA that was provided. No specific compensation or incentive has been provided to sway our analysis or opinions. Standard compensation was limited to the service of genome sequencing and analysis along with generation of this report.

Molecular Methods for Genome Sequencing

PAC BIO Long Reads

The library for *L. fermentum* LfQi6 was prepared using the SMRTbell DNA Damage Repair Kit (Pacific Biosciences) following the manufacturer's user guide. The initial concentration of gDNA was valuated (Table 1) using the Qubit® dsDNA HS Assay Kit (ThermoFisher Scientific). The sample was sheared (Table 1) using the Covaris G-tube (Covaris Inc.), and 1 µg of the sample was used to enter into the SMRTbell DNA Damage Repair protocol.

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Table 1. Initial DNA Concentration, 260/280 and 260/230 ratios

Sample	DNA concentration (ng/uL)	260/280	260/230	Avg library size (bp)
L. fermentum LfQi6	8.0	1.75	1.03	8651

During library preparation, *L. fermentum* LfQi6 underwent DNA damage and end repair as well as barcode adapter ligation. Subsequent to adapter ligation, the sample went through Exonuclease III and VII digestion to remove the failed ligation products. Following library preparation, the final concentration of the library (Table 2) was measured using the Qubit® dsDNA HS Assay Kit (ThermoFisher Scientific), and the average library size of (Table 2) was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The library pool was then sequenced using the 10-hour movie time on the PacBio Sequel (Pacific Biosciences).

Table 2. Final Library Concentration and Size

Sample	DNA Concentration (ng/uL)	Avg. library size (bp)
L. fermentum LfQi6	19.1	8821

De Novo Assembly of PAC BIO data was accomplished using the SMRT Analysis Hierarchical Genome Assembly Process (HGAP). HGAP consists of three primary processes: Preassembly, Assembly, and Consensus Polishing. The single pass reads were mapped against the seed reads, which averaged 9.2Kb in length. The average depth of coverage for Lactbacillus-Fermentum-Qi6DNA was 115x (Figure 1). The percent of bases successfully realigned to the draft assembly was 88.6% with a mean concordance of 88.4% (Figure 2).

Figure 1. *L. fermentum* LfQi6 DNA Depth of Coverage Distribution

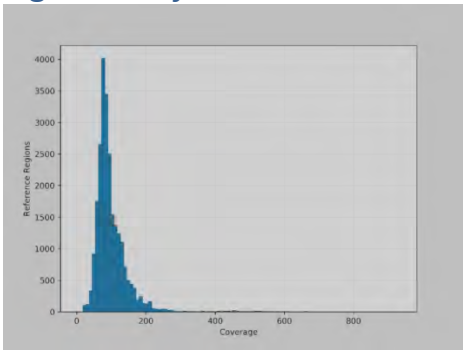

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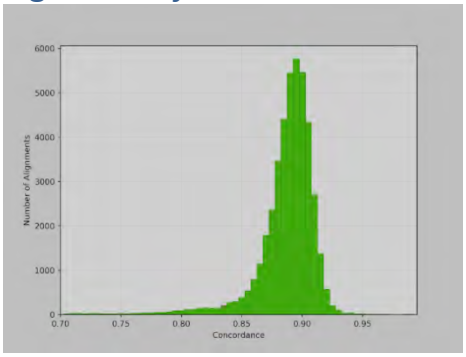
Figure 2. *L. fermentum* LfQi6 Realigned Subread Concordance



Results of the Falcon Assembler paired with the Arrow polishing algorithm can be seen below (Table 3).

Table 3. Polished Assembly Metrics

Sample	# of Polished Contigs	N50 Contig Length	Sum of Contig Lengths
L. fermentum LfQi6	24	218,570	1,849,311

ILLUMINA Paired End Sequencing

The concentration of DNA was evaluated using the Qubit® dsDNA HS Assay Kit (Life Technologies). The library was prepared using Nextera DNA Flex library preparation kit (Illumina) following the manufacturer's user guide. 50 ng DNA was used to prepare the library. The sample underwent the simultaneous fragmentation and addition of adapter sequences. These adapters are utilized during a limited-cycle (6 cycles) PCR in which unique index was added to the sample. Following the library preparation, the final concentration of the library (Table 4) was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies), and the average library size (Table 4) was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The library was diluted (to 0.9nM) and sequenced paired end for 500 cycles using the NovaSeq system (Illumina).

Table 4. DNA, final library concentration, and average library size.

