26 January 2023

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

Dear Dr. Gaynor:

Re: GRAS Notice for *Lactobacillus rhamnosus* MP108

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Glac Biotech Co. Ltd, as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that *Lactobacillus rhamnosus* MP108, is GRAS on the basis of scientific procedures, for use in conventional food and beverage products, including those intended for infants, across multiple categories. These food uses of *Lactobacillus rhamnosus* MP108, are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Glac Biotech Co. Ltd's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Sheng-Hung Huang
General Manager
Glac Biotech Co. Ltd

Email: michael.huang@glac.com.tw
Tel: +886-2-26558108#118

GRAS NOTICE FOR USE OF *LACTOBACILLUS RHAMNOSUS* MP108 IN CONVENTIONAL FOOD AND BEVERAGE PRODUCTS, INCLUDING THOSE INTENDED FOR INFANTS, IN THE UNITED STATES

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

SUBMITTED BY:
Glac Biotech Co., Ltd.
3F-2, No.17, Guoji Rd.
Xinshi Dist., Tainan City
74442 Taiwan

DATE:
29 December 2022

GRAS Notice for Use of *Lactobacillus rhamnosus* MP108 in Conventional Food and Beverage Products in the United States

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GRAS Notice for Use of *Lactobacillus rhamnosus* MP108 in Conventional Food and Beverage Products in the United States

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Glac Biotech Co. Ltd., (Glac Biotech) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of *Lactobacillus rhamnosus* MP108, as manufactured by Glac Biotech, in various conventional food and beverage products as described in Section 1.3 below, are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Glac Biotech's view that these notified uses of *L. rhamnosus* MP108 are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Glac Biotech, the undersigned hereby certifies that all data and information presented in this notice represents a complete and balanced submission that is representative of the generally available literature. Glac Biotech considered all unfavorable as well as favorable information that is publicly available and/or known to Glac Biotech and that is pertinent to the evaluation of the safety and GRAS status of *L. rhamnosus* MP108 as a food ingredient for addition to *L. rhamnosus* MP108 food products, as described herein.

Signed,

Sheng-Hung Huang,

General Manager, Glac Biotech Co., Ltd

Dec. 29th. 2022.
Date

1.1 Name and Address of Notifier

Glac Biotech Co., Ltd.
3F-2, No.17, Guoji Rd.
Xinshi Dist., Tainan City
74442 Taiwan

1.2 Common Name of Notified Substance

Current name: Lacticaseibacillus rhamnosus MP108

Homotypic synonym: Lactobacillus rhamnosus MP108

It should be noted that the International Journal of Systematic and Evolutionary Microbiology released a new classification system for the *Lactobacillaceae* family (Zheng *et al.*, 2020). Based on several nucleotide and amino acid identity approaches and phylogenic, metabolic, and identifying genes, *Lactobacillaceae* was split into 26 genera. The *Lactobacillus rhamnosus* is now referred to as *Lacticaseibacillus rhamnosus*, but

based on the long history of use under its homotypic synonym *Lactobacillus rhamnosus*, it will be referred to as such for the remainder of this document.

1.3 Conditions of Use

L. rhamnosus MP108 is intended for use as an ingredient in food and beverage products from several categories, including beverages, cereals, dairy and dairy analogues, grain products, confections, and food intended for infants (excluding infant formula) intended for the U.S. marketplace. The ingredient is intended for use at a maximum level of 1.0 x 10^9 CFU/serving in all products. A summary of the food categories in which *L. rhamnosus* MP108 is intended for use is provided in Table 1.3-1, organized according to 21 CFR §170.3 (U.S. FDA, 2021). The ingredient is not subject to 21 §170.270 as it is not intended for use in meat and poultry or meat and poultry containing products that are subject to U.S. Department of Agriculture (USDA) and the USDA Food Safety Inspection Service (FSIS) oversight.

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021)	Food Uses*	Maximum Intended Use Level (CFUx10^9/serving)
Beverages and Beverage Bases	Energy Drinks	1.0
	Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.0
	Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	1.0
	Sports Drinks	1.0
	Bottled tea	1.0
Breakfast Cereals	Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	1.0
	Ready-to-Eat Breakfast Cereals	
	Puffed Cereals	1.0
	High-Fiber Cereals	1.0
	Biscuit-Type Cereals	1.0
Cheeses	Cheeses	1.0
Chewing Gum	Chewing Gum	1.0
Dairy Product Analogs	Non-Dairy Milk (soy-based drinks)	1.0
Gelatins, Puddings, and Fillings	Milk-Based Desserts	1.0
Grain Products and Pastas	Cereal and Granola Bars	1.0
	Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.0
Hard Candy	Hard Candy	1.0
Milk Products	Buttermilk	1.0
	Evaporated, Condensed, and/or Dry Milks	1.0
	Fermented Milks, Plain	1.0
	Flavored Milks, Milk Drinks, and Mixes	1.0
	Milk Shakes	1.0
	Milk-Based Meal Replacement, Nutrition, and Protein Beverages[a]	1.0
	Plain or Flavored Yogurt	1.0

Table 1.3-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021)	Food Uses*	Maximum Intended Use Level ($CFUx10^9$/serving)
	Yogurt Drinks	1.0
Plant Protein products	Soy-based Food	1.0
Processed Fruits and Fruit Juices	Fruit Drinks and Ades Including Smoothies	1.0
	Fruit Juices	1.0
	Fruit Nectars	1.0
Soft Candy	Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	1.0
Other – Baby Food	Baby food: Cereals	
	Dry Instant	1.0
	Prepared, Ready-to-Serve	1.0
	Baby food: Ready-to-Eat cereals	1.0
	Baby food: Fruits or Vegetables (strained)	1.0
	Baby food: Fruit Juice	1.0

CFR = *Code of Federal Regulations*; CFU = colony-forming units; U.S. = United States.
* *L. rhamnosus* MP108 is intended for use in unstandardized products and not in foods where standards of identity exist and do not permit its addition.
[a] Includes ready-to-drink and powdered forms.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2018b), Glac Biotech has concluded that the intended uses of *L. rhamnosus* MP108 as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Glac Biotech Co., Ltd.
3F-2, No.17, Guoji Rd.
Xinshi Dist., Tainan City
74442 Taiwan
email: michael.huang@glac.com.tw

Should the FDA have any questions or additional information requests regarding this Notification, Glac Biotech will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Glac Biotech's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and

therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Description of the Ingredient

Glac Biotech's *Lactobacillus rhamnosus* MP108 ingredient is a lyophilized powder, white to light brown with a fermented smell, containing not less than ≥1.0 x 10^{11} CFU/g. The ingredient is manufactured by large scale, batch culture to propagate the cells to a cell density of 1 x 10^{9} CFU/ml, and cells are separated from the culture medium by centrifugation prior to lyophilization. Final processing of the product includes the addition of maltodextrin derived from non-GM corn starch (maize) as a cryoprotectant to not less than 97% *L. rhamnosus* MP108.

2.1.2 Name and Taxonomy

Common Name: *Lactobacillus rhamnosus* MP108

Taxonomic Lineage:

Kingdom: *Bacteria*
Phylum: *Firmicutes*
Class: *Bacilli*
Order: *Lactobacillales*
Family: *Lactobacillaceae*
Genus: *Lactobacillus*
Species: *rhamnosus*
Strain: MP108

2.1.3 Classification of *L. rhamnosus*

L. rhamnosus is identified by clustered, rod-shaped bacteria formations, lactic acid producing, facultative heterofermentation activity, and 16S rDNA sequencing (Collins *et al.,* 1989; Zheng *et al.,* 2020). The *Lactobacillus* genus has undergone an evolution of classification through advances in molecular techniques and the use of 16S rDNA gene sequencing. *L. rhamnosus* was formerly considered a subspecies of *L. casei*; however, taxonomic characterization by Collins *et al*. (1989) resulted in designation of *L. rhamnosus* as a separate species. More recent polyphasic taxonomic characterization studies by Zheng *et al*. (2020) have resulted in reclassification of the genus *Lactobacillus* into 25 genera, and *Lactobacillus rhamnosus* was renamed *Lacticaseibacillus rhamnosus*. Despite this official name change, the nomenclature *Lactobacillus* remains valid, and use of the updated nomenclature is, at this moment, not widespread due to the familiarity of the original naming convention. Accordingly, the name *Lactobacillus rhamnosus (L. rhamnosus)* will be used throughout this dossier.

2.1.4 Phenotypic Identity

L. rhamnosus is a commensal, non-motile, rod-shaped, Gram-positive, aerotolerant anaerobe, non-spore forming, facultative heterofermenter bacteria tolerant of the environmental conditions in the gastrointestinal (GI) tract (*e.g.,* low pH, anaerobic fermentation). This species is also a member of a large classification of Lactic Acid-producing Bacteria (LAB), which as the name suggests, are capable of producing lactic acid as a metabolic end product of carbohydrate catabolism. Several other distinct but related genera cover species that qualify as LAB, such as *Lactococcus* and *Streptococcus* (Quinto *et al.,* 2014).

2.1.5 Genotypic Identity

The *L. rhamnosus* MP108 strain, isolated from infant feces, was initially identified by 16S rRNA and phenylalanyl-tRNA synthetase *alpha* subunit (pheS) gene sequencing. The strain was deposited in the Bioresource Collection and Research Center (Taiwan) under BCRC 19616. More recently, the genome of the organism was sequenced using bacterial *de novo* sequencing to generate an assembly map of the genome (Table 2.1.5-1).

Table 2.1.5-1 Overview of the Genome for *L. rhamnosus* MP108

Strain	MP108
Genome size (bp)	2.925 Million
GC (%)	47.47
Gene (CDS)	2,884
Clustered Gene	2,828
Trna	59
Plasmid	0
Prophage	0

bp = base pairs; CDS = coding sequence; GC = guanine-cytosine; tRNA = transfer ribonucleic acid.

2.2 Manufacturing

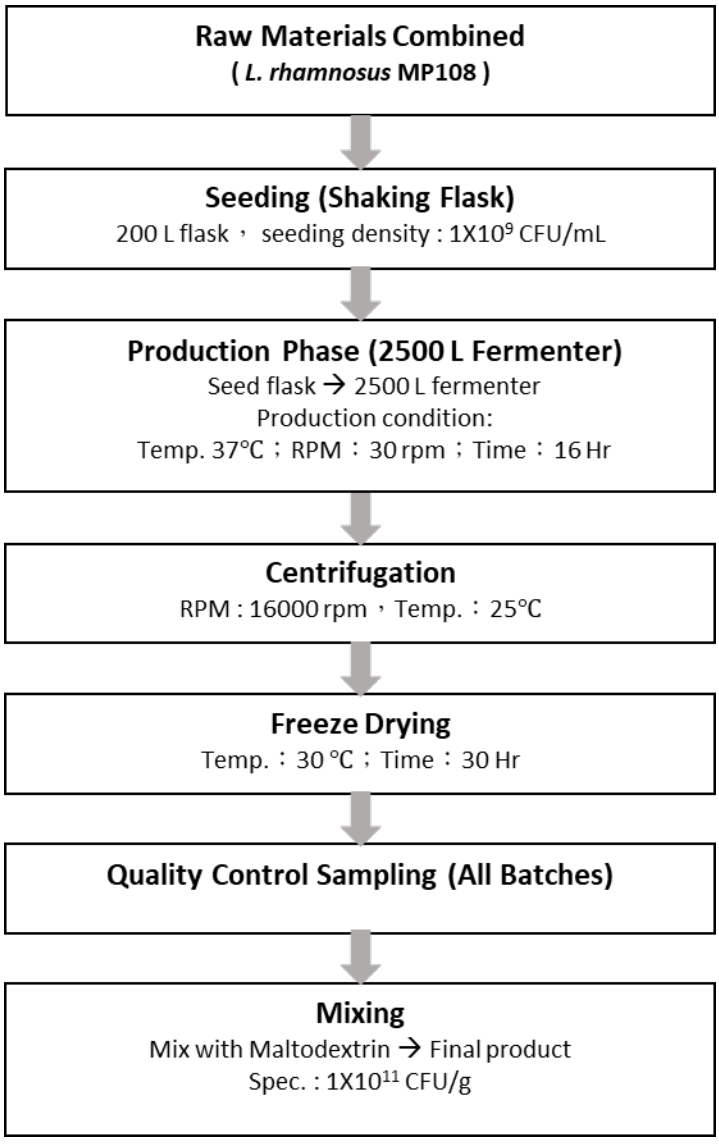
2.2.1 Raw Materials/Processing Aids

The growth medium contains nutrient sources and ingredients that are commonly used in microbial growth and fermentation media. All processing aids and ingredients used in the fermentation process are food-grade and are permitted for their respective use by federal regulation or have previously been determined to be GRAS for their intended uses. Glucose is used as a carbon source and skim milk powder, whey powder, soybean protein hydrolysate and soybean protein isolate are used as nitrogen sources. The protein sources are hydrolyzed to peptides and amino acids using an alkaline protease enzyme. Analyses of the final ingredient for residues of intact allergenic protein using validated ELISA assay test kits for food matrices did not identify evidence for transfer of allergenic protein to the finished product. The risk of allergenicity by individuals sensitized to milk or soybean protein allergens following consumption of food products containing the ingredient were considered low; however, Glac Biotech's *L. rhamnosus* MP108 will be subject to the allergen labeling requirements of the U.S. Food Allergen Consumer Labeling Protection Act. Glac Biotech's *L. rhamnosus* MP108 ingredient is manufactured in compliance with U.S. cGMP for food at an ISO22000:2005 certified facility.

2.2.2 Description of the Production Process

Glac Biotech's *L. rhamnosus* MP108 ingredient is manufactured using an optimized microbial fermentation process followed by live microbe isolation and freeze drying. Briefly, sterilized growth media is prepared for the 200 L seed culture started with a 16S rRNA-verified *L. rhamnosus* MP108 inoculum, which is grown in a shaking flask at 37˚C to a cell density of 1 x 10^9 CFU/ml. The seed culture is used to inoculate the 2,500 L production culture which is grown at 37°C, stirred at low RPM, for 16 hours. Cells are isolated by centrifugation at 25°C and 16,000 RPM. Isolated cells are then freeze dried and mixed with maltodextrin to a final concentration of 1 x 10^{11} CFU/g for packaging.

Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for *L. rhamnosus* MP108



2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

The chemical specifications for *L. rhamnosus* MP108 are presented in Table 2.3.1-1.

Table 2.3.1-1 Product Specifications for *L. rhamnosus* MP108

Specification Parameter	Specification Limit	Method of Analysis
Physiochemical		
Appearance & Odor	Light yellow to light brown	Sensory evaluation
L. rhamnosus MP108	≥1.0 x 10^{11} CFU/g	MOHWM0013.01
Moisture	≤7%	CNS5033
Water Activity (Aw)	≤0.25	CNS5255
Heavy Metals		
Lead	≤0.1 ppm	MOHWM0014.03
Arsenic	≤0.02 ppm	MOHWM0014.03
Microbiological		
Coliforms	(<0.3 MPN/50 g)	MOHWM0015.01
Escherichia coli	Negative (CFU/50 g)	MOHWM0023.01
Yeast and Mold	≤1 x 10^{2} CFU/g	MOHWM0008.01
Salmonella	Negative (CFU/g)	MOHWM0025.01
Staphylococcus aureus	Negative (CFU/g)	MOHWM0002.02
Listeria monocytogenes	Negative (CFU/25 g)	MOHWM0026.03
Cronobacter spp. *Enterobacter sakazakii*	Negative (MPN/g)	MOHW0004.02

CFU = colony-forming units; MOHW = Ministry of Health and Welfare; MPN = most probable number.

2.3.2 Batch Analysis

Analysis of 3 non-consecutive lots of *L. rhamnosus* MP108 powder demonstrates that the manufacturing process as described in Section 2.2 produces a consistent product that meets specifications. A summary of the chemical analysis for the 3 lots of *L. rhamnosus* MP108 is presented in Table 2.3.2-1.