Sample	DNA concentration (ng/µL)	Final library DNA concentration (ng/µL)	Average library size (bp)
L. fermentum LfQi6	9.1	46.60	659

HYBRID Assembly of Genome

A combined assembly of PAC bio reads and Illumina paired end data was performed using NGEN V16 using the PAC BIO assembly as a guided reference to produce scaffolds under process of error correction and gap closure.

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Genome Report

The full annotation and optimized draft assembly of the Qi6 genome along with hybrid contigs, PAC BIO sequel primary assembly, raw data and annotations (consisting of GenBank file, transcriptome, proteome, contigs and spreadsheet) are provided as separate files.

Genome Overview

A final genome was derived of estimated size 1,831,981 base pairs, with GC content of 51%, an N50 of 223380 and an L50 of 3 all of which indicate a very high quality draft genome. The closest genome to this was determined as *Lactobacillus fermentum* IFO 3956 on RAST genome annotation server. Annotation was performed using RAST classic (rast.nmpdr.org). The top 4 closest neighbors are indicated in the following table (Table 5).

Table 5. Closest Neighbor Genome ID, Match Score, Genome Name

334390.3	545	Lactobacillus fermentum IFO 3956
334390.5	534	Lactobacillus fermentum IFO 3956
575599.3	524	Lactobacillus fermentum 28-3-CHN
525325.3	434	Lactobacillus fermentum ATCC 14931

It is the opinion of Molecular Research LP that this is a genome consistent with classification as a *Lactobacillus fermentum*. Genome full 16s sequence (see below for the assembled complete *L. fermentum* LfQi6 16s genome) was analysed using NCBI BLAST. The top 100 hits were all identified as *L. fermentum*, further indicating that this is a *L. fermentum* strain.

***L. fermentum* LfQi6 Complete 16s Sequence Used for NCBI BLAST Analysis**

ttttatatgagagtttgatcctggctcaggatgaacgccggcggtgtgcctaatacatgcaagtcgaacgcgttggcccgattgattgatggtgcttgcacctgattgattttggtcgccaac
gagtggcggacgggtgagtaacacgtaggtaacctgcccagaagcgggggacaacatttggaaacagatgctaataccgcataacaacgttgttcgcatgaacaacgcttaaaagatg
gcttctcgctatcacttctggatggacctgcggtgcattagcttgttggtggggtaacggcctaccaaggcgatgatgcatagccgagttgagagactgatcggccacaatgggactgaga
cacggcccatactcctacgggaggcagcagtagggaatcttccacaatgggcgcaagcctgatggagcaacaccgcgtgagtgaagaagggtttcggctcgtaaagctctgttgttaaa
gaagaacacgtatgagattaactgttcatacgttgacggtatttaaccagaaagtcacggctaactacgtgccagcagccgcggtaatacgtaggtggcaagcgttatccggatttattg
ggcgtaaagagagtgcaggcggttttctaagtctgatgtgaaagccttcggcttaaccggagaagtgcatcggaaactggataacttgagtgcagaagagggtagtggaactccatgtg
tagcggtggaatgcgtagatatatggaagaacaccagtggcgaaggcggctacctggtctgcaactgacgctgagactcgaaagcatgggtagcgaacaggattagataccctggtag
tccatgccgtaaacgatgagtgctaggtgttggagggtttccgcccttcagtgccggagctaacgcattaagcactccgcctggggagtacgaccgcaaggttgaaactcaaaggaattg
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gacaaaccggaggaaggtggggacgacgtcagatcatcatgcccctttatgacctgggctacacacgtgctacaatggacggtacaacgagtcgcgaactcgcgagggcaagcaaatct