Table 2.3.2-1 Summary of the Batch Analysis for 3 Lots of *L. rhamnosus* MP108

Specification Parameter	Specification	Manufacturing Lot		
		51020200261	**51020200288**	**51020210057**
Physiochemical				
Appearance & Odor	Light yellow to light brown	Complies	Complies	Complies
L. rhamnosus MP108	≥1.0 x 10^{11} CFU/g	4.8 x 10^{11}	4.7 x 10^{11}	5.7 x 10^{11}
Moisture	≤7%	2.1	2.4	5.4
Water Activity (Aw)	≤0.25	0.05	0.004	0.04
Heavy Metals				
Lead (L.O.D. 0.01 ppm)	≤0.1 ppm	B.D.	B.D.	B.D.

Table 2.3.2-1 Summary of the Batch Analysis for 3 Lots of *L. rhamnosus* MP108

Specification Parameter	Specification	Manufacturing Lot		
		51020200261	51020200288	51020210057
Arsenic (L.O.D. 0.01 ppm)	≤0.02 ppm	B.D.	B.D.	B.D.
Microbiological				
Coliforms	<0.3 MPN/50 g	B.L.	B.L.	B.L.
Escherichia coli	Negative (CFU/50 g)	Negative	Negative	Negative
Yeast and Mold	≤1 x 10^2 CFU/g	<10	<10	<10
Salmonella	Negative (CFU/g)	Negative	Negative	Negative
Staphylococcus aureus	Negative (CFU/g)	Negative	Negative	Negative
Listeria monocytogenes	Negative (CFU/25 g)	Negative	Negative	Negative
Cronobacter spp. *Enterobacter sakazakii* (L.O.D. 0.003 MPN/g)	Negative (MPN/g)	Negative	Negative	Negative

B.D. = below detection; B.L. = below limit; CFU = colony-forming units; L.O.D. = limit of detection; MPN = most probable number.

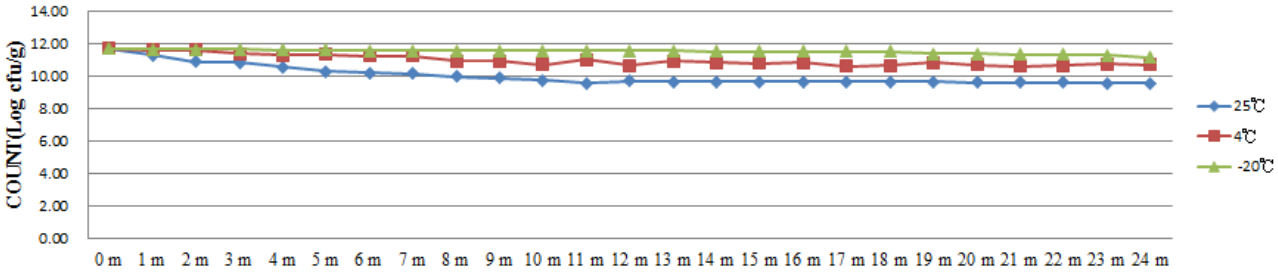
2.4 Stability

When stored in its original unopened container, *L. rhamnosus* MP108 powder is stable at 25°C, 60% relative humidity for 24 months. The stability of *L. rhamnosus* MP108 was tested under 3 temperature and relative humidity conditions. The first set of stability data provided by Glac Biotech demonstrate *L. rhamnosus* MP108 is stable at 4°C for 24 weeks, with cell viability greater than 1 x 10^{11} CFU/g; however, *L. rhamnosus* MP108 stored at 25°C showed significant reduction in cell viability after 3 months (10^{11} CFU/g to 10^4 CFU/g). Therefore, this data shows that *L. rhamnosus* MP108 is stable when stored at 4°C for greater than 24 weeks and at 25°C for 3 months.

The second set of stability data provided by Glac Biotech demonstrate *L. rhamnosus* MP108 is stable at 4°C for 7 months, with cell viability greater than 1 x 10^{11} CFU/g, and while at 25°C there was less of a reduction in cell viability (2.18 x 10^{11} CFU/g to 8.39 x 10^9 CFU/g) after 7 months compared to the first study. However, the second set of stability data provided by Glac Biotech still showed the cell viability of *L. rhamnosus* MP108 at 25°C dropped below the specification of greater than 1 x 10^{11} CFU/g after 1 month (9.59 x 10^{10} CFU/g). The current data supports the stability of the *L. rhamnosus* MP108 ingredient when stored at 4°C for up to 7 months.

Glac Biotech conducted a study to evaluate the stability of the *L. rhamnosus* MP108 ingredient during long-term storage. The stability of the ingredient, quantified by cell viability, was observed for 24 months under 3 distinct storage conditions with varying temperature and relative humidity: 1) 25°C, 60% RH; 2) 4°C; and 3) -20°C. Viability was assessed as a logarithmic function of CFU/g each month during the 24-month storage period and was found to decrease in each test condition by log(2.12), log(0.98), and log(0.51), respectively, as illustrated in Figure 2.4-1. The viability of each sample was within product specifications detailed in Table 2.3.1-1. Glac Biotech concluded from these data that *L. rhamnosus* MP108 ingredient is stable for 24 months when stored at -20°C and 4°C, and for 3 months at 25°C with 60% relative humidity.

Figure 2.4-1 Viability Stability Testing of *L. rhamnosus* MP108



Part 3. §170.235 Dietary Exposure

3.1 Estimated Intake of *L. rhamnosus* MP108

3.1.1 Methods

An assessment of the anticipated intake of *L. rhamnosus* MP108 as an ingredient under the intended conditions of use was conducted using data available in the 2017-2018 cycle of the U.S. National Center for Health Statistics (NHANES) (CDC, 2021a,b; USDA, 2021). A detailed description of the survey and methodology employed in the intake assessment of *L. rhamnosus* MP108 is provided in Appendix A, while an abbreviated summary along with the pertinent results is presented herein.

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2017-2018. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2021a,b; USDA, 2021). The NHANES data were employed to assess the mean and 90th percentile intake of *L. rhamnosus* MP108 for each of the following population groups:

- Infants, ≤6 months;
- Infants, 7 to 12 months;
- Young children, 13 to 24 months;
- Children, ages 2 to 5 years;
- Children, ages 6 to 11 years;
- Female teenagers, ages 12 to 19 years;
- Male teenagers, ages 12 to 19 years;
- Female adults, ages 20 years and up;
- Male adults, ages 20 years and up; and
- Total population (ages 2 years and older, and both gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of *L. rhamnosus* MP108 by the U.S. population.[1] Estimates for the daily intake of *L. rhamnosus* MP108 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2017-2018; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of *L. rhamnosus* MP108 averaged over all individuals surveyed, regardless of whether they consumed food products in which *L. rhamnosus* MP108 is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*., those who reported no intake of food products containing *L. rhamnosus* MP108 during the 2 survey days). "Consumer-only" intake refers to the estimated intake of *L. rhamnosus* MP108 by those individuals who reported consuming food products in which the use of *L. rhamnosus* MP108 is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which *L. rhamnosus* MP108 is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of *L. rhamnosus* MP108 were generated using the maximum use level indicated for each intended food use together with food consumption data available from the 2017-2018 NHANES datasets. The results for these assessments are presented in Section 3.1.2.

3.1.2 Intake Estimates for *L. rhamnosus* MP108

A summary of the estimated daily intake of *L. rhamnosus* MP108 from proposed food uses is provided in Table 3.1.2-1 on an absolute basis (CFUx10^9/person/day), and in Table 3.1.2-2 on a body weight basis (CFUx10^8/kg body weight/day).

The percentage of consumers was lowest in infants up to 6 months of age, at 34.7%, while the percentage of consumers was high among all other age groups evaluated in the current intake assessment; greater than 90.6% of the population groups consisted of consumers of food products in which *L. rhamnosus* MP108 is currently proposed for use. Children (ages 2 to 5 years) had the greatest proportion of consumers at 99.4%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of *L. rhamnosus* MP108 were determined to be 2.9 and 6.3 CFUx10^9/person/day, respectively. Of the individual population groups, infants ages 7 and 12 months were determined to have the greatest mean and 90th percentile consumer-only intakes of *L. rhamnosus* MP108 on an absolute basis, at 6.0 and 14.6 CFUx10^9/person/day, respectively, while female teenagers had the lowest mean and 90th percentile consumer-only intakes of 2.4 and 4.6 CFUx10^9/person/day, respectively (3.1.2-1).

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3.1.2-1 Summary of the Estimated Daily Intake of *L. rhamnosus* MP108 from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to ≤6 months	1.3	3.7*	34.7	68	3.7	10.6*
Infants	7 to 12 months	5.7	14.5	94.6	126	6.0	14.6
Young Children	13 to 24 months	3.1	6.3	98.6	146	3.2	6.3
Children	2 to 5 years	3.0	5.4	99.4	466	3.0	5.4
Children	6 to 11 years	2.9	5.3	98.6	670	2.9	5.3
Female Teenagers	12 to 19 years	2.3	4.6	93.7	420	2.4	4.6
Male Teenagers	12 to 19 years	2.7	5.9	95.2	409	2.9	6.0
Female Adults	20 years and older	2.6	5.8	93.4	1,980	2.7	5.9
Male Adults	20 years and older	2.9	6.8	90.6	1,760	3.2	7.2
Total Population	2 years and older	2.7	6.0	93.2	5,705	2.9	6.3

CFU = colony-forming units; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table 3.1.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group	*Per Capita* Intake (CFUx10^8/kg bw/day)		Consumer-Only Intake (CFUx10^8/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants	0 to ≤6 months	1.8	5.1*	34.7	68	5.2	13.9*
Infants	7 to 12 months	6.3	16.5	94.6	126	6.6	16.5
Young children	13 to 24 months	2.8	6.2	98.6	144	2.8	6.2
Children	2 to 5 years	1.8	3.3	99.5	459	1.8	3.3
Children	6 to 11 years	0.9	1.8	98.6	668	0.9	1.8
Female Teenagers	12 to 19 years	0.4	0.8	93.6	413	0.4	0.9
Male Teenagers	12 to 19 years	0.4	0.9	95.1	406	0.4	0.9
Female Adults	20 years and older	0.4	0.8	93.4	1,962	0.4	0.8
Male Adults	20 years and older	0.3	0.8	90.6	1,746	0.4	0.9
Total Population	2 years and older	0.5	1.1	93.2	5,654	0.5	1.1

Table 3.1.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group	*Per Capita* Intake ($CFUx10^8$/kg bw/day)		Consumer-Only Intake ($CFUx10^8$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile

bw = body weight; CFU = colony-forming units; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

3.1.3 Summary and Conclusions

Consumption data and information pertaining to *L. rhamnosus* MP108 were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain *L. rhamnosus* MP108 at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that *L. rhamnosus* MP108 will have 100% market penetration in all identified food categories.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of *L. rhamnosus* MP108 by the total U.S. population from proposed food uses in the U.S. were estimated to be 2.9 $CFUx10^9$/person/day (0.5 $CFUx10^8$/kg body weight/day) and 6.3 $CFUx10^9$/person/day (1.1 $CFUx10^8$/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of *L. rhamnosus* MP108 were determined to be 6.0 $CFUx10^9$/person/day (6.6 $CFUx10^8$/kg body weight/day) and 14.6 $CFUx10^9$/person/day (16.5 $CFUx10^8$/kg body weight/day), as identified among infants ages to 7 and 12 months, respectively. Female teenagers had the lowest mean and 90th percentile consumer-only intakes of 2.4 $CFUx10^9$/person/day (0.4 $CFUx10^8$/person/day) and 4.6 $CFUx10^9$/person/day (0.9 $CFUx10^8$/person/day), respectively. On a body weight basis, teenagers and adults (both genders) had the lowest mean consumer-only intakes of 0.4 $CFUx10^8$/kg body weight/day while female adults had the lowest 90th percentile of 0.8 $CFUx10^8$/kg body weight/day.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with *L. rhamnosus* MP108.

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Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

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Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

The GRAS evaluation of *L. rhamnosus* MP108 was conducted using scientific procedure and was modeled following consideration of the EFSA QPS guidelines (EFSA, 2007), the guidelines for the Evaluation of Probiotics in Food (FAO/WHO, 2002) issued by the Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food, and the safety decision tree for evaluating microbial cultures intended for human and animal consumption published by Pariza *et al.* (2015).

The MP108 strain does not have a history of consumption in the U.S. It is available for use in infant and children's food in China following an approval by the National Health Commission (NHC) of China. In the EU, *L. rhamnosus* has been granted Qualified Presumption of Safety (QPS) status by EFSA which indicates that *"strains should not harbor and acquired antimicrobial resistance genes to clinically relevant antimicrobials"* (EFSA, 2018).

A comprehensive toxicological assessment of strain MP108 published by Zhang *et al.* (2021) included a reverse bacterial mutation assay, an *in vitro* chromosome aberration assay in Chinese hamster ovary cells, and an *in vivo* mouse micronucleus assay to evaluate genotoxicity and mutagenicity. A 90-day *in vivo* toxicology study in rats certified by the Food Safety National Standard (China), which adheres to standards similar to the relevant Organisation for Economic Co-operation and Development (OECD) Test Guidelines, was also reported by Zhang *et al.* (2021). In these toxicological studies, no genotoxic effects of *L. rhamnosus* MP108 were observed at the maximum dose tested, 5.6 g/kg body weight, and a no-observed-adverse-effect-level (NOAEL) of 1,500 mg/kg body weight/day, equivalent to >1.5 x 10^{11} CFU/kg body weight/day, in rats was established (Zhang *et al.,* 2021). Additional animal studies evaluating acute (single dose) and subchronic (28-day) consumption of *L. rhamnosus* MP108 in rats were conducted on behalf of Glac Biotech by Chuangyi Biotech Co., Ltd. (2012a,b); these unpublished data are corroborative and not pivotal to this safety evaluation. A product-specific, clinical study conducted in infants by Wu *et al.* (2017), included in Section 6.4.1, was also supportive of the safe use of *L. rhamnosus* MP108 in infants for up to 8 weeks at 3.5 x 10^{10} CFU/day. In line with other GRAS notices for microbial ingredients, the decision tree for determining the safety of microbial food cultures published by Pariza *et al.* (2015) was applied to *L. rhamnosus* MP108, and the strain was concluded to be safe for human consumption when used in the manufacture of food, probiotics, and dietary supplements. In addition, discussion of the metabolic fate of *L. rhamnosus* MP108 pertaining to bacterial translocation from the GI tract and the potential for gut colonization has been included in Section 6.2. The potential for antibiotic resistance transfer, virulence, and pathogenicity were investigated using both empirical (see Section 6.5.1) and bioinformatics (see Section 6.5.2) approaches.

6.2 Absorption, Distribution, Metabolism and Excretion

Unlike other small molecule or biologically sourced food and beverage ingredients, the consumption of microorganisms from natural sources (*e.g.,* fermented foods) or from food and beverage products to which a microorganism has been added, has the potential to impact the gut microbiome and gut health by colonization of the GI tract and contribution to the metabolic function of the gut (*e.g.,* short-chain fatty acid production). The metabolic fate of a live organisms consumed from food is difficult to define clinically due to the extremely complex nature of microbial metabolism and the immune system response in the GI tract, which is further confounded by significant interpatient variation in gut microbiome health and activity. A discussion of the potential for translocation to systemic circulation, pathogenicity, major metabolic products, and colonization of the GI tract is presented for *L. rhamnosus* MP108 below.

6.2.1 Bacterial Translocation and Pathogenicity

The translocation of live microorganisms from the lumen of the GI tract to circulation and extraintestinal sites is not common and often associated with increased GI permeability due to compromised integrity of the GI barrier. The resulting transport of bacteria to the mesenteric lymph nodes, liver, spleen, kidney, and systemic circulation may lead to the development of bacteremia, sepsis, and/or multiple organ failure (Ishibashi and Yamazaki, 2001; Lichtman, 2001; MacFie, 2004; Liong, 2008).