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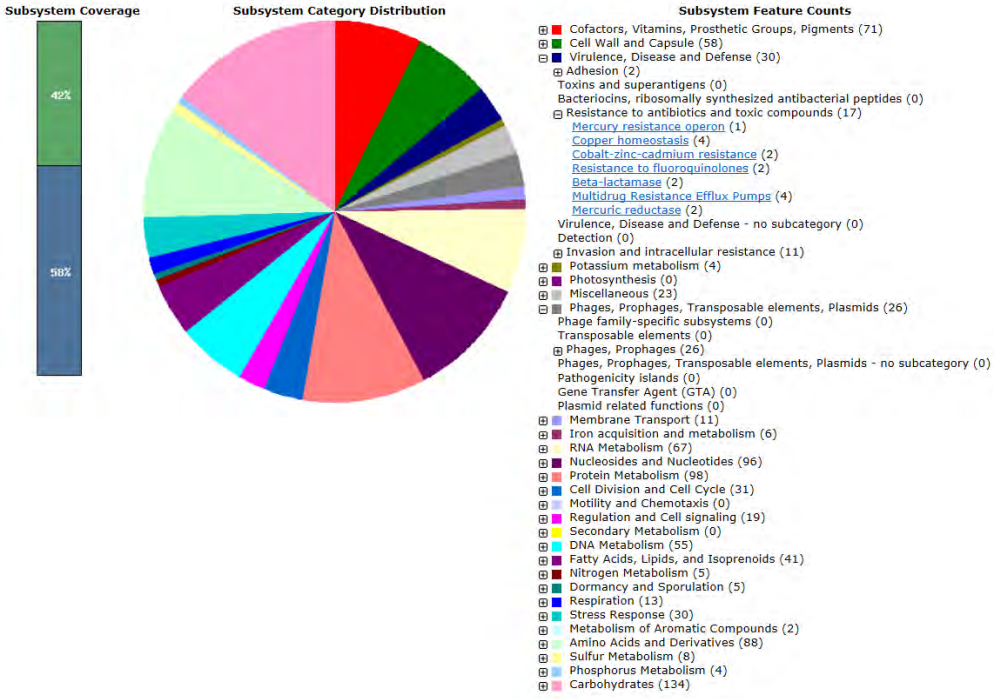
cttaaaaccgttctcagttcggactgcaggctgcaactcgcctgcacgaagtcggaatcgctagtaatcgcggatcagcatgccgcggtgaatacgttcccgggccttgtacacaccgccc
gtcacaccatgagagtttgtaacacccaaagtcggtggggtaacctttttaggagccagccgcctaaggtgggacagatgattagggtgaagtcgtaacaaggtagccgtaggagaa

Evaluation for Presence of Virulence Factors, Antibiotic Resistance or Transposable Elements

Based upon the RAST annotation, there were no identified virulence factors found within the genome. No transposable elements were found or no pathogenicity islands identified. Standard prophage elements were identified as follows: phage tail proteins (3), phage replication elements (5), phage packaging machinery (7), phage tail proteins (5), phage capsid proteins (6). Please refer to RAST subsystem details below (Table 6).

Table 6. RAST-Generated Subsystems in *L. fermentum* LfQi6 (Top) and *L. fermentum* CECT 5716 (Bottom)



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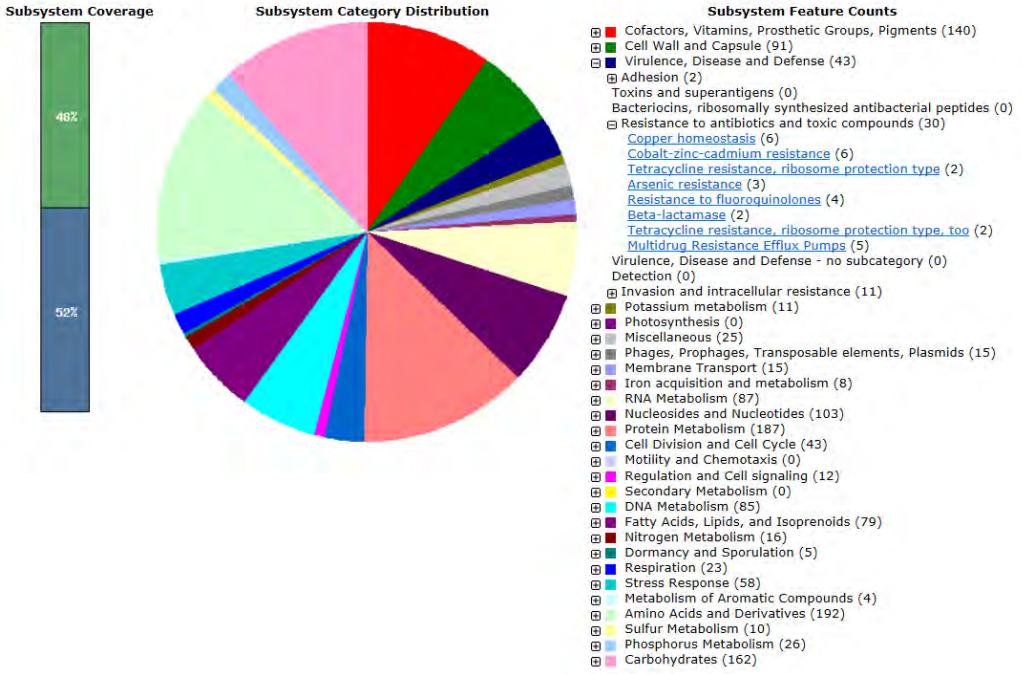