The safety of lactic acid bacteria and their occurrence in human clinical infections was reviewed by Gasser (1994) in which the infections were categorized as endocarditis, hematological infections, and extraintestinal, localized infections. Cases of infections from *L. rhamnosus* strains were identified in each category. A total of 33 cases were evaluated in the review; 23 cases of endocarditis (including *L. casei* spp. *rhamnosus* pre-1989*)*, 5 cases of blood stream infections, 4 chest infections, and 1 GI infection. In cases of endocarditis attributed to *L. rhamnosus* strains, no clinical features to differentiate these infections from endocarditis caused by other microorganisms were observed, and frequency of occurrence was low. The reviewed cases were largely associated with prior endocarditis or predisposing poor dental health or recent dental procedures. This suggests that the infections, which "*represent infections of extreme rarity",* are not related to translocation of *L. rhamnosus* out of the GI tract (Gasser, 1994). An overview of the safety of dietary microorganisms, in which the focused discussion of a panel of experts, with a variety of expertise in fields required for the effective study of microbial ingredient safety, evaluating evidence from available clinical data, was published by Borriello *et al.* (2003). The following conclusion was stated:

> "*Current evidence suggests that the risk of infection with […] lactobacilli or bifidobacteria is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers* [...]" (Borriello *et al.,* 2003).

A review was published by Goldstein *et al.* (2015) in which the authors further evaluated the taxonomic complexity observed between species of the *Lactobacillus* genus and how those species may be characterized from a perspective of risk potential and safety from infection and antibiotic resistance. Clinical reports of several infection types, including bacteremia, endocarditis, meningitis, and peritonitis, were evaluated for significance and relatedness to consumption of *L. rhamnosus* species from a variety of food items. The available data from empirical MIC testing of the reported strains was published in those reports. Similar analyses were conducted on reviews reporting antibiotic resistance and cases of *Lactobacillus* infections published by Salminen *et al.* (2006) and Gouriet *et al.* (2012), as well as 2 clinical reports of infections in children confirmed by 16S rRNA sequencing (Vahabnezhad *et al.,* 2013; Robin *et al.,* 2010). The infections associated with *L. rhamnosus* strains represent the largest species group of *Lactobacillus* infections, most of which are localized in the abdomen, but as *L. rhamnosus* strains, particularly *L. rhamnosus* GG, are the most consumed *Lactobacilli* species, this was not unexpected. Additionally, cases of infection caused by strains of *L. rhamnosus* were associated with prior infection, immunocompromised, or compromised oral health and dental procedures, echoing the conclusions reported by Borriello *et al.* (2003), Gouriet *et al.* (2012), and Goldstein *et al.* (2015), establishing the rarity of these infections and supporting the safety of consumption of *L. rhamnosus* strains as dietary microbes. Such consumption is safe and unlikely to result in adverse events; the toxicology assessment published by Zhang *et al.* (2021) concluded that *"[based] on information on Lactobacillus spp., and on L. rhamnosus spp. in particular, there appears to be minimal concern regarding translocation and pathogenicity, at least in healthy populations […]*" This minimal concern for translocation and pathogenicity can be extended to the product strain *L. rhamnosus* MP108. A conclusion supported by repeat-dose studies in rats and clinical study data that indicates no translocation or pathogenicity of the strain, as discussed in Section 6.3.

6.2.2 Gastrointestinal Colonization and Survival of *L. rhamnosus* MP108

There are 4 clinical studies identified in the literature, that while reporting safety endpoints also evaluated colonization and survivability of other strains of *L. rhamnosus* (Firmesse *et al.,* 2008; Dommels *et al.,* 2009; Verdenelli *et al.,* 2009; de Andrade Pires *et al.,* 2020). These studies enlisted healthy human subjects {Firmesse *et al.* 2008; Dommels *et al.* 2009; de Andrade Pires *et al.* 2020}, and elderly subjects, {Verdenelli *et al.* 2009}, for treatment schedules ranging from 2 to 4 weeks or 90 days, respectively. Although the daily dose varied among the studies, the lowest dose was 1 x 10^8 CFU *L. rhamnosus*/day administered for 4 weeks in healthy human subjects and a dose of 2 x 10^9 CFU/day *L. rhamnosus* for 90 days in elderly subjects. In all studies, fecal concentrations of the administered strains were significantly elevated following treatment (p<0.05) which persisted for a short period but returned to baseline control in samples collected weeks after cessation. These data indicate that while *L. rhamnosus* survives passage of the GI tract and persists for up to 2 weeks following treatment, colonization is transient as observed by the return of fecal *L. rhamnosus* concentrations to baseline levels in all cases.

6.3 Toxicological Studies

Toxicology studies conducted using the test article *L. rhamnosus* MP108 were conducted to evaluate the mutagenic and genotoxic potential and the oral subchronic toxicity of the ingredient. Studies pivotal to the safety evaluation of *L. rhamnosus* MP108 were compiled and published in a comprehensive toxicological evaluation of *L. rhamnosus* MP108 powder (Zhang *et al.*, 2021). Additional single dose and 28-day studies was conducted on behalf of Glac Biotech by Chuangyi Biotech Co., Ltd., the results of which are described in Section 6.3.1 and 6.3.2, respectively (Chuangyi Biotech Co., Ltd., 2012a,b). Unpublished study data were deemed corroborative of safety as the high dose single administration does not provide data relevant to the proposed use levels in humans and the 28-day rat study had a shorter administration phase and lower maximum dose than the published 90-day subchronic oral toxicity study (Zhang *et al.,* 2021). Thus, they are supportive but not necessary for a GRAS conclusion for *L. rhamnosus* MP108.

6.3.1 Single Dose Studies

The acute toxicity of *L. rhamnosus* MP108 powder was evaluated by Chuangyi Biotech Co., Ltd. (2012a) in Sprague-Dawley rats in accordance with OECD Test Guideline 425 [*Acute oral toxicity: up-and-down procedure (UDP)*] (OECD, 2008). Rats (5 animals/sex/group) received a single gavage dose of 5 g *L. rhamnosus* MP108 powder/kg body weight dissolved in reverse osmosis water by gavage at 10 ml/kg body weight and observed for 14 days. All animals survived the duration of the observation period and gained weight normally. At the end of the observation period, animals were sacrificed and underwent macroscopic examination. No gross lesions were observed. Based on these results, the median lethal dose (LD_{50}) of *L. rhamnosus* MP108 powder is greater than 5 g/kg body weight in rats (Chuangyi Biotech Co., Ltd. (2012a). These unpublished data are corroborative and not pivotal to this safety evaluation.

6.3.2 Repeat Dose Studies

The subacute toxicity of *L. rhamnosus* MP108 powder was evaluated in Sprague-Dawley rats according to an OECD-comparable Taiwanese Department of Health (DOH) guidance. Rats (10 animals/sex/group) received gavage doses of *L. rhamnosus* MP108 powder (0, 250, 500, or 1,000 mg/kg body weight/day), dissolved in reverse osmosis water to an administration dose of 10 ml/kg, consecutively for 28 days (Chuangyi Biotech Co., Ltd., 2012b). Clinical evaluation for signs of toxicity were conducted daily; body weights and food intake were assessed weekly. Necropsy was conducted after 28 days for pathology examination and blood and

organs were collected for hematological analysis and serum biochemical analysis. Treated rats exhibited no abnormal clinical symptoms and no significant effects on body weight gain. No abnormalities were noted during ophthalmological examination. No significant differences were observed in urinalysis, hematological examination, and serum biochemistry between dose groups and controls and no treatment-related lesions were observed during macroscopic or histopathological examinations. The NOAEL was determined to be 1,000 mg/kg body weight/day, the highest dose tested (Chuangyi Biotech Co., Ltd. 2012b). These unpublished data are corroborative and not pivotal to this safety evaluation.

In a 90-day study (Food Safety National Standard [China]; equivalent to OECD TG 408) published by Zhang *et al*. (2021), Sprague Dawley rats (<6 weeks old) divided into 3 treatment groups (n=10/sex/group) were administered *L. rhamnosus* MP108 at varying doses, and 1 group to serve as a placebo control, for 90 days. The doses for low, mid, and high dose groups were 250, 500, and 1,500 mg/kg body weight/day, respectively, were administered by gavage; test item was dissolved in sterile water immediately prior to administration. There were 2 satellite groups (n=5/sex/group), in addition to the 90-day study, in which 1 group was administered a solvent control and the other 1,500 mg/kg body weight/day for 45 days. Clinical observations were made daily. Food consumption and body weights were measured weekly throughout the study. Blood samples for hematology and biochemistry were collected at study initiation and 1 week prior to termination; ophthalmologic observations were made on the same schedule. No treatment-related adverse clinical findings were observed during the study; all test animals exhibited normal activity, growth, and food consumption, as compared to control. There were no significant changes in hematology or blood biochemistry metrics in any treatment group compared to control. At study termination, necropsy of animals in all treatment groups, both 45- and 90-day treated, did not reveal any significant changes in organ weights or other macroscopic observations compared to control. The results of histological examination of high-dose animals did not differ significantly from those of control animals.

The results of the toxicology studies evaluating the safety of *L. rhamnosus* MP108 demonstrate that the administration of up to 1,500 mg/kg body weight/day, equivalent to > 1.5 x 10^{11} CFU/kg body weight/day, in rats for 90 days did not cause adverse reactions.

6.3.3 Genotoxicity and Mutagenicity

Bacterial Reverse Mutation Test

The mutagenic potential of *L. rhamnosus* MP108 powder was assessed using *Salmonella* Typhimurium strains TA97, TA98, TA100, TA102, and TA1535 in an *in vitro* bacterial reverse mutation test according to OECD Test Guideline 471 (*Bacterial reverse mutation test*) (OECD, 1997a). Dose-range finding tests indicated no toxicity at concentrations up to 5.0 mg/plate. The concentrations of *L. rhamnosus* MP108 powder for the definitive mutagenicity test were 0 (DMSO, negative control), 0.3125, and 0.625, 1.25, 2.5, and 5.0 mg/plate. Sodium azide, 4-nitroquinoline-N-oxide, mitomycin C, benzo[a]pyrene, and 2-aminofluorene were used as positive controls. Tests were run in triplicate in both the presence and absence of rat liver S9 mix. *L. rhamnosus* MP108 powder was considered negative in this test as all concentrations did not significantly increase the number of revertant colonies as compared to the negative control. In contrast, significant increases in the number of revertant colonies were seen with the positive substances. *L. rhamnosus* MP108 powder was non-mutagenic under the conditions of this assay (Zhang *et al*., 2021).

Chromosome Aberration Test

The genotoxicity of *L. rhamnosus* MP108 powder was evaluated in an *in vitro* mammalian cell chromosome test in Chinese hamster ovary cells (CHO-K1) in accordance with OECD Test Guideline 473 (*In Vitro*

mammalian chromosome aberration test) (OECD, 1997b). The maximum dose for the chromosome abnormality test was established based on preliminary cytotoxicity tests. Precipitation of the test substance occurred at 2.5 mg/mL in water. A concentration of 1.25 mg/mL was used as the maximum concentration for the chromosome aberration test. Three treatment approaches were utilized. Specifically, cells were treated for 3 hours with S9 and for 3 hours and 20 hours without S9 enzymes, at concentrations of 0 (DMSO, negative control), 0.3125 mg/mL, 0.625 mg/mL, and 1.25 mg/mL. Mitomycin C (-S9) and cyclophosphamide(+S9) served as positive controls. No statistically significant difference in the number of chromosomal abnormal cells in any of the test groups compared to the negative control group were observed (p>0.05). Therefore, *L. rhamnosus* MP108 powder was non-genotoxic under the conditions of this assay (Zhang *et al*., 2021).

In Vivo Micronucleus Test

Powdered *L. rhamnosus* MP108 was evaluated in an *in vivo* micronucleus test in ICR mice in accordance with OECD Test Guideline 474 (*Mammalian erythrocyte micronucleus test*) (OECD, 1997c). Male mice (5 animals/group) received a single dose of 0 (water), or 1.25 g, 2.5, or 5.0 g/kg body weight of *L. rhamnosus* MP108 *via* oral gavage. Blood was collected 48 and 72 hours after administration and used to assess the number of reticulocytes in the peripheral blood and the micronucleus rate of reticulocytes. The animals exhibited no sign of toxicity during the test and no significant differences in body weight were seen. The numbers of reticulocytes and micronucleated reticulocytes were comparable across treatment groups and were not significantly different from the negative control group (p>0.05). In contrast, the count of reticulocytes of mice in the positive control (cyclophosphamide) group was significantly reduced (p<0.05) and the count of micronucleated reticulocytes was significantly increased (p<0.05) compared to the negative control. Based on these results, *L. rhamnosus* MP108 powder was found to be non-genotoxic under the conditions of this assay (Zhang *et al*., 2021).

6.4 Human Studies

The safety of *L. rhamnosus* MP108 in humans was evaluated in a double-blind, randomized, placebo-controlled study conducted in children (4 to 48 months) with atopic dermatitis over 8 weeks (Wu *et al.,* 2017). Children in the supplemented group (n=30; 1.4 ± 1.1 years, Male 80%, 10.5 ± 3.0 kg, 77.1 ± 12.7 cm) received 1 daily capsule of *L. rhamnosus* MP108 powder [ComProbi - 350 mg *L. rhamnosus* MP108 (≥1.0 x 10^{11} CFU/g) and maltodextrin] and the control group (n=32; 1.8 ± 1.1 years, Male 56.3%, 11.6 ± 3.0 kg, 83.0 ± 11.8 cm) received a placebo containing maltodextrin only. The primary end point was a Scoring of Atopic Dermatitis (SCORAD) index using the Hanifin and Rajka criteria (Tada, 2002) at baseline compared to Week 4 and Week 8. A significant difference in SCORAD (decrease) was observed between supplemented (-23.20 ± 15.24) and control (-12.35 ± 12.82) groups (p=0.002) over 8 weeks of MP108 consumption. The safety assessment included the clinical observations of blood pressure, heart and respiratory rate, and ear temperature at 0, 4, and 8 weeks of treatment. No significant changes were observed in any of the safety parameters measured. Adverse events were reported in 42.42% (n=35) of supplemented subjects and 45.45% (n=37) of control subjects, but "*showed no relation to [the] study products*" (Wu *et al.,* 2017). Based on the results, administration of *L. rhamnosus* MP108 is safe for consumption in children ages 4 to 48 months for up to 8 weeks at ingested quantities of 175 mg of test article providing approximately 3.5 x 10^{10} CFU/day. These data were acknowledged as pivotal by Glac Biotech; the study by Wu *et al.* (2017) supports the safe use of *L. rhamnosus* MP108 as an ingredient in food and beverage products in the U.S. containing no more than 1 x 10^{9} CFU/serving.

6.5 Assessment for Antibiotic Resistance and Virulence Potential

Based on the findings presented below, consumption of the *L. rhamnosus* MP108 strain as an ingredient in food is not expected to cause toxicity or exhibit pathogenic effects and is not expected lead to gene transfer resulting in antibiotic resistance or pathogenicity.

6.5.1 Antibiotic Resistance Analysis – Minimum Inhibitory Concentration

Glac Biotech conducted antibiotic testing of *L. rhamnosus* MP108 to ensure that genes conferring resistance to clinically important antibiotics were not present in the genome and/or are not at risk of transfer to pathogenic microorganisms once introduced to the human gut. The minimum inhibitor concentration (MIC) of a variety of clinically relevant antibiotics against the product strain was determined in compliance with ISO 10932 guidelines for the microdilution method (ISO 10932: 2010 Milk and milk products – Determination of the minimal inhibitory concentration of antibiotics applicable to bifidobacterial and non-enterococcal lactic acid bacteria – ISO, 2010). The MICs determined for *L. rhamnosus* MP108 were compared to EFSA breakpoint values to assign a grade of resistant or susceptible (EFSA, 2012). These data are presented below in Table 6.5.1-1. *L. rhamnosus* MP108 was sensitive to all antibiotics tested; however, the MIC levels for erythromycin and chloramphenicol were marginally higher than the EFSA breakpoint values for *L. rhamnosus*, indicating low-level resistance to these antibiotics.

Glac Biotech notes that chloramphenicol is no longer widely used in clinical practice in the U.S. due to toxicity concerns related to bone marrow aplasia (Scholar, 2007). The apparent low-level chloramphenicol resistance was therefore not considered of clinical significance from a risk assessment perspective.

A bioinformatic assessment of the strain, described in Section 6.5, was supportive of these data, finding no unique genes that confer anti-microbial properties (AMP) in the product strain. In addition, a study on another strain of *L. rhamnosus* in the human intestinal tract found that base substitutions in the 23S rRNA genes disrupted macrolide (*e.g.,* erythromycin) activity by decreasing its affinity for ribosomes, effectually conferring resistance in those species carrying the mutation (Vester & Douthwaite, 2001; Flórez *et al.,* 2007). Other reported mechanisms for resistance to macrolides include a variety of efflux systems, methylases that disrupt ribosomal binding, and inactivating enzymes for which over 40 genes have been identified (Poehlsgaard, *et al.,* 2005; Leclercq, 2002). The resistance exhibited by the MP108 strain is indicative of an intrinsic mechanism and it not likely to undergo genetic transfer to other microbes in the human gut.

Table 6.5.1-1 Minimum Inhibitory Concentration of Antibiotics for *L. rhamnosus* MP108

Antibiotic	MIC Value (µg/mL)		
	***Lactobacillus rhamnosus* MP108 (Product strain) (Bioflag, 2021a,b)**	**EFSA Breakpoint Values *L. rhamnosus* (EFSA, 2012)**	**Susceptible (S) Resistant (R)**
Gentamicin	8	16	S
Kanamycin	64	64	S
Streptomycin	16	32	S
Tetracycline	4	8	S
Erythromycin	2	1	R
Clindamycin	1	1	S
Chloramphenicol	8	4	R
Ampicillin	1	4	S

EFSA = European Food Safety Authority; MIC = minimum inhibitory concentration.

6.5.2 Bioinformatic Analyses

At the request of Glac Biotech, Beijing Genomics Institute (BGI, Shenzhen, China) conducted a microbial genome sequence and functional annotation assessment of the product strain *L. rhamnosus* MP108, accompanied by relevant virulence factor and antimicrobial resistance analyses to confirm the strain identity and the absence of pathogenic and antimicrobial resistance risk factors; the results are summarized below.

The genome of the product strain *L. rhamnosus* MP108 has been sequenced and annotated, see report (Appendix B). The complete genome sequence is 2,925,062 base pairs, 2,884 genes, has a guanine-cytosine (GC) content of 47.47%, and is absent of plasmids or prophage elements (Table 6.5.2-1).

Low quality data were excluded, remaining raw data were treated, and polymerase reads were analyzed by PacBio RS II platform and Illumina HiSeq 4000 platform. Four SMRT cells Zero-Mode Waveguide arrays of sequencing were used by the PacBio platform to generate the subreads set. PacBio subreads (length <1 kb) were removed. The program Pbdagcon (https://github.com/PacificBiosciences/pbdagcon) was used for self-correction. Draft genomic contigs, which are uncontested groups of fragments, were assembled using the Celera Assembler against a high-quality corrected circular consensus sequence subreads set. To improve the accuracy of the genome sequences, GATK (Genome Analysis Tool Kit) (https://www.broadinstitute.org/gatk/) and SOAP (Short Oligonucleotide Alignment Program) packages (SOAP2, SOAPsnp, SOAPindel) were used to make single-base corrections. To trace the presence of any plasmid, the filtered Illumina reads were mapped using SOAP to the bacterial plasmid database.
Protein sequences, derived from the sequenced genome, were compared to several databases to identify biologically relevant alignments. The databases and number of annotated genes identified by each are listed in Table 6.5.2-1, below. Analysis of the *L. rhamnosus* MP108 genome was conducted to screen for genetic risk factors associated with antimicrobial resistance, virulence factors, and pathogenicity using a variety of homology tools. Of significance to this GRAS notice were the functional annotation results from Basic Local Alignment Search Tool (BLAST) search against the following databases: the virulence factors of pathogenic bacteria (VFDB), the Comprehensive Antibiotic Resistance Database (CARD), pathogen-host interaction database (PHI), and the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Table 6.5.2-1 Number of Functional Annotated Genes in *L. rhamnosus* MP108

Database	Number of Annotated Genes	Proportion of Total Annotated Genes (%)
Total Genes	**2,884**	**100**
VFDB	94	3.25
CARD	35	1.21
PHI	145	5.02
KEGG	1,659	57.52
Antimicrobial Drug Resistance	32	1.11
Bacterial Infectious Disease	11	0.38

CARD = Comprehensive Antibiotic Resistance Database; KEGG = Kyoto Encyclopedia of Genes and Genomes; PHI = pathogen host interactions; VFDB = virulence factor database.

Interrogation of the genome annotation from comparison to the KEGG database identified 32 genes related to antimicrobial resistance and 11 genes associated with infectious disease. A core-pan gene analysis was conducted using the genomes of 5 closely related strains of *L. rhamnosus* to evaluate the number and identity of genes not common to the species that may confer the phenotypic differences observed between strains. In this analysis, “clustered” genes that are common to all members of the group are distinguished from “unclustered” genes which are unique to each strain. The clustered genes presumably contribute to essential processes required for normal growth and metabolism of the microorganism, whereas the unclustered genes are responsible for the phenotypic differences observed between strains, such as abnormal antimicrobial resistance or alternate metabolic products. The functional annotation of the *L. rhamnosus* MP108 strain genome and absence of plasmid DNA indicates that antibiotic resistance to erythromycin is chromosomal and demonstrates no concerns of virulence or horizontal antibiotic resistance gene transfer exist from consumption of the product strain.

6.6 Application of the Decision Tree Approach (Pariza *et al.,* 2015

The decision tree for determining the safety of microbial cultures to be consumed by humans or animals published by Pariza *et al.* (2015) was applied as follows to evaluate the safety *L. rhamnosus* MP108 for human consumption:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding).

Answer: YES

Taxonomic identity of L. rhamnosus *MP108 was confirmed by 16S rRNA and phenylalanyl-tRNA synthetase alpha subunit (pheS) gene sequencing and whole-genome sequencing and annotation, differentiating the strain from other characterized strains of L. rhamnosus*.

2. Has the strain genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.)

Answer: YES

3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? (If YES, go to 4. If NO, go to 15.)

Answer: YES

While interrogation of the genome sequence identified potential genes similar to known virulence factors, none were determined a risk factor due to insufficient sequence identity or where demonstrated to be highly conserved with the species and therefore not likely to represent virulence factors.

4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? (If YES, go to 5. If NO, go to 15.)

Answer: YES

5. Does the strain produce antimicrobial substances? (If NO, go to 6. If YES, go to 15.)

Answer: NO

The observed resistance to erythromycin and chloramphenicol exhibited by the MP108 strain appears to be intrinsic and not the direct result of production of a known antibiotic resistance compound.

6. Has the strain been genetically modified using rDNA techniques? (If YES, go to 7a or 7b. If NO, go to 8a or 8b.)

Answer: NO

7a. Do the expressed products that are encoded by the introduced DNA have a history of safe use in food? (If YES, go to 8a. If NO, the expressed products must be shown to be safe before proceeding to 8a.)

Answer: Not Applicable

8a. Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')? (If YES, go to 9a. If NO, go to 13a.)

Answer: NO

13a. Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? (If YES, go to 15. If NO, go to 14a.)

Answer: NO

In a 90-day study in rats by Zhang et al. (2021), the NOAEL was determined by the authors to be 1,500 mg/kg body weight/day, equivalent to (> 1.5 x 10^{11} CFU/kg body weight/day), the highest dose tested.

14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

6.7 General Recognition Standard - GRAS Panel Evaluation

Glac Biotech has concluded that *L. rhamnosus* MP108 is GRAS for use in non-exempt term infant formula and specified conventional food products, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of *L. rhamnosus* MP108, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The GRAS Panel consisted of the following qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Michael W. Pariza, Ph.D. (University of Wisconsin-Madison), and I. Glenn Sipes, Ph.D. (University of Arizona College of Medicine).

The GRAS Panel, convened by Glac Biotech independently and critically evaluated all data and information presented herein, and also concluded that *L. rhamnosus* MP108 is GRAS for use in conventional food products as described in Section 1.3, based on scientific procedures (Appendix C). A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of *L. rhamnosus* MP108 has been presented herein. .

6.8 Conclusion

Based on the above data and information presented herein, Glac Biotech has concluded that *L. rhamnosus* MP108 is GRAS, on the basis of scientific procedures, for use in food and beverage products as described in Section 1.3. General recognition of Glac Biotech's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of *L. rhamnosus* MP108 in food, who similarly concluded that the proposed uses of that *L. rhamnosus* MP108 are GRAS on the basis of scientific procedures.

L. rhamnosus MP108 therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the *Code of Federal Regulations*.

Part 7. §170.255 List of Supporting Data and Information

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
131—Milk and Cream	131.125	Nonfat dry milk
169—Food dressings and flavorings	169.181	Vanilla-vanillin flavoring
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.840	Polysorbate 80
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
	182.1778	Sodium phosphate
	182.3041	Erythorbic acid
	182.6285	Dipotassium phosphate
	182.8985	Zinc chloride
	182.8988	Zinc gluconate
	182.8991	Zinc oxide
	182.8994	Zinc stearate
	182.8997	Zinc sulfate
184—Direct food substances affirmed as generally recognized as safe	184.1143	Ammonium sulfate
	184.1207	Calcium lactate
	184.1271	L-Cysteine
	184.1400	Lecithin
	184.1443	Magnesium sulfate
	184.1444	Maltoxetrin
	184.1553	Peptones
	184.1751	Sodium citrate
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APPENDIX A
Intake Assessment Report

ESTIMATED DAILY INTAKE OF *LACTOBACILLUS RHAMNOSUS* MP108 BY THE U.S. POPULATION FROM PROPOSED FOOD USES (2017-2018 NHANES)

Estimated Daily Intake of *L. rhamnosus* MP108 by the U.S. Population from Proposed Food Uses (2017-2018 NHANES)

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Estimated Daily Intake of *L. rhamnosus* MP108 by the U.S. Population from Proposed Food Uses (2017-2018 NHANES)

1.0 INTRODUCTION

Lactobacillus rhamnosus MP108 is proposed for use in the United States (U.S.) in a variety of foods and beverages, for consumption by infants and the general population, such as ready-to-drink beverages, breakfast cereals, dairy products/analogues, chewing gum, confectionary, processed fruits and fruit juices, and baby foods.

Estimates for the intake of *L. rhamnosus* MP108 were based on the proposed food uses and use levels for *L. rhamnosus* MP108 in conjunction with food consumption data from the U.S. National Center for Health Statistics' National Health and Nutrition Examination Surveys (NHANES) 2017-2018. Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food uses of *L. rhamnosus* MP108 and the percentage of consumers was determined. Similar calculations were used to estimate the intake of *L. rhamnosus* MP108 resulting from each individual proposed food use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- Infants and young children, <2 years;
- Children, ages 2 to 5;
- Children, ages 6 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (ages 2 years and older, and both gender groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES are available for public use (CDC, 2021a,b; USDA, 2021a,b). The NHANES are conducted as continuous, annual surveys, and they are released in 2-year cycles. During each year of the ongoing NHANES program, individuals from the U.S. are sampled from up to 30 different study locations in a complex multi-stage probability design intended to ensure the data are a nationally representative sample of the U.S. population. The current analysis uses data from the NHANES 2017-2018.

NHANES 2017-2018 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting primary sampling units (PSUs), which were counties throughout the U.S., of which 30 PSUs are visited per year. Smaller contiguous counties were combined to attain a minimum population size. These PSUs were segmented, and households were chosen within each segment. One or

more participants within a household were interviewed. For NHANES 2017-2018, 16,211 individuals were selected for the sample, 9,254 were interviewed (51.9%), and 8,704 were examined (48.8%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2017-2018 collected socio-economic, physiological, and demographic information from individual participants in the survey, such as sex, age, body weight, and other variables (such as height and race-ethnicity) that may be useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The primary sample design for NHANES 2017-2018 includes an oversample of non-Hispanic Asian persons, Hispanic persons, non-Hispanic black persons, non-Hispanic white and "other" older persons (≥ 80 years), and non-Hispanic low income white and "others" persons (≤ 185% of the Department of Health and Human Services poverty guidelines); however, sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2021a,b; USDA, 2021a,b).

2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of *L. rhamnosus* MP108 by the U.S. population[1]. Estimates for the daily intake of *L. rhamnosus* MP108 represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2017-2018 (*i.e.*, a value was established for each person). From these average amounts, a distribution was established from which the mean and percentile intake estimates for the cohort of interest were determined, which incorporated survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of *L. rhamnosus* MP108 averaged over all individuals surveyed, regardless of whether they consumed food products in which *L. rhamnosus* MP108 is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, including individuals who reported no intake of food products containing *L. rhamnosus* MP108 during the 2 survey days). "Consumer-only" intake refers to the estimated intake of *L. rhamnosus* MP108 by only those individuals who reported consuming food products of interest on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of *L. rhamnosus* MP108 based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

3.0 FOOD USAGE DATA

The proposed food uses and use levels for *L. rhamnosus* MP108 employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food use were chosen from the NHANES 2017-2018 (CDC, 2021b). Food codes were grouped in food use categories according to Title 21, Section §170.3 of the *Code of Federal Regulations* (U.S. FDA, 2021a). If necessary, product-specific adjustment factors were developed for composite foods/mixtures based on data provided in the Food and Nutrient Database for Dietary Studies (USDA ARS, 2021a,b) or the Food Commodity Intake Database (U.S. EPA & USDA, 2021). All food codes included in the current intake assessment are listed in Appendix C.

Table 3-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Food Uses*	Proposed Use Level ($CFUx10^9$/Serving)	RACC[a] (g or mL)	Maximum Intended Use Level ($CFUx10^9$/100 g)
Beverages and Beverage Bases	Energy Drinks	1.0	360	0.28
	Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.0	360	0.28
	Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	1.0	240	0.42
	Sports Drinks	1.0	360	0.28
	Bottled tea	1.0	360	0.28
Breakfast Cereals	Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	1.0	40 to 55	2.50
	Ready-to-Eat Breakfast Cereals			
	Puffed Cereals	1.0	15	6.67
	High-Fiber Cereals	1.0	40	2.50
	Biscuit-Type Cereals	1.0	60	1.67
Cheeses	Cheeses	1.0	30 to 110	3.33
Chewing Gum	Chewing Gum	1.0	3	33.33
Dairy Product Analogs	Non-Dairy Milk (soy-based drinks)	1.0	240	0.42
Gelatins, Puddings, and Fillings	Milk-Based Desserts	1.0	130 to 150	0.77
Grain Products and Pastas	Cereal and Granola Bars	1.0	40	2.50
	Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.0	40	2.50
Hard Candy	Hard Candy	1.0	15	6.67

Table 3-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Food Uses*	Proposed Use Level (CFUx10^9/Serving)	RACC[a] (g or mL)	Maximum Intended Use Level (CFUx10^9/100 g)
Milk Products	Buttermilk	1.0	240	0.42
	Evaporated, Condensed, and/or Dry Milks	1.0	30	3.33
	Fermented Milks, Plain	1.0	240	0.42
	Flavored Milks, Milk Drinks, and Mixes	1.0	240	0.42
	Milk Shakes	1.0	240	0.42
	Milk-Based Meal Replacement, Nutrition, and Protein Beverages[b]	1.0	240	0.42
	Plain or Flavored Yogurt	1.0	170	0.59
	Yogurt Drinks	1.0	93 to 207[c]	1.08
Plant Protein products	Soy-based Food	1.0	85	1.18
Processed Fruits and Fruit Juices	Fruit Drinks and Ades Including Smoothies	1.0	240	0.42
	Fruit Juices	1.0	240	0.42
	Fruit Nectars	1.0	240	0.42
Soft Candy	Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	1.0	30	3.33
Other – Baby Food	Baby food: Cereals			
	Dry Instant	1.0	15	6.67
	Prepared, Ready-to-Serve	1.0	110	0.91
	Baby food: Ready-to-Eat cereals	1.0	100	1.00
	Baby food: Fruits or Vegetables (strained)	1.0	125	0.80
	Baby food: Fruit Juice	1.0	120	0.83

CFR = *Code of Federal Regulations*; CFU = colony forming units; RACC = Reference Amounts Customarily Consumed; U.S. = United States.