Safety Comparison of L. fermentum Strains LfQi6 and CECT 5716

Lactobacillus fermentum CECT 5716 is a well-characterized, Generally Recognized as Safe (GRAS) probiotic strain isolated from human milk and is currently used in commercial infant formulas. This strain has been identified as a beneficial and safe probiotic documented to be safe for human clinical use in adults at doses up to $1x1^{10}$ CFU/day.

The genome of *L. fermentum* LfQi6 was compared against *L. fermentum* CECT 5716 using the function-based comparison tool in the SEED viewer, an online genomic database for rapid genomic annotation. For the purposes of this study, 'features' are defined as a region of DNA that generally encodes for a single gene product, and a 'subsystem' is a set of functional roles. There may be multiple copies of a gene with a single feature present in a bacterial strain. Strain *L. fermentum* LfQi6 contained 1,859 genetic features, 235 subsystems and 1,785 coding sequences with 74 RNAs. *L. fermentum* CECT 5716 had 2191 features, 320 subsystems and 2119 coding sequences with 72 RNAs (Table 6). Genomes of the two strains were of very similar size. Sequence comparison of the 1,616 genes shared by each organism showed 95.6% identity across all these features. Using SEED analysis, both *L. fermentum* CECT 5716 and *L. fermentum* LfQi6 had closest global identity to *L. fermentum* IFO 3956, another well characterized *Lactobacillus* strain.

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Functional subsystem analysis using SEED revealed a very high level of homogeneity between *L. fermentum* strains LfQi6 and CECT 5716. Specifically, the following functional genome segments were very similar between the two strains: amino acid and derivatives, cell division and cell cycle, cell wall and capsule, clustering-based subsystems, cofactors, vitamins, prosthetic groups, pigments, dormancy and sporulation, fatty acids, lipids and isoprenoids, iron acquisition and metabolism, membrane transport, metabolism of aromatic compounds, miscellaneous, nitrogen metabolism, nucleosides and nucleotides, phosphorus metabolism, potassium metabolism, protein metabolism, regulation and cell signaling, regulons, respiration, RNA metabolism, stress response, sulfur metabolism, and virulence, disease and defense. Both strains are indicated by SEED analysis to have *gyra* and *gyrb* genes, which have been associated with fluoroquinolone resistance, but both of these strains have been tested and found to be phenotypically sensitive to this antibiotic.

There are limited subsystems which are variants between *L. fermentum* strains LfQi6 and CECT 5716 (Table 8). *L. fermentum* LfQi6 has 4 subsystems not present in CECT 5716 which are related to cyclic AMP signaling, citrate metabolism, resistance to mercury, and the RelB/StbD replicon stabilization protein (transcription regulation). These few variants are not expected to impact safety to the host. *L. fermentum* CECT has 61 subsystems not found in the genome of *L. fermentum* LfQi6, including resistance to tetracycline and arsenic.

Conclusion

Molecular Research is of the opinion that based on RAST analysis no virulence or transposable elements were identified. The genome of *L. fermentum* LfQi6 showed high similarity to that of CECT 5716 with the majority of genetic differences occurring in subsystems pertaining to carbohydrate utilization and DNA metabolism. There were no significant differences detected between the two genomes related to virulence, disease, and defense and as such the safety of these two strains is determined to be similar.

Based on genetic comparison to the well-known probiotic organism *L. fermentum* CECT 5716, the current organism, *L. fermentum* LfQi6 has no identified unique subsystems associated with known safety issues related to probiotic organisms. Additionally, the safety of *L. fermentum* CECT 5716 has been documented in human clinical trials at doses up to $1x10^{10}$ CFU/day in adults. In summary, based on genetic analysis and genetic similarity to *L. fermentum* CECT 5716, results indicate that *L. fermentum* LfQi6 does not contain any significant potential risks present that are expected to impact safety.