* *L. rhamnosus* MP108 is intended for use in unstandardized products and not in foods where standards of identity exist and do not permit its addition.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2021b). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

[b] Includes ready-to-drink and powdered forms.

[c] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently on the U.S. market.

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of *L. rhamnosus* MP108 from proposed food uses are provided in Section 4.1. Estimates for the daily intake of *L. rhamnosus* MP108 from individual proposed food uses in the U.S. are summarized in Section 4.2 and presented in Tables A-1 to A-8 and B-1 to B-8 of Appendices A and B, respectively.

4.1 Estimated Daily Intake of *L. rhamnosus* MP108 from All Proposed Food Uses in the U.S.

Table 4.1-1 summarizes the estimated total intake of *L. rhamnosus* MP108 (CFUx10^9/person/day) from all the proposed food uses by U.S. population groups. Table 4.1-2 presents this data on a per kilogram body weight basis (CFUx10^8/kg body weight/day). The percentage of consumers was high among all age groups evaluated, with greater than 76.8% of the population groups consuming food products in which *L. rhamnosus* MP108 is currently being proposed for use (Table 3-1). The greatest proportion of consumers was observed in 2 to 5 year old children (99.4%). The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (ages 2 and older), the mean and 90th percentile consumer-only intakes of *L. rhamnosus* MP108 were determined to be 2.9 and 6.3 CFUx10^9/person/day, respectively. Of the individual population groups, infants and young children were determined to have the greatest mean and 90th percentile consumer-only intakes of *L. rhamnosus* MP108 on an absolute basis, at 4.1 and 9.8 CFUx10^9/person/day, respectively, while female teenagers had the lowest mean and 90th percentile consumer-only intakes of 2.4 and 4.6 CFUx10^9/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of *L. rhamnosus* MP108 from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to <2	3.1	7.7	76.8	338	4.1	9.8
Children	2 to 5	3.0	5.4	99.4	466	3.0	5.4
Children	6 to 11	2.9	5.3	98.6	670	2.9	5.3
Female Teenagers	12 to 19	2.3	4.6	93.7	420	2.4	4.6
Male Teenagers	12 to 19	2.7	5.9	95.2	409	2.9	6.0
Female Adults	20 and older	2.6	5.8	93.4	1,980	2.7	5.9
Male Adults	20 and older	2.9	6.8	90.6	1,760	3.2	7.2
Total Population	2 and older	2.7	6.0	93.2	5,705	2.9	6.3

CFU = colony forming units; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (ages 2 and older) mean and 90th percentile consumer-only intakes of *L. rhamnosus* MP108 were determined to be 0.5 and 1.1 CFUx10^8/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes, 4.3 and 10.0 CFUx10^8/kg body weight/day, respectively. Teenagers and adults (both genders) had the lowest mean consumer-only intakes of 0.4 CFUx10^8/kg body weight/day while female adults had the lowest 90th percentile of 0.8 CFUx10^8/kg body weight/day (Table 4.1-2).

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (CFUx10^8/kg bw/day)		Consumer-Only Intake (CFUx10^8/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	
Infants and Young Children	0 to <2	3.3	8.0	76.7	336	4.3	10.0
Children	2 to 5	1.8	3.3	99.5	459	1.8	3.3
Children	6 to 11	0.9	1.8	98.6	668	0.9	1.8
Female Teenagers	12 to 19	0.4	0.8	93.6	413	0.4	0.9
Male Teenagers	12 to 19	0.4	0.9	95.1	406	0.4	0.9
Female Adults	20 and older	0.4	0.8	93.4	1,962	0.4	0.8
Male Adults	20 and older	0.3	0.8	90.6	1,746	0.4	0.9
Total Population	2 and older	0.5	1.1	93.2	5,654	0.5	1.1

bw = body weight; CFU = colony forming units; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

4.2 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses in the U.S.

Estimates for the mean and 90th percentile daily intakes of *L. rhamnosus* MP108 from each individual food category are summarized in Tables A-1 to A-8 and B-1 to B-8 on a CFUx10^9/day and CFUx10^9/kg body weight/day basis, respectively. In terms of consumer-only percentage contribution to total mean intake of *L. rhamnosus*, infants and young children were identified as being significant consumers of baby fruits or vegetables (strained) (26% consumers), cheeses (25% consumers), and ready-to-eat (RTE) breakfast cereals, high-fiber cereals (23% consumers). Meanwhile, the total U.S. population (ages 2 and older) was identified as being a significant consumer of fruit juices (24 to 59% consumers), cheeses (46 to 56% consumers), RTE breakfast cereals, high-fiber cereals (19 to 51% consumers), and soft candy, chocolate, gummies, mints, nougat, and toffees (25 to 45% consumers).

In terms of contribution to total mean intake in the total population of *L. rhamnosus* MP108, baby cereals, dry instants, contributed 54% to total mean intakes of infants and young children, while hot breakfast cereals contributed 12% and baby fruits or vegetables (strained) 6.1%. Hot breakfast cereals (which contributed 5 to 21% to total mean intakes), cheeses (which contributed 10 to 20% to total mean intakes), fruit juices (which contributed 6 to 17% to total mean intakes), and fruit drinks and ades including smoothies (which contributed 10 to 14% to total mean intakes) were the 4 main sources of intake across total population groups (ages 2 and older). Non-milk-based meal replacement, protein, and nutritional beverages; buttermilk; fermented milks, plain; fruit nectars; and baby ready-to-eat cereals all individually

contributed ≤0.4% to total mean *L. rhamnosus* MP108 intakes across all population groups (see Tables A-1 to A-8 and B-1 to B-8 for further details).

5.0 SUMMARY AND CONCLUSIONS

Consumption data and information pertaining to the *L. rhamnosus* MP108 were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain *L. rhamnosus* MP108 at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that *L. rhamnosus* MP108 will have 100% market penetration in all identified food categories.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of *L. rhamnosus* MP108 by the total U.S. population (ages 2 and older) from proposed food uses in the U.S. were estimated to be 2.9 CFUx10^9/person/day (0.5 CFUx10^8/kg body weight/day) and 6.3 CFUx10^9/person/day (1.1 CFUx10^8/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of *L. rhamnosus* MP108 were determined to be 4.1 CFUx10^9/person/day (4.3 CFUx10^8/kg body weight/day) and 9.8 CFUx10^9/person/day (10.0 CFUx10^8/kg body weight/day), as identified among infants and young children, respectively. Female teenagers had the lowest mean and 90th percentile consumer-only intakes of 2.4 CFUx10^9/person/day (0.4 CFUx10^8/person/day) and 4.6 CFUx10^9/person/day (0.9 CFUx10^8/person/day), respectively. On a body weight basis, teenagers and adults (both genders) had the lowest mean consumer-only intakes of 0.4 CFUx10^8/kg body weight/day while female adults had the lowest 90th percentile of 0.8 CFUx10^8/kg body weight/day. There is currently limited data available regarding the safety of *L. rhamnosus* in the general population; however, a clinical study in children (Wu *et al.*, 2017) reported that 175 mg (~3.5x10^{10} CFU/day) for 8 weeks was safe in children aged 4 to 48 months. In the current study, infants and young children (0 to 2 years) were estimated to consume the equivalent of 0.41 CFUx10^{10}/person/day at the mean intake level, and 0.98 CFUx10^{10}/person/day at the high-level intakes, which is below the intakes reported as safe in Wu *et al.* 2017 for this age group. Furthermore, young children aged 2 to 5 years also presented estimated intakes below this safety threshold, 0.3 CFUx10^{10}/person/day and 0.54 CFUx10^{10}/person/day, for mean intakes and high-level intakes, respectively.

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APPENDIX A
Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Different Population Groups within the U.S. (2017-2018 NHANES Data)

Table A-1 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Infants and Young Children Aged 0 to <2 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	3.1	7.7	76.8	338	4.1	9.8
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	0.2	<0.1*	na	1.6	4	0.3*	0.4*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0	0	0	0	0	0	0
Sports Drinks	1.0	<0.1*	na	6.0	22	0.5*	1.0*
Bottled tea	0.1	<0.1*	na	1.0	4	0.2*	0.3*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	11.7	0.4	1.5*	13.4	60	2.7	6.0*
RTE Breakfast Cereals, Puffed Cereals	0.6	<0.1*	na	2.8	16	0.7*	1.3*
RTE Breakfast Cereals, High-Fiber Cereals	2.9	0.1	0.3	23.3	88	0.4	0.9
RTE Breakfast Cereals, Biscuit-Type Cereals	<0.1	<0.1*	na	0.2	1	<0.1*	<0.1*
Cheeses							
Cheeses	3.7	0.1	0.5	24.8	81	0.5	0.8
Chewing Gum							
Chewing Gum	0	0	0	0	0	0	0
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	1.2	<0.1*	na	1.8	10	2.0*	2.9*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.1	<0.1*	na	1.6	5	0.2*	0.2*
Grain Products and Pastas							
Cereal and Granola Bars	0.4	<0.1*	na	2.5	9	0.5*	0.7*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.1	<0.1*	na	0.7	2	0.5*	0.5*
Hard Candy							
Hard Candy	0.3	<0.1*	na	1.4	10	0.5*	0.7*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	1.6	<0.1*	na	0.7	3	7.3*	7.5*
Fermented Milks, Plain	0.1	<0.1*	na	1.0	1	0.3*	0.3*
Flavored Milks, Milk Drinks, and Mixes	0.9	<0.1*	na	4.0	15	0.7*	1.2*
Milk Shakes	<0.1	<0.1*	na	0.2	1	0.2*	0.2*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	<0.1	<0.1*	na	0.3	1	0.3*	0.3*

Table A-1 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Infants and Young Children Aged 0 to <2 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	2.4	0.1	0.3*	17.4	63	0.4	0.8*
Yogurt Drinks	0.1	<0.1*	na	0.6	3	0.3*	0.4*
Plant Protein products							
Soy-based Food	0.1	<0.1*	na	2.4	5	0.1*	0.1*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	2.8	0.1	0.4*	17.0	65	0.5	1.0*
Fruit Juices	6.0	0.2	0.7	29.0	122	0.6	1.4
Fruit Nectars	<0.1	<0.1*	na	0.1	1	0.1*	0.1*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	1.5	<0.1	na	10.5	38	0.4	0.8*
Other – Baby Food							
Baby Cereals, Dry Instant	54.4	1.7	5.6	21.0	121	8.1	17.5
Baby Cereals, Prepared, Ready-to-Serve	1.0	<0.1*	na	5.7	28	0.5*	0.8*
Baby Ready-to-Eat cereals	<0.1	<0.1*	na	0.8	2	0.1*	0.2*
Baby Fruits or Vegetables (strained)	6.1	0.2	0.8	26.1	136	0.7	1.6
Baby Fruit Juice	0.9	<0.1*	na	5.3	24	0.5*	0.8*

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-2 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 2 to 5 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	3.0	5.4	99.4	466	3.0	5.4
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	0.5	<0.1*	na	2.0	11	0.7*	1.1*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.1*	na	0.4	1	0.1*	0.1*
Sports Drinks	1.5	<0.1	na	8.0	37	0.6	1.1*
Bottled tea	0.8	<0.1*	na	5.4	19	0.5*	0.9*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	15.8	0.5	1.9	14.6	81	3.2	6.1
RTE Breakfast Cereals, Puffed Cereals	3.1	0.1	na	9.5	45	1.0	1.4*
RTE Breakfast Cereals, High-Fiber Cereals	8.6	0.3	0.8	51.2	240	0.5	0.9
RTE Breakfast Cereals, Biscuit-Type Cereals	0.1	<0.1*	na	1.5	9	0.2*	0.4*
Cheeses							
Cheeses	10.2	0.3	0.9	53.4	235	0.6	1.1
Chewing Gum							
Chewing Gum	0.5	<0.1*	na	2.7	9	0.6*	1.2*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.1	<0.1*	na	0.4	5	0.7*	1.0*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.1	<0.1*	na	1.1	7	0.3*	0.4*
Grain Products and Pastas							
Cereal and Granola Bars	2.1	0.1	0.2*	11.3	49	0.5	1.1**
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.5	<0.1*	na	3.0	11	0.5*	0.8
Hard Candy							
Hard Candy	2.0	0.1	0.2*	12.3	63	0.5	0.9*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	1.4	<0.1*	na	0.5	5	7.9*	12.7*
Fermented Milks, Plain	0	0	0	0	0	0	0
Flavored Milks, Milk Drinks, and Mixes	9.8	0.3	1.0	34.0	126	0.9	2.0
Milk Shakes	0.3	<0.1*	na	2.5	10	0.4*	0.5*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	0.3	<0.1*	na	2.0	10	0.4*	0.6*

Table A-2 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 2 to 5 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	4.2	0.1	0.5	26.7	114	0.5	0.7
Yogurt Drinks	1.9	0.1*	na	6.3	22	0.9*	1.8*
Plant Protein products							
Soy-based Food	0.1	<0.1*	na	1.4	7	0.2*	0.6*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	9.9	0.3	0.9	43.2	194	0.7	1.3
Fruit Juices	16.6	0.5	1.2	59.1	290	0.8	1.5
Fruit Nectars	0.1	<0.1*	na	0.7	4	0.5*	0.7*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	8.1	0.2	0.8	45.1	201	0.5	1.0
Other – Baby Food							
Baby Cereals, Dry Instant	1.1	<0.1*	na	0.7	4	4.7*	5.4*
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0.1	<0.1*	na	0.7	3	0.2*	0.4*
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-3 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 6 to 11 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.9	5.3	98.6	670	2.9	5.3
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.4	<0.1	na	9.5	37	0.4	1.0*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.1*	na	0.4	1	0.2*	0.2*
Sports Drinks	2.8	0.1	0.2*	10.4	66	0.8	1.3*
Bottled tea	1.4	<0.1	na	7.3	46	0.6	1.1*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	5.1	0.1	na	5.4	46	2.7	4.1*
RTE Breakfast Cereals, Puffed Cereals	6.7	0.2	na	9.7	58	2.0	4.2*
RTE Breakfast Cereals, High-Fiber Cereals	12.0	0.3	1.0	46.0	352	0.7	1.4
RTE Breakfast Cereals, Biscuit-Type Cereals	0.6	<0.1*	na	3.7	17	0.5*	0.7*
Cheeses							
Cheeses	11.7	0.3	0.9	54.8	316	0.6	1.2
Chewing Gum							
Chewing Gum	1.6	<0.1*	na	3.5	26	1.3*	3.2*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.1	<0.1*	na	0.6	4	0.4*	0.4*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.4	<0.1*	na	2.7	18	0.5*	0.8*
Grain Products and Pastas							
Cereal and Granola Bars	1.8	0.1	0.3*	11.2	59	0.5	0.7*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.4	<0.1*	na	5.5	18	0.7*	1.1*
Hard Candy							
Hard Candy	4.5	0.1	0.3	14.4	100	0.9	2.7
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	<0.1	<0.1*	na	0.1	2	0.4*	0.4*
Fermented Milks, Plain	<0.1	<0.1*	na	0.1	1	0.3*	0.3*

Table A-3 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 6 to 11 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Flavored Milks, Milk Drinks, and Mixes	9.1	0.3	1.0	34.0	242	0.8	1.4
Milk Shakes	0.6	<0.1*	na	2.7	15	0.6*	0.9*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	0.4	<0.1*	na	1.5	9	0.8*	1.3*
Plain or Flavored Yogurt	3.8	0.1	0.5	23.0	122	0.5	0.9
Yogurt Drinks	0.9	<0.1*	na	4.5	18	0.6*	0.8*
Plant Protein products							
Soy-based Food	0.1	<0.1*	na	2.3	13	0.1*	0.2*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	13.3	0.4	1.0	42.8	319	0.9	1.8
Fruit Juices	10.7	0.3	0.9	42.6	313	0.7	1.4
Fruit Nectars	0.2	<0.1*	na	1.1	7	0.6*	0.9*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.3	0.3	0.9	39.5	267	0.7	1.4
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-4 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.3	4.6	93.7	420	2.4	4.6
Beverages and Beverage Bases							
Energy Drinks	0.6	<0.1*	na	1.6	7	0.9*	1.3*
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.1	<0.1*	na	3.6	16	0.7*	1.1*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.3	<0.1*	na	0.2	1	3.7*	3.7*
Sports Drinks	2.9	0.1	na	8.4	37	0.8	1.3*
Bottled tea	5.3	0.1	0.5*	17.1	54	0.7	1.3*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	9.1	0.2	na	6.1	39	3.4	6.1*
RTE Breakfast Cereals, Puffed Cereals	3.8	0.1*	na	6.0	25	1.4*	2.4*
RTE Breakfast Cereals, High-Fiber Cereals	12.8	0.3	1.0	30.8	130	0.9	1.9
RTE Breakfast Cereals, Biscuit-Type Cereals	0.6	<0.1*	na	3.8	14	0.4*	0.8*
Cheeses							
Cheeses	14.3	0.3	1.1	47.3	190	0.7	1.6
Chewing Gum							
Chewing Gum	1.4	<0.1*	na	2.4	16	1.4*	1.8*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.1*	na	0.9	6	0.5*	0.5*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.4	<0.1*	na	0.6	6	1.4*	1.9*
Grain Products and Pastas							
Cereal and Granola Bars	3.6	0.1	0.3*	14.2	48	0.6	1.0*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.4	<0.1*	na	1.3	4	0.6*	0.8*
Hard Candy							
Hard Candy	2.6	0.1	na	9.1	48	0.6	1.3*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	<0.1	<0.1*	na	<0.1	1	0.4*	0.4*
Fermented Milks, Plain	<0.1	<0.1*	na	0.2	1	0.3*	0.3*
Flavored Milks, Milk Drinks, and Mixes	4.4	0.1	0.5*	12.6	69	0.8	1.3*
Milk Shakes	0.8	<0.1*	na	2.7	11	0.7*	1.0*

Table A-4 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	1.4	<0.1*	na	4.7	10	0.7*	0.8*
Plain or Flavored Yogurt	2.2	<0.1	na	7.1	31	0.7	1.1*
Yogurt Drinks	0.3	<0.1*	na	0.8	2	0.9*	1.0*
Plant Protein products							
Soy-based Food	0.2	<0.1*	na	1.4	7	0.3*	1.2*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	14.4	0.3	1.0	33.0	163	1.0	1.8
Fruit Juices	7.4	0.2	0.7	23.6	118	0.7	1.4
Fruit Nectars	0.3	<0.1*	na	0.8	4	0.8*	1.3*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.2	0.2	0.9	28.3	139	0.7	1.3
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	<0.1	<0.1*	na	0.1	1	0.4*	0.4*
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-5 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.7	5.9	95.2	409	2.9	6.0
Beverages and Beverage Bases							
Energy Drinks	0.9	<0.1*	na	3.3	8	0.7*	0.9*
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.1	<0.1*	na	3.5	12	0.9*	1.9*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.1*	na	<0.1	1	1.6*	1.6*
Sports Drinks	6.5	0.2	0.8*	17.7	67	1.0	1.8*
Bottled tea	3.7	0.1	0.3*	12.2	49	0.8	1.5*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	8.0	0.2*	na	4.5	21	4.9*	11.9*
RTE Breakfast Cereals, Puffed Cereals	4.8	0.1	na	6.2	37	2.1	3.1*
RTE Breakfast Cereals, High-Fiber Cereals	12.3	0.3	1.2	35.4	150	1.0	1.7
RTE Breakfast Cereals, Biscuit-Type Cereals	1.3	<0.1*	na	3.2	9	1.1*	1.6*
Cheeses							
Cheeses	12.7	0.3	0.8	45.7	177	0.8	1.4
Chewing Gum							
Chewing Gum	1.4	<0.1*	na	4.8	19	0.8*	1.3*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.3	<0.1*	na	1.0	5	0.8*	1.5*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.2	<0.1*	na	0.7	5	0.6*	0.8*
Grain Products and Pastas							
Cereal and Granola Bars	1.9	0.1	na	8.5	34	0.6	1.3*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.8	<0.1*	na	2.3	14	0.9*	1.7*
Hard Candy							
Hard Candy	0.9	<0.1*	na	4.7	22	0.6*	0.8*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	0.1	<0.1*	na	0.1	1	4.4*	4.4*
Fermented Milks, Plain	0	0	0	0	0	0	0
Flavored Milks, Milk Drinks, and Mixes	7.3	0.2	0.7	24.4	93	0.8	1.4
Milk Shakes	1.0	<0.1*	na	2.6	11	1.0*	1.5*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.3	0.1*	na	6.3	21	1.0*	1.5*

Table A-5 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	1.0	<0.1*	na	3.7	16	0.7*	1.1*
Yogurt Drinks	0	0	0	0	0	0	0
Plant Protein products							
Soy-based Food	<0.1	<0.1*	na	0.1	2	0.5*	0.5*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	12.8	0.4	1.1	31.6	134	1.1	1.9
Fruit Juices	10.1	0.3	1.0	26.9	138	1.0	2.3
Fruit Nectars	0.3	<0.1*	na	1.2	2	0.7*	0.7*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	8.5	0.2	0.8	24.8	122	0.9	2.2
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-6 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.6	5.8	93.4	1,980	2.7	5.9
Beverages and Beverage Bases							
Energy Drinks	0.6	<0.1	na	2.5	42	0.6	1.0*
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	2.5	0.1	na	7.2	122	0.9	1.6
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.3	<0.1*	na	1.2	11	0.6*	1.1*
Sports Drinks	1.1	<0.1	na	3.0	70	0.9	1.8*
Bottled tea	3.7	0.1	0.1	10.0	197	1.0	1.9
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	20.6	0.5	2.3	14.3	388	3.7	7.1
RTE Breakfast Cereals, Puffed Cereals	1.9	<0.1	na	3.3	50	1.5	2.7*
RTE Breakfast Cereals, High-Fiber Cereals	6.2	0.2	0.6	19.2	412	0.8	1.5
RTE Breakfast Cereals, Biscuit-Type Cereals	1.4	<0.1	na	5.2	95	0.7	1.6
Cheeses							
Cheeses	19.7	0.5	1.2	56.2	1,025	0.9	1.9
Chewing Gum							
Chewing Gum	1.4	<0.1	na	4.9	81	0.7	1.3
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.1*	na	1.2	29	0.5*	1.0*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.6	<0.1	na	2.4	55	0.7	1.3*
Grain Products and Pastas							
Cereal and Granola Bars	1.7	<0.1	na	8.1	134	0.6	1.0
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.8	<0.1	na	5.0	64	1.0	2.3*
Hard Candy							
Hard Candy	0.8	<0.1	na	4.0	116	0.5	1.0
Milk Products							
Buttermilk	<0.1	<0.1*	na	0.1	4	0.4*	0.6*
Evaporated, Condensed, and/or Dry Milks	0.3	<0.1	na	0.7	34	1.3	2.9*
Fermented Milks, Plain	<0.1	<0.1*	na	0.1	4	0.3*	0.4*
Flavored Milks, Milk Drinks, and Mixes	2.1	0.1	na	6.5	166	0.8	1.6
Milk Shakes	0.3	<0.1*	na	1.3	27	0.7*	1.4*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.0	0.1	na	5.5	100	0.9	1.8

Table A-6 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	3.6	0.1	0.4	15.1	280	0.6	1.1
Yogurt Drinks	0.2	<0.1*	na	0.4	12	1.2*	2.0*
Plant Protein products							
Soy-based Food	0.8	<0.1	na	3.5	82	0.6	1.5
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	10.3	0.3	0.8	21.5	504	1.2	2.2
Fruit Juices	6.0	0.2	0.5	25.4	571	0.6	1.2
Fruit Nectars	0.1	<0.1*	na	0.5	22	0.6*	1.2*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.6	0.2	0.8	32.6	609	0.8	1.7
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-7 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.9	6.8	90.6	1,760	3.2	7.2
Beverages and Beverage Bases							
Energy Drinks	3.0	0.1	na	6.3	84	1.4	2.7
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	2.8	0.1	na	6.7	88	1.2	2.1
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.1*	na	<0.1	1	1.1*	1.1*
Sports Drinks	4.4	0.1	na	9.0	155	1.5	2.9
Bottled tea	3.6	0.1	na	9.7	193	1.1	2.8
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	19.7	0.6	2.3	12.5	298	4.6	8.5
RTE Breakfast Cereals, Puffed Cereals	1.7	0.1	na	3.0	50	1.7	3.5*
RTE Breakfast Cereals, High-Fiber Cereals	6.5	0.2	0.8	19.9	391	1.0	1.7
RTE Breakfast Cereals, Biscuit-Type Cereals	1.6	<0.1	na	5.9	99	0.8	1.5
Cheeses							
Cheeses	17.1	0.5	1.4	48.3	815	1.0	2.0
Chewing Gum							
Chewing Gum	1.0	<0.1	na	2.4	48	1.3	2.0*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.1*	na	1.1	25	0.5*	0.7*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.7	<0.1	na	2.6	49	0.8	1.7*
Grain Products and Pastas							
Cereal and Granola Bars	1.6	<0.1	na	7.4	98	0.6	1.0
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.7	<0.1	na	4.6	53	1.1	1.9*
Hard Candy							
Hard Candy	1.1	<0.1	na	4.7	84	0.7	2.4
Milk Products							
Buttermilk	<0.1	<0.1*	na	<0.1	1	0.8*	0.8*
Evaporated, Condensed, and/or Dry Milks	0.5	<0.1*	na	0.9	28	1.9*	4.8*
Fermented Milks, Plain	<0.1	<0.1*	na	0.2	3	0.2*	0.2
Flavored Milks, Milk Drinks, and Mixes	2.3	0.1	na	5.4	110	1.3	2.1
Milk Shakes	0.6	<0.1	na	1.9	33	0.9	1.1*
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.3	0.1	na	5.9	94	1.1	1.7

Table A-7 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	2.1	0.1	na	9.2	146	0.7	1.1
Yogurt Drinks	0.1	<0.1*	na	0.3	3	1.1*	1.2*
Plant Protein products							
Soy-based Food	0.3	<0.1	na	2.0	39	0.4	1.0*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	9.9	0.3	1.0	21.5	432	1.4	2.7
Fruit Juices	6.5	0.2	0.7	23.7	488	0.8	1.6
Fruit Nectars	0.4	<0.1*	na	0.9	20	1.2*	1.7*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	8.3	0.2	0.8	26.3	444	0.9	2.4
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table A-8 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by the U.S. Population Aged 2 Years and Older (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/day)		Consumer-Only Intake ($CFUx10^9$/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	2.7	6.0	93.2	5,705	2.9	6.3
Beverages and Beverage Bases							
Energy Drinks	1.4	<0.1	na	3.5	141	1.1	2.7
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	2.3	0.1	na	6.6	286	1.0	2.0
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.1	<0.1*	na	0.5	16	0.6*	1.1*
Sports Drinks	2.9	0.1	na	7.1	432	1.1	2.0
Bottled tea	3.4	0.1	na	9.9	558	0.9	2.0
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	17.5	0.5	1.7	12.0	873	4.0	7.5
RTE Breakfast Cereals, Puffed Cereals	2.5	0.1	na	4.3	265	1.6	3.0
RTE Breakfast Cereals, High-Fiber Cereals	7.6	0.2	0.8	24.7	1,675	0.8	1.5
RTE Breakfast Cereals, Biscuit-Type Cereals	1.3	<0.1	na	5.0	243	0.7	1.5
Cheeses							
Cheeses	16.9	0.5	1.2	52.0	2,758	0.9	1.9
Chewing Gum							
Chewing Gum	1.2	<0.1	na	3.6	199	0.9	2.0
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.1	na	1.0	74	0.5	1.0*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.6	<0.1	na	2.2	140	0.7	1.4
Grain Products and Pastas							
Cereal and Granola Bars	1.8	<0.1	na	8.6	422	0.6	1.0
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.6	<0.1	na	4.4	164	1.0	1.9
Hard Candy							
Hard Candy	1.3	<0.1	na	5.8	433	0.6	1.6
Milk Products							
Buttermilk	<0.1	<0.1*	na	<0.1	5	0.5*	0.8*
Evaporated, Condensed, and/or Dry Milks	0.4	<0.1	na	0.6	71	1.9	4.1*
Fermented Milks, Plain	<0.1	<0.1*	na	0.1	9	0.3*	0.4*
Flavored Milks, Milk Drinks, and Mixes	3.6	0.1	0.3	11.0	806	0.9	1.8
Milk Shakes	0.5	<0.1	na	1.8	107	0.8	1.1
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	1.9	0.1	na	5.2	244	1.0	1.7

Table A-8 Estimated Daily Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by the U.S. Population Aged 2 Years and Older (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/day)		Consumer-Only Intake (CFUx10^9/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Plain or Flavored Yogurt	2.8	0.1	0.4	13.1	709	0.6	1.1
Yogurt Drinks	0.3	<0.1	na	1.0	57	0.8	1.8*
Plant Protein products							
Soy-based Food	0.4	<0.1	na	2.5	150	0.5	1.3
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	10.7	0.3	1.0	25.5	1,746	1.1	2.1
Fruit Juices	7.5	0.2	0.8	27.9	1,918	0.7	1.5
Fruit Nectars	0.2	<0.1	na	0.8	59	0.9	1.6*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	8.9	0.2	0.8	30.8	1,782	0.8	1.7
Other – Baby Food							
Baby Cereals, Dry Instant	0.1	<0.1*	na	<0.1	4	4.7*	5.4*
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	<0.1	<0.1*	na	<0.1	4	0.3*	0.4*
Baby Fruit Juice	0	0	0	0	0	0	0

CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

APPENDIX B

Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Different Population Groups within the U.S. (2017-2018 NHANES Data)

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Infants and Young Children Aged 0 to <2 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.33	0.80	76.7	336	0.43	1.00
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	0.2	<0.01*	na	1.6	4	0.04*	0.04*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0	0	0	0	0	0	0
Sports Drinks	0.9	<0.01*	na	6.0	22	0.05*	0.10*
Bottled tea	0.1	<0.01*	na	1.0	4	0.02*	0.02*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	10.2	0.03	0.12*	13.4	60	0.25	0.47*
RTE Breakfast Cereals, Puffed Cereals	0.5	<0.01*	na	2.8	16	0.06*	0.09*
RTE Breakfast Cereals, High-Fiber Cereals	2.4	0.01	0.03	23.0	86	0.03	0.07
RTE Breakfast Cereals, Biscuit-Type Cereals	<0.1	<0.01*	na	0.2	1	<0.01*	<0.01*
Cheeses							
Cheeses	3.2	0.01	0.04	25.0	81	0.04	0.09
Chewing Gum							
Chewing Gum	0	0	0	0	0	0	0
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	1.0	<0.01*	na	1.8	10	0.18*	0.27*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.1	<0.01*	na	1.6	5	0.01*	0.02*
Grain Products and Pastas							
Cereal and Granola Bars	0.4	<0.01*	na	2.5	9	0.05*	0.07*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.1	<0.01*	na	0.7	2	0.04*	0.05*
Hard Candy							
Hard Candy	0.2	<0.01*	na	1.4	10	0.05*	0.07*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	1.6	0.01*	na	0.7	3	0.76*	0.68*
Fermented Milks, Plain	0.1	<0.01*	na	1.1	1	0.03*	0.03*
Flavored Milks, Milk Drinks, and Mixes	0.9	<0.01*	na	4.0	15	0.07*	0.12*
Milk Shakes	<0.1	<0.01*	na	0.2	1	0.01*	0.01*