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Table 7. Subsystems Unique to *L. fermentum* LfQi6 and *L. fermentum* CECT 5716

Subsystems Unique to L. fermentum LfQi6 (3)	Subsystems Unique to L. fermentum CECT (61)
cAMP signaling in bacteria	5-FCL-like protein
Citrate Metabolism, Transport, and Regulation	A Gram-positive cluster that relates ribosomal protein L28P to a set of uncharacterized proteins
Mercuric reductase	Acetoin, butanediol metabolism
Toxin-antitoxin replicon stabilization systems.	Arginine Biosynthesis -- gjo
	Arsenic resistance
	Bacterial Cell Division
	Bacterial signal recognition particle (SRP)
	Benzoate degradation
	Broadly distributed proteins not in subsystems
	CBSS-138119.3.peg.2719
	CBSS-269482.1.peg.1294
	CBSS-272943.3.peg.1367
	CBSS-279010.5.peg.587
	CBSS-312309.3.peg.1965
	CBSS-349161.4.peg.2417
	CBSS-370552.3.peg.1240
	CBSS-393130.3.peg.794
	CBSS-56780.10.peg.1536
	CBSS-83331.1.peg.3039
	Cluster containing CofD-like protein and co-occurring with DNA repair
	Conserved gene cluster associated with Met-tRNA formyltransferase
	Creatine and Creatinine Degradation
	Dehydrogenase complexes
	Denitrifying reductase gene clusters
	D-gluconate and ketogluconates metabolism
	DNA recombination and repair protein RecO
	DNA repair, bacterial DinG and relatives
	DNA replication strays
	DNA structural proteins, bacterial
	DNA-binding regulatory proteins, strays
	dTDP-rhamnose synthesis
	F0F1-type ATP synthase
	Glutamate and Aspartate uptake in Bacteria

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	Glycerolipid and Glycerophospholipid Metabolism in Bacteria
	Glycyl-tRNA synthetase
	GroEL GroES
	Heat shock dnaK gene cluster extended
	High affinity phosphate transporter and control of PHO regulon
	Histidine Biosynthesis
	Hyperosmotic potassium uptake
	Iojap protein
	Isoprenoid Biosynthesis
	Leucine Biosynthesis
	Lysine Biosynthesis DAP Pathway
	Macromolecular synthesis operon
	Menaquinone and Phylloquinone Biosynthesis
	NusA-TFII Cluster
	Phosphate metabolism
	Restriction-Modification System
	Rhamnose containing glycans
	Ribosome recycling related cluster
	S-methylmethionine
	Tetracycline resistance, ribosome protection type
	Threonine and Homoserine Biosynthesis
	Transport of Nickel and Cobalt
	tRNA aminoacylation, Asp and Asn
	tRNA aminoacylation, His
	tRNA aminoacylation, Pro
	tRNA nucleotidyltransferase
	Universal GTPases
	Xylose utilization

APPENDIX II

JENEIL BIOTECH INC.

400 North Dekora Woods Blvd. Saukville, WI 53080 TEL: (262)268-6815 FAX: (262)-268-6820

CERTIFICATE OF ANALYSIS

PRODUCT NAME: ***Lactobacillus fermentum*** **LfQi6**
LOT NUMBER: 180423
MANUFACTURE DATE: April 23, 2018
BEST IF USED BY: April 23, 2019
COUNTRY OF ORIGIN: U.S.A.

	STANDARD SPECIFICATIONS	RESULTS	TEST METHOD
Lactobacillus fermentum **LfQi6**			
Enumeration	NLT 10.0 X 10^{10}/g	23.0 X 10^{10}/g	SMEDP 17th edition ISO 7889; 2003
ANALYTICAL			
Moisture %	6% Maximum	4.0%	AOAC #2008.06
Arsenic	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Cadmium	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Lead	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Mercury	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
MICROBIOLOGICAL			
Yeast & Mold	NMT 100 CFU /g	<10 CFU /g	BAM CH. 18
Coliform	NMT 10 CFU /g	<10 CFU /g	BAM CH. 4
E.coli	None Detected	None Detected	BAM CH. 4
Staphylococci (Coag. Pos.)	None Detected	None Detected	AOAC #975.55
Salmonella	None Detected	None Detected	AOAC #999.08
Listeria	None Detected	None Detected	AOAC #996.14
P.aeruginosa	None Detected	None Detected	Internal Method
ALLERGENS			
Beta-Lactoglobulin	<0.1 ppm	None Detected	ELISA Systems
Casein	<0.28 ppm	None Detected	ELISA Systems
Soy	<2.5 ppm	None Detected	ELISA Systems

Reviewed By:

August 15, 2019
Date:

JENEIL BIOTECH INC.
400 North Dekora Woods Blvd. Saukville, WI 53080 TEL: (262)268-6815 FAX: (262)-268-6820

CERTIFICATE OF ANALYSIS

PRODUCT NAME: ***Lactobacillus fermentum*** **LfQi6**
LOT NUMBER: 180329
MANUFACTURE DATE: March 29, 2018
BEST IF USED BY: March 29, 2019
COUNTRY OF ORIGIN: U.S.A.