Table B-1 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Infants and Young Children Aged 0 to <2 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	<0.1	<0.01*	na	0.3	1	0.03*	0.03*
Plain or Flavored Yogurt	2.1	0.01	0.03*	17.5	63	0.04	0.07*
Yogurt Drinks	0.1	<0.01*	na	0.6	3	0.03*	0.04*
Plant Protein products							
Soy-based Food	0.1	<0.01*	na	2.4	5	0.01*	0.01*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	2.2	0.01	0.03*	16.7	64	0.04	0.08*
Fruit Juices	5.1	0.02	0.06	29.0	121	0.06	0.11
Fruit Nectars	<0.1	<0.01*	na	0.1	1	0.01*	0.01*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	1.2	<0.01	na	10.5	38	0.04	0.07*
Other – Baby Food							
Baby Cereals, Dry Instant	59.1	0.20	0.58	21.1	121	0.92	2.64
Baby Cereals, Prepared, Ready-to-Serve	1.0	<0.01*	na	5.8	28	0.06*	0.08*
Baby Ready-to-Eat cereals	<0.1	<0.01*	na	0.8	2	0.01*	0.02*
Baby Fruits or Vegetables (strained)	6.7	0.02	0.10	26.2	136	0.08	0.19
Baby Fruit Juice	0.9	<0.01*	na	5.3	24	0.05*	0.08*

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 2 to 5 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.18	0.33	99.5	459	0.18	0.33
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	0.5	<0.01*	na	2.0	11	0.04*	0.08*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.01*	na	0.4	1	0.01*	0.01*
Sports Drinks	1.5	<0.01	na	7.7	35	0.04	0.08*
Bottled tea	0.7	<0.01*	na	5.5	19	0.02*	0.05*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	16.1	0.03	0.11*	14.4	79	0.20	0.37*
RTE Breakfast Cereals, Puffed Cereals	3.2	0.01	na	9.6	45	0.06	0.10*
RTE Breakfast Cereals, High-Fiber Cereals	8.5	0.02	0.04	51.0	236	0.03	0.05
RTE Breakfast Cereals, Biscuit-Type Cereals	0.1	<0.01*	na	1.5	9	0.01*	0.02*
Cheeses							
Cheeses	10.2	0.02	0.05	53.8	234	0.03	0.07
Chewing Gum							
Chewing Gum	0.4	<0.01*	na	2.8	9	0.03*	0.04*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.1	<0.01*	na	0.3	4	0.03*	0.04*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.1	<0.01*	na	1.1	7	0.02*	0.03*
Grain Products and Pastas							
Cereal and Granola Bars	2.0	<0.01	0.01*	11.5	49	0.03	0.07*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.5	<0.01*	na	3.0	11	0.03*	0.04*
Hard Candy							
Hard Candy	1.9	<0.01	0.01*	12.4	62	0.03	0.05*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	2.0	<0.01*	na	0.5	5	0.66*	1.19*
Fermented Milks, Plain	0	0	0	0	0	0	0
Flavored Milks, Milk Drinks, and Mixes	9.7	0.02	0.06	34.4	125	0.05	0.11
Milk Shakes	0.3	<0.01*	na	2.5	10	0.02*	0.03*

Table B-2 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 2 to 5 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	0.3	<0.01*	na	2.1	10	0.03*	0.05*
Plain or Flavored Yogurt	4.2	0.01	0.03	27.0	113	0.03	0.05
Yogurt Drinks	2.1	<0.01*	na	6.3	21	0.06*	0.11*
Plant Protein products							
Soy-based Food	0.1	<0.01*	na	1.4	7	0.01*	0.05*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	9.6	0.02	0.05	43.5	191	0.04	0.08
Fruit Juices	17.0	0.03	0.08	59.3	287	0.05	0.10
Fruit Nectars	0.1	<0.01*	na	0.7	4	0.03*	0.04*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	7.6	0.01	0.04	45.3	198	0.03	0.06
Other – Baby Food							
Baby Cereals, Dry Instant	1.2	<0.01*	na	0.7	4	0.30*	0.37*
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0.1	<0.01*	na	0.7	3	0.02*	0.02*
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 6 to 11 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.09	0.18	98.6	668	0.09	0.18
Beverages and Beverage Bases							
Energy Drinks	0	0	0	0	0	0	0
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.3	<0.01	na	9.5	37	0.01	0.02*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.01*	na	0.4	1	0.01*	0.01
Sports Drinks	2.7	<0.01	0.01*	10.4	66	0.02	0.04*
Bottled tea	1.3	<0.01	na	7.3	46	0.02	0.03*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	5.1	<0.01	na	5.4	46	0.09	0.14*
RTE Breakfast Cereals, Puffed Cereals	7.3	0.01	na	9.7	58	0.07	0.16*
RTE Breakfast Cereals, High-Fiber Cereals	11.7	0.01	0.03	46.0	351	0.02	0.04
RTE Breakfast Cereals, Biscuit-Type Cereals	0.5	<0.01*	na	3.7	17	0.01*	0.02*
Cheeses							
Cheeses	11.4	0.01	0.03	54.7	314	0.02	0.04
Chewing Gum							
Chewing Gum	1.7	<0.01*	na	3.5	26	0.05*	0.14*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.1	<0.01*	na	0.6	4	0.01*	0.01*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.4	<0.01*	na	2.7	18	0.01*	0.02*
Grain Products and Pastas							
Cereal and Granola Bars	1.8	<0.01	0.01*	11.2	59	0.01	0.02*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.3	<0.01*	na	5.5	18	0.02*	0.04*
Hard Candy							
Hard Candy	5.4	<0.01	0.01	14.4	100	0.03	0.12
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	<0.1	<0.01*	na	0.1	2	0.01*	0.01*
Fermented Milks, Plain	<0.1	<0.01*	na	0.1	1	0.01*	0.01*
Flavored Milks, Milk Drinks, and Mixes	9.2	0.01	0.03	34.0	242	0.02	0.05
Milk Shakes	0.6	<0.01*	na	2.7	15	0.02*	0.03*

Table B-3 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Children Aged 6 to 11 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	0.4	<0.01*	na	1.5	9	0.02*	0.03*
Plain or Flavored Yogurt	3.9	<0.01	0.01	23.0	121	0.02	0.03
Yogurt Drinks	1.1	<0.01*	na	4.5	18	0.02*	0.03*
Plant Protein products							
Soy-based Food	0.1	<0.01*	na	2.3	13	<0.01*	0.01*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	12.3	0.01	0.03	42.9	318	0.03	0.05
Fruit Juices	11.0	0.01	0.03	42.6	312	0.02	0.04
Fruit Nectars	0.2	<0.01*	na	1.1	7	0.02*	0.02*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.3	0.01	0.03	39.6	267	0.02	0.05
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.04	0.08	93.6	413	0.04	0.09
Beverages and Beverage Bases							
Energy Drinks	0.6	<0.01*	na	1.6	7	0.01*	0.02*
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.1	<0.01*	na	3.7	16	0.01*	0.02*
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.3	<0.01*	na	0.2	1	0.06*	0.06*
Sports Drinks	2.8	<0.01	na	8.6	37	0.01	0.02*
Bottled tea	5.4	<0.01	0.01*	17.4	54	0.01	0.02*
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	7.6	<0.01	na	5.7	37	0.05	0.08*
RTE Breakfast Cereals, Puffed Cereals	4.0	<0.01*	na	5.8	24	0.03*	0.05*
RTE Breakfast Cereals, High-Fiber Cereals	12.7	<0.01	0.02	30.4	127	0.02	0.04
RTE Breakfast Cereals, Biscuit-Type Cereals	0.7	<0.01*	na	3.9	14	0.01*	0.01*
Cheeses							
Cheeses	14.4	0.01	0.02	47.0	185	0.01	0.03
Chewing Gum							
Chewing Gum	1.4	<0.01*	na	2.4	16	0.02*	0.04*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.01*	na	0.9	6	0.01*	0.01*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.4	<0.01*	na	0.6	6	0.02*	0.03*
Grain Products and Pastas							
Cereal and Granola Bars	4.0	<0.01	0.01*	14.4	48	0.01	0.02*
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	0.4	<0.01*	na	1.3	4	0.01*	0.01*
Hard Candy							
Hard Candy	2.7	<0.01	na	9.3	48	0.01	0.02*
Milk Products							
Buttermilk	0	0	0	0	0	0	0
Evaporated, Condensed, and/or Dry Milks	<0.1	<0.01*	na	<0.1	1	0.01*	0.01*
Fermented Milks, Plain	<0.1	<0.01*	na	0.2	1	<0.01*	<0.01*
Flavored Milks, Milk Drinks, and Mixes	5.1	<0.01	0.01*	12.8	69	0.02	0.04*
Milk Shakes	0.8	<0.01*	na	2.7	11	0.01*	0.02*

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	1.8	<0.01*	na	4.7	10	0.01*	0.02*
Plain or Flavored Yogurt	2.2	<0.01*	na	6.9	29	0.01*	0.02*
Yogurt Drinks	0.3	<0.01*	na	0.8	2	0.01*	0.01*
Plant Protein products							
Soy-based Food	0.2	<0.01*	na	1.5	7	0.01*	0.02*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	13.8	0.01	0.02	33.2	161	0.02	0.03
Fruit Juices	7.8	<0.01	0.01	23.7	117	0.01	0.02
Fruit Nectars	0.3	<0.01*	na	0.8	4	0.01*	0.03*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.2	<0.01	0.01	28.4	137	0.01	0.02
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	<0.1	<0.01*	na	0.1	1	0.01*	0.01*
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-4 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	1.8	<0.01*	na	4.7	10	0.01*	0.02*
Plain or Flavored Yogurt	2.2	<0.01*	na	6.9	29	0.01*	0.02*
Yogurt Drinks	0.3	<0.01*	na	0.8	2	0.01*	0.01*
Plant Protein products							
Soy-based Food	0.2	<0.01*	na	1.5	7	0.01*	0.02*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	13.8	0.01	0.02	33.2	161	0.02	0.03
Fruit Juices	7.8	<0.01	0.01	23.7	117	0.01	0.02
Fruit Nectars	0.3	<0.01*	na	0.8	4	0.01*	0.03*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.2	<0.01	0.01	28.4	137	0.01	0.02
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	<0.1	<0.01*	na	0.1	1	0.01*	0.01*
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Teenagers Aged 12 to 19 Years within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.1	<0.01*	na	6.3	21	0.01*	0.02*
Plain or Flavored Yogurt	0.9	<0.01*	na	3.7	16	0.01*	0.01*
Yogurt Drinks	0	0	0	0	0	0	0
Plant Protein products							
Soy-based Food	<0.1	<0.01*	na	0.1	2	0.01*	0.01*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	14.1	0.01	0.02	31.6	133	0.02	0.04
Fruit Juices	9.8	<0.01	0.01	26.5	136	0.02	0.05
Fruit Nectars	0.4	<0.01*	na	1.2	2	0.01*	0.01*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.0	<0.01	0.01	24.7	121	0.02	0.03
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.04	0.08	93.4	1,962	0.04	0.08
Beverages and Beverage Bases							
Energy Drinks	0.6	<0.01	na	2.5	42	0.01	0.02*
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	2.5	<0.01	na	7.2	120	0.01	0.02
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.3	<0.01*	na	1.2	11	0.01*	0.02*
Sports Drinks	1.0	<0.01	na	3.0	70	0.01	0.03*
Bottled tea	3.4	<0.01	na	10.1	197	0.01	0.03
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	21.5	0.01	0.03	14.2	383	0.05	0.11
RTE Breakfast Cereals, Puffed Cereals	1.5	<0.01	na	3.3	50	0.02	0.03*
RTE Breakfast Cereals, High-Fiber Cereals	6.3	<0.01	0.01	19.2	406	0.01	0.02
RTE Breakfast Cereals, Biscuit-Type Cereals	1.5	<0.01	na	5.2	94	0.01	0.02
Cheeses							
Cheeses	19.0	0.01	0.02	56.2	1,016	0.01	0.02
Chewing Gum							
Chewing Gum	1.4	<0.01	na	5.0	81	0.01	0.02
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.3	<0.01*	na	1.2	29	0.01*	0.02*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.6	<0.01	na	2.4	55	0.01	0.02*
Grain Products and Pastas							
Cereal and Granola Bars	1.8	<0.01	na	8.1	134	0.01	0.01
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.9	<0.01	na	5.0	64	0.01	0.03*
Hard Candy							
Hard Candy	0.7	<0.01	na	4.0	115	0.01	0.01
Milk Products							
Buttermilk	<0.1	<0.01*	na	0.1	4	0.01*	0.01*
Evaporated, Condensed, and/or Dry Milks	0.3	<0.01	na	0.7	33	0.02	0.04*
Fermented Milks, Plain	<0.1	<0.01*	na	0.1	4	<0.01*	0.01*
Flavored Milks, Milk Drinks, and Mixes	2.2	<0.01	na	6.4	164	0.01	0.02
Milk Shakes	0.3	<0.01*	na	1.3	27	0.01*	0.02*

Table B-6 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Female Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.1	<0.01	na	5.5	100	0.01	0.02
Plain or Flavored Yogurt	3.6	<0.01	0.01	15.1	277	0.01	0.01
Yogurt Drinks	0.2	<0.01*	na	0.4	12	0.02*	0.03*
Plant Protein products							
Soy-based Food	0.9	<0.01	na	3.5	82	0.01	0.03
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	10.2	<0.01	0.01	21.6	501	0.02	0.03
Fruit Juices	5.8	<0.01	0.01	25.3	564	0.01	0.02
Fruit Nectars	0.1	<0.01*	na	0.5	22	0.01*	0.02*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	10.1	<0.01	0.01	32.5	603	0.01	0.02
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.03	0.08	90.6	1,746	0.04	0.09
Beverages and Beverage Bases							
Energy Drinks	2.8	<0.01	na	6.3	84	0.01	0.02
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	2.8	<0.01	na	6.7	88	0.01	0.02
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	<0.1	<0.01*	na	<0.1	1	0.01*	0.01*
Sports Drinks	4.5	<0.01	na	9.0	155	0.02	0.04
Bottled tea	3.4	<0.01	na	9.7	191	0.01	0.02
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	20.4	0.01	0.03	12.5	296	0.06	0.10
RTE Breakfast Cereals, Puffed Cereals	1.8	<0.01	na	3.0	49	0.02	0.04*
RTE Breakfast Cereals, High-Fiber Cereals	6.6	<0.01	0.01	19.9	388	0.01	0.02
RTE Breakfast Cereals, Biscuit-Type Cereals	1.7	<0.01	na	5.9	99	0.01	0.02
Cheeses							
Cheeses	16.0	0.01	0.01	48.2	806	0.01	0.02
Chewing Gum							
Chewing Gum	1.1	<0.01	na	2.4	48	0.02	0.03*
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.01*	na	1.1	25	0.01*	0.01*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.8	<0.01	na	2.6	48	0.01	0.02**
Grain Products and Pastas							
Cereal and Granola Bars	1.5	<0.01	na	7.4	97	0.01	0.01
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.8	<0.01	na	4.6	52	0.01	0.03*
Hard Candy							
Hard Candy	1.2	<0.01	na	4.8	84	0.01	0.04
Milk Products							
Buttermilk	<0.1	<0.01*	na	<0.1	1	0.01*	0.01*
Evaporated, Condensed, and/or Dry Milks	0.6	<0.01*	na	0.9	28	0.02*	0.07*
Fermented Milks, Plain	<0.1	<0.01*	na	0.2	3	<0.01*	<0.01*
Flavored Milks, Milk Drinks, and Mixes	2.4	<0.01	na	5.5	110	0.02	0.02
Milk Shakes	0.6	<0.01	na	1.9	33	0.01	0.02*

Table B-7 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by Male Adults Aged 20 Years and Older within the U.S. (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	2.3	<0.01	na	5.9	93	0.01	0.02
Plain or Flavored Yogurt	2.1	<0.01	na	9.2	146	0.01	0.01
Yogurt Drinks	0.1	<0.01*	na	0.3	3	0.01*	0.02*
Plant Protein products							
Soy-based Food	0.3	<0.01	na	2.1	39	<0.01	0.01*
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	9.7	<0.01	0.01	21.3	425	0.02	0.03
Fruit Juices	6.6	<0.01	0.01	23.7	483	0.01	0.02
Fruit Nectars	0.4	<0.01*	na	0.9	20	0.01*	0.02*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	8.5	<0.01	0.01	26.4	442	0.01	0.03
Other – Baby Food							
Baby Cereals, Dry Instant	0	0	0	0	0	0	0
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	0	0	0	0	0	0	0
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

Table B-8 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by the U.S. Population Aged 2 Years and Older (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (CFUx10^9/kg bw/day)		Consumer-Only Intake (CFUx10^9/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	0.05	0.11	93.2	5,654	0.05	0.11
Beverages and Beverage Bases							
Energy Drinks	1.0	<0.01	na	3.6	141	0.01	0.02
Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.9	<0.01	na	6.6	284	0.01	0.02
Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	0.1	<0.01*	na	0.5	16	0.01*	0.02*
Sports Drinks	2.6	<0.01	na	7.1	429	0.02	0.04
Bottled tea	2.7	<0.01	na	9.9	555	0.01	0.02
Breakfast Cereals							
Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	16.4	0.01	0.03	11.9	861	0.06	0.13
RTE Breakfast Cereals, Puffed Cereals	3.1	<0.01	na	4.3	262	0.03	0.07
RTE Breakfast Cereals, High-Fiber Cereals	8.2	<0.01	0.01	24.7	1,658	0.02	0.03
RTE Breakfast Cereals, Biscuit-Type Cereals	1.1	<0.01	na	5.0	242	0.01	0.02
Cheeses							
Cheeses	14.8	0.01	0.02	52.0	2,730	0.01	0.03
Chewing Gum							
Chewing Gum	1.2	<0.01	na	3.7	199	0.02	0.03
Dairy Product Analogs							
Non-Dairy Milk (soy-based drinks)	0.2	<0.01	na	1.0	73	0.01	0.02*
Gelatins, Puddings, and Fillings							
Milk-Based Desserts	0.5	<0.01	na	2.2	139	0.01	0.02
Grain Products and Pastas							
Cereal and Granola Bars	1.9	<0.01	na	8.6	421	0.01	0.02
Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.4	<0.01	na	4.5	163	0.01	0.03
Hard Candy							
Hard Candy	1.9	<0.01	na	5.8	431	0.02	0.04
Milk Products							
Buttermilk	<0.1	<0.01*	na	<0.1	5	0.01*	0.01*
Evaporated, Condensed, and/or Dry Milks	0.6	<0.01	na	0.6	70	0.05	0.07*
Fermented Milks, Plain	<0.1	<0.01*	na	0.1	9	<0.1*	0.01*
Flavored Milks, Milk Drinks, and Mixes	5.2	<0.01	0.01	11.1	803	0.02	0.05
Milk Shakes	0.5	<0.01	na	1.9	107	0.01	0.03

Table B-8 Estimated Daily Per Kilogram Body Weight Intake of *L. rhamnosus* MP108 from Individual Proposed Food Uses by the U.S. Population Aged 2 Years and Older (2017-2018 NHANES Data)

Food Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake ($CFUx10^9$/kg bw/day)		Consumer-Only Intake ($CFUx10^9$/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Milk-Based Meal Replacement, Nutrition, and Protein Beverages	1.5	<0.01	na	5.2	243	0.01	0.02
Plain or Flavored Yogurt	3.2	<0.01	0.01	13.2	702	0.01	0.02
Yogurt Drinks	0.7	<0.01	na	1.0	56	0.03	0.08*
Plant Protein products							
Soy-based Food	0.4	<0.01	na	2.5	150	0.01	0.02
Processed Fruits and Fruit Juices							
Fruit Drinks and Ades Including Smoothies	10.6	<0.01	0.02	25.5	1,729	0.02	0.04
Fruit Juices	9.2	<0.01	0.01	27.8	1,899	0.02	0.03
Fruit Nectars	0.2	<0.01	na	0.8	59	0.01	0.02*
Soft Candy							
Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	9.0	<0.01	0.01	30.8	1,768	0.01	0.03
Other – Baby Food							
Baby Cereals, Dry Instant	0.2	<0.01*	na	<0.1	4	0.30*	0.37*
Baby Cereals, Prepared, Ready-to-Serve	0	0	0	0	0	0	0
Baby Ready-to-Eat cereals	0	0	0	0	0	0	0
Baby Fruits or Vegetables (strained)	<0.1	<0.01*	na	<0.1	4	0.02*	0.02*
Baby Fruit Juice	0	0	0	0	0	0	0

bw = body weight; CFU = colony forming units; n = sample size; na = not available; NHANES = National Health and Nutrition Examination Surveys; RTE = ready-to-eat; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements (mean n<30; 90th percentile n<80).

APPENDIX C
Representative Food Codes for Proposed Food Uses of *L. rhamnosus* MP108 in the U.S. (2017-2018 NHANES Data)

Representative Food Codes for Proposed Food Uses of *L. rhamnosus* MP108 in the U.S. (2017-2018 NHANES Data)

Beverages and Beverage Bases

Energy Drinks
[*L. rhamnosus* MP108] = 0.28 CFUx10^9/100 g

95310200	Energy drink (Full Throttle)
95310400	Energy drink (Monster)
95310500	Energy drink (Mountain Dew AMP)
95310550	Energy drink (No Fear)
95310555	Energy drink (No Fear Motherload)
95310560	Energy drink (NOS)
95310600	Energy drink (Red Bull)
95310700	Energy drink (Rockstar)
95310750	Energy drink (SoBe Energize Energy Juice Drink)
95310800	Energy drink (Vault)
95311000	Energy Drink
95312400	Energy drink, low calorie (Monster)
95312410	Energy drink, sugar free (Monster)
95312500	Energy drink, sugar free (Mountain Dew AMP)
95312550	Energy drink, sugar free (No Fear)
95312555	Energy drink, sugar-free (NOS)
95312560	Energy drink (Ocean Spray Cran-Energy Juice Drink)
95312600	Energy drink, sugar-free (Red Bull)
95312700	Energy drink, sugar free (Rockstar)
95312800	Energy drink, sugar free (Vault)
95312900	Energy drink (XS)
95312905	Energy drink (XS Gold Plus)
95313200	Energy drink, sugar free

Enhanced, Flavored, Carbonated, or Fortified Water Beverages
[*L. rhamnosus* MP108] = 0.28 CFUx10^9/100 g

92410110	Carbonated water, sweetened
92410210	Carbonated water, unsweetened
92410250	Carbonated water, sweetened, with low-calorie or no-calorie sweetener
94100200	Water, bottled, sweetened, with low calorie sweetener
94100300	Water, bottled, flavored (Capri Sun Roarin' Waters)
94210100	Water, bottled, flavored (Propel Water)
94210200	Water, bottled, flavored (Glaceau Vitamin Water)
94210300	Water, bottled, flavored (SoBe Life Water)
94220215	Water, bottled, flavored, sugar free (Glaceau Vitamin Water)

94220310 Water, bottled, flavored, sugar free (SoBe)

Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages
[*L. rhamnosus* MP108 = 0.42 CFUx10^9/100 g

95120050 Nutritional drink or shake, liquid, soy-based

Foods adjusted for being present in dried form
Reconstitution factor of 7
[*L. rhamnosus* MP108] = 2.94 CFUx10^9/100 g

95201300 Nutritional powder mix (EAS Soy Protein Powder)
95230010 Nutritional powder mix, protein, soy based, NFS

Sports Drinks
[*L. rhamnosus* MP108] = 0.28 CFUx10^9/100 g

95320200 Sports drink (Gatorade G)
95320500 Sports drink (Powerade)
95321000 Sports drink, NFS
95322200 Sports drink, low calorie (Gatorade G2)
95322500 Sports drink, low calorie (Powerade Zero)
95323000 Sports drink, low calorie
95330100 Fluid replacement, electrolyte solution
95330500 Fluid replacement, 5% glucose in water

Foods adjusted for being present in dried form
Reconstitution factor of 16.625
[*L. rhamnosus* MP108] = 4.66 CFUx10^9/100 g

92900300 Sports drink, dry concentrate, not reconstituted

Bottled Tea
[*L. rhamnosus* MP108] = 0.28 CFUx10^9/100 g

92307500 Iced Tea / Lemonade juice drink
92307510 Iced Tea / Lemonade juice drink, light
92307520 Iced Tea / Lemonade juice drink, diet
92309000 Tea, iced, bottled, black
92309010 Tea, iced, bottled, black, decaffeinated
92309020 Tea, iced, bottled, black, diet
92309030 Tea, iced, bottled, black, decaffeinated, diet
92309040 Tea, iced, bottled, black, unsweetened
92309050 Tea, iced, bottled, black, decaffeinated, unsweetened

92309500 Tea, iced, bottled, green
92309510 Tea, iced, bottled, green, diet
92309520 Tea, iced, bottled, green, unsweetened

Breakfast Cereals

Hot Breakfast Cereals (*e.g.*, Oatmeal, Grits)
[*L. rhamnosus* MP108] = 2.50 CFUx10^9/100 g

56200300 Cereal, cooked, NFS
56200990 Grits, NS as to regular, quick, or instant, NS as to fat
56201000 Grits, NS as to regular, quick, or instant, no added fat
56201040 Grits, NS as to regular, quick, or instant, fat added
56201050 Grits, regular or quick, made with water, NS as to fat
56201051 Grits, regular or quick, made with water, no added fat
56201052 Grits, regular or quick, made with water, fat added
56201055 Grits, regular or quick, made with milk, NS as to fat
56201056 Grits, regular or quick, made with milk, no added fat
56201057 Grits, regular or quick, made with milk, fat added
56201065 Grits, regular or quick, made with non-dairy milk, NS as to fat
56201066 Grits, regular or quick, made with non-dairy milk, no added fat
56201067 Grits, regular or quick, made with non-dairy milk, fat added
56201090 Grits, with cheese, NS as to fat
56201091 Grits, with cheese, no added fat
56201092 Grits, with cheese, fat added
56201210 Grits, instant, made with water, no added fat
56201220 Grits, instant, made with water, fat added
56201230 Grits, instant, made with water, NS as to fat
56201340 Grits, instant, made with milk, fat added
56201342 Grits, instant, made with milk, no added fat
56201344 Grits, instant, made with milk, NS as to fat
56201350 Grits, instant, made with non-dairy milk, NS as to fat
56201355 Grits, instant, made with non-dairy milk, no added fat
56201360 Grits, instant, made with non-dairy milk, fat added
56201515 Cornmeal mush, NS as to fat
56201516 Cornmeal mush, no added fat
56201517 Cornmeal mush, fat added
56201540 Cornmeal, Puerto Rican Style
56202900 Oatmeal, from fast food, plain
56202905 Oatmeal, from fast food, maple flavored
56202910 Oatmeal, from fast food, fruit flavored
56202920 Oatmeal, from fast food, other flavors
56202960 Oatmeal, NS as to regular, quick, or instant, NS as to fat

56203000 Oatmeal, NS as to regular, quick, or instant, no added fat
56203040 Oatmeal, NS as to regular, quick, or instant, fat added
56203055 Oatmeal, regular or quick, made with water, NS as to fat
56203056 Oatmeal, regular or quick, made with water, no added fat
56203057 Oatmeal, regular or quick, made with water, fat added
56203065 Oatmeal, regular or quick, made with milk, NS as to fat
56203066 Oatmeal, regular or quick, made with milk, no added fat
56203067 Oatmeal, regular or quick, made with milk, fat added
56203075 Oatmeal, regular or quick, made with non-dairy milk, NS as to fat
56203076 Oatmeal, regular or quick, made with non-dairy milk, no added fat
56203077 Oatmeal, regular or quick, made with non-dairy milk, fat added
56203085 Oatmeal, instant, plain, made with water, NS as to fat
56203086 Oatmeal, instant, plain, made with water, no added fat
56203087 Oatmeal, instant, plain, made with water, fat added
56203095 Oatmeal, instant, plain, made with milk, NS as to fat
56203096 Oatmeal, instant, plain, made with milk, no added fat
56203097 Oatmeal, instant, plain, made with milk, fat added
56203105 Oatmeal, instant, plain, made with non-dairy milk, NS as to fat
56203106 Oatmeal, instant, plain, made with non-dairy milk, no added fat
56203107 Oatmeal, instant, plain, made with non-dairy milk, fat added
56203125 Oatmeal, instant, maple flavored, NS as to fat
56203130 Oatmeal, instant, maple flavored, no added fat
56203135 Oatmeal, instant, maple flavored, fat added
56203150 Oatmeal, instant, fruit flavored, NS as to fat
56203155 Oatmeal, instant, fruit flavored, no added fat
56203160 Oatmeal, instant, fruit flavored, fat added
56203170 Oatmeal, instant, other flavors, NS as to fat
56203175 Oatmeal, instant, other flavors, no added fat
56203180 Oatmeal, instant, other flavors, fat added
56203500 Oatmeal, reduced sugar, plain, NS as to fat
56203510 Oatmeal, reduced sugar, plain, no added fat
56203520 Oatmeal, reduced sugar, plain, fat added
56203540 Oatmeal, made with milk and sugar, Puerto Rican style
56203550 Oatmeal, reduced sugar, flavored, NS as to fat
56203555 Oatmeal, reduced sugar, flavored, no added fat
56203560 Oatmeal, reduced sugar, flavored, fat added
56203600 Oatmeal, multigrain, NS as to fat
56203610 Oatmeal, multigrain, no added fat
56203620 Oatmeal, multigrain, fat added
56205050 Rice, cream of, cooked, no added fat
56205080 Rice, creamed, made with milk and sugar, Puerto Rican style
56205090 Rice, cream of, cooked, fat added
56205092 Rice, cream of, cooked, NS as to fat

56205094 Rice, cream of, cooked, made with milk
56205101 Congee
56206990 Cream of wheat, NS as to regular, quick, or instant, NS as to fat
56207000 Cream of wheat, NS as to regular, quick, or instant, no added fat
56207005 Cream of wheat, NS as to regular, quick, or instant, fat added
56207015 Cream of wheat, regular or quick, made with water, NS as to fat
56207016 Cream of wheat, regular or quick, made with water, no added fat
56207017 Cream of wheat, regular or quick, made with water, fat added
56207021 Cream of wheat, regular or quick, made with milk, NS as to fat
56207022 Cream of wheat, regular or quick, made with milk, no added fat
56207023 Cream of wheat, regular or quick, made with milk, fat added
56207025 Cream of wheat, regular or quick, made with non-dairy milk, NS as to fat
56207026 Cream of wheat, regular or quick, made with non-dairy milk, no added fat
56207027 Cream of wheat, regular or quick, made with non-dairy milk, fat added
56207030 Cream of wheat, instant, made with water, no added fat
56207050 Wheat, cream of, cooked, made with milk and sugar, Puerto Rican style
56207060 Cream of wheat, instant, made with water, fat added
56207070 Cream of wheat, instant, made with water, NS as to fat
56207094 Cream of wheat, instant, made