	STANDARD SPECIFICATIONS	RESULTS	TEST METHOD
Lactobacillus fermentum **LfQi6**			
Enumeration	NLT 10.0 X 10^{10}/g	36.2 X 10^{10}/g	SMEDP 17th edition ISO 7889; 2003
ANALYTICAL			
Moisture %	6% Maximum	3.9%	AOAC #2008.06
Arsenic	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Cadmium	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Lead	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
Mercury	≤ 1 ppm	≤ 1 ppm	EPA 3050/6020 USP730
MICROBIOLOGICAL			
Yeast & Mold	NMT 100 CFU /g	<10 CFU /g	BAM CH. 18
Coliform	NMT 10 CFU /g	<10 CFU /g	BAM CH. 4
E.coli	None Detected	None Detected	BAM CH. 4
Staphylococci (Coag. Pos.)	None Detected	None Detected	AOAC #975.55
Salmonella	None Detected	None Detected	AOAC #999.08
Listeria	None Detected	None Detected	AOAC #996.14
P.aeruginosa	None Detected	None Detected	Internal Method
ALLERGENS			
Beta-Lactoglobulin	<0.1 ppm	None Detected	ELISA Systems
Casein	<0.28 ppm	None Detected	ELISA Systems
Soy	<2.5 ppm	None Detected	ELISA Systems

[redacted]
Reviewed By:

August 15, 2019
Date:

JENEIL BIOTECH INC.
400 North Dekora Woods Blvd. Saukville, WI 53080 TEL: (262)268-6815 FAX: (262)-268-6820

CERTIFICATE OF ANALYSIS

PRODUCT NAME: ***Lactobacillus fermentum*** **LfQi6**
LOT NUMBER: 170705
MANUFACTURE DATE: July 5, 2017
BEST IF USED BY: July 5, 2018
COUNTRY OF ORIGIN: U.S.A.

	STANDARD SPECIFICATIONS	RESULTS	TEST METHOD
Lactobacillus fermentum **LfQi6**			
Enumeration	NLT 10.0 X 10^{10}/g	27.4 X 10^{10}/g	SMEDP 17th edition ISO 7889; 2003
ANALYTICAL			
Moisture %	6% Maximum	3.9%	AOAC #2008.06
Arsenic	≤ 1 ppm	0.13 ppm	EPA 3050/6020 USP730
Cadmium	≤ 1 ppm	0.021 ppm	EPA 3050/6020 USP730
Lead	≤ 1 ppm	0.03 ppm	EPA 3050/6020 USP730
Mercury	≤ 1 ppm	0.006 ppm	EPA 3050/6020 USP730
MICROBIOLOGICAL			
Yeast & Mold	NMT 100 CFU /g	<10 CFU /g	BAM CH. 18
Coliform	NMT 10 CFU /g	<10 CFU /g	BAM CH. 4
E.coli	None Detected	None Detected	BAM CH. 4
Staphylococci (Coag. Pos.)	None Detected	None Detected	AOAC #975.55
Salmonella	None Detected	None Detected	AOAC #999.08
Listeria	None Detected	None Detected	AOAC #996.14
P.aeruginosa	None Detected	None Detected	Internal Method
ALLERGENS			
Beta-Lactoglobulin	<0.1 ppm	None Detected	ELISA Systems
Casein	<0.28 ppm	None Detected	ELISA Systems
Soy	<2.5 ppm	None Detected	ELISA Systems

[redacted]
Reviewed By:

August 15, 2019
Date:

Dear Dr. Highbarger, November 24, 2021

Quorum Innovations has received your additional questions regarding our Evaluation of the Generally recognized as Safe (GRAS) Status of *Lactobacillus Fermentum LfQi6* for as a Food Ingredient. In this response I have included your specific questions in quotes and Quorum's responses just underneath. We discussed the parameters of the responses that the agency required and I am hopeful that after that discussion we had, these answers meet the agencies expectations. The information herein is from our Contract Manufacturer who performed the specific testing. I enjoyed our earlier conversation and as always if there are any additional questions, please do not hesitate to contact me.

"Dear Dr. Monsul,

The Office of Food Additive Safety has a few additional questions that we would like you to address prior to completing your GRAS Notice 988 for the use of *Lactobacillus fermentum* LfQi6 as an ingredient in dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; and chewing gum at levels up to 2 x 10^8 colony forming unit (CFU)/serving."

1. "For the administrative record, we ask that you remove the use of *Lactobacillus Fermentum LfQi6* for functional/nutritional products since this does not fall under the purview of the GRAS Notification program."
   Ans: In the document Evaluation of the Generally recognized as Safe (GRAS) Status of *Lactobacillus Fermentum LfQi6* for as a Food Ingredient, Quorum Innovations will remove from page 4 of 40, Section 1.4, "… and functional/nutritional products."

2. "Please specify that all analytical methods are validated for their intended use."
   Ans: Quorum Innovations states that all tests used in the evaluation of LfQi6 have been validated, including but not limited to tests identified on pages 11 of 40, Table 4, in the document, Evaluation of the Generally recognized as Safe (GRAS) Status of *Lactobacillus Fermentum LfQi6* for as a Food Ingredient Specifications of *Lactobacillus fermentum* LfQi6 powder. Refer to the attached documents labelled QC Testing Methods (2021) and Lactobacillus fermentum LfQi6 Spec and C of As for lot 170705 180329 180423 (4 pgs) .

3. "Please provide the source of maltodextrin."
   Ans: Maltodextrin is used as an adjuvant sourced from Identity preserved corn, botanical source *Zea mays*. The Maltodextrin does not contain allergens.

4. "Please provide a statement to demonstrate that the fermentation medium used in the manufacturing of *Lactobacillus Fermentum* LfQi6 does not contain any major food allergens."
   Ans: The fermentation medium does not contain any major food allergens, and has been tested for the allergens listed on page 11 of 40, Table 4, in the document Evaluation of the Generally recognized as Safe (GRAS) Status of *Lactobacillus Fermentum LfQi6* for as a Food

Ingredient. The fermentation media does not contain milk or soy products. Please refer to the allergen statement attached for *Lactobacillus fermentum.*

5. "Additionally, I forgot to add some additional questions – specifically, what are the gram quantities of the microorganisms tested in you specifications."
   Ans: The sample sizes of LfQi6 powder used for microbiological tests listed on the Certificate of Analysis are: 10 grams for coliform, *Escherichia coli, Staphylococcus* (coagulase positive), Yeast and Mold, and *Pseudomonas aeruginosa*. For Listeria and Salmonella testing, a 25 gram sample is used.

6. "We ask that you please respond to these questions within 10-working days.
   Ans: After discussion with Dr. Highbarger, we agreed to provide the specific response of week ending Nov 26th, 2021.

Thank you for your attention and if there are any further questions, please do not hesitate to contact me either by email (nickmonsulmd@quoruminnovations.comn) or phone (941) 951-1026.

All the Best,

Nicholas Monsul, MD

Dear Dr. Highbarger, January 19, 2022

Thank you for your comments regarding our GRN 998 Application. Our responses to the specific questions are included in this document, but in blue.

Hi Nick,

We have two additional quick questions before we can complete our review of GRN 988 for the use of *Lactobacillus fermentum* LfQi6 in food.

1. Intended use of *L. fermentum* LfQi6

In **Section 1.4. Intended conditions of use** (p. 4), the notice states: "The subject of this GRAS, *Lactobacillus fermentum* LfQi6, a standardized powder, is intended for use as a food ingredient for consumers in the following food categories: dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum; and functional/nutritional products."

However, in **Section 6.3. GRAS Panel Evaluation, Summary and Discussion** (p. 28-29), the notice states that "…. for use as a food ingredient in selected food products such as bakery products (biscuits, pastries, cookies, brownies, crackers), chocolate (also includes gummies-candy), yogurt and flavored dairy beverages at use levels up to $2x10^8$ cfu/serving".

Please clarify the discrepancy between **Section 1.4** and **Section 6.3** and confirm the uses and use levels of *L. fermentum* LfQi6.

Quorum Innovations asserts, in section 1.4 Intended conditions of use, to **remove** the "functional/nutritional products" from the list of intended uses. Furthermore, the intended food categories in Section 1.4, "… dairy products (fluid milk and milk drinks, milk-based desserts and meal replacements, dry and powdered milk, yogurt, and cheese); ready-to-eat cereals; fruit juices, nectars, ades, and drinks; confections; chewing gum …" will be included in Section 6.3 **GRAS Panel Evaluation, Summary and Discussion** (p. 28-29) for consistency of application.

2. Dietary Exposure to *L. fermentum* LfQi6

Generally, in the absence of stated use levels in specific food categories, estimates of dietary exposure are based on the maximum intended use level for an ingredient. We note that, based on the statements provided in GRN 000988, the estimated dietary exposure to *L. fermentum* LfQi6 (2×10^9 cfu/d) on p. 13 of the notice is based on the level intended (2×10^8 cfu/serving) rather than the maximum use level(s) (up to 10^{10} cfu/serving) added to achieve the level intended (2×10^8 cfu/serving) at the point of consumption. Further you cite the dietary exposure estimate, calculated using the minimum use level, in your concluding statement "For safety assessment purposes, the high intake of $2x10^9$ cfu of *L. fermentum* LfQi6/person/day is considered." We request the following clarifications to address the higher use levels in your dietary exposure estimate.

a) Please provide an estimated dietary exposure for *L. fermentum* LfQi6 that is based on the highest use level (10^{10} cfu/serving).

b) Please confirm that you considered the use level of up to 10^{10} cfu/serving and the resulting estimates of dietary exposure in your safety evaluation.

Quorum Innovation has shown that *L. fermentum* LfQi6 is non-toxic, lacks potentially harmful metabolic activities, does not produce toxic biogenic amines, lacks mucin degradation, various enzymatic activities and non-pathogenic hemolytic activity. Additionally, *L. fermentum* LfQi6 lacks the virulence factors and transposable elements further concluding that *L. fermentum* LfQi6 is non-toxic. Quorum has determined that consumption is safe at levels of $8x10^{10}$/serving/day and is consistent with other approved usages of other similar *L. fermentum* strains, CECT5716 at $1x10^{10}$ cfu/day, ME-3 DSM-14241 at $3x10^{11}$ cfu/day, SBS-1 at $2x10^{9}$ cfu/day.

Quorum Innovations thanks you for your attention to our application. We would welcome any additional questions should they arise.

All the Best

Nicholas T. Monsul, M.D.

Thank you for your attention to this matter.

If you have any additional questions, do not hesitate to contact me.

Lane A. Highbarger, Ph.D. (he/him)
Microbiology and Regulatory Review

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Nicholas Monsul, M.D.
Quorum Innovations

Re: GRAS Notice No. GRN 000988

February 25, 2022

Dear Dr Highbarger;

Thank you for your latest correspondence and conference call with your colleague on February 23, 2022 to clarify the three items listed below. After our discussion that day, we mutually agreed to the three points listed below. This note, therefore, is to confirm in writing our mutual understanding. Quorum's return comments are noted in a different font and italicized to highlight our responses and to confirm that we are in agreement.

1. Please confirm that the intended use of *Limosilactobacillus fermentum* LfQi6 is to provide 2 x 10^8 colony forming unit (CFU)/serving in fluid milk and milk drinks, milk-based desserts and meal replacements, flavored dairy beverages, dry and powdered milk, yogurt, cheese, ready-to-eat cereals, fruit juices, nectars, ades, and drinks, confections, biscuits, pastries, cookies, brownies, crackers, chocolate, gummies-candy, and chewing gum.

   Quorum response to 1.: confirmed.

   a. Additionally, please confirm that *L. fermentum* LfQi6 will be added up to maximum use level of 10^{10} CFU/serving.

      Quorum response to 1a: confirmed.

2. You stated in your 12 Jan 2022 response:

   > *Quorum has determined that consumption is safe at levels of **8x10^{10}/serving/day** and is consistent with other approved usages of other similar L. fermentum strains, CECT5716 at 1x10^{10} cfu/day, ME-3 DSM-14241 at 3x10^{11} cfu/day, SBS-1 at 2x10^{9} cfu/day**.**

   Please clarify you meant **8x10^{10} CFU/day**.

   Quorum response to 2: confirmed, 8x10^{10} CFU/day.

3. It is our understanding that you have concluded that the dietary exposure to the ingredient is safe at use levels up to 8x10^{10} CFU/day. Based on the use level and the safety narrative that you have presented in the notice we understand that this use level would correspond to a consumption of 8 servings of food per day. Please confirm.

   Quorum response: confirmed.

Sincerely,

Nicholas T. Monsul, M.D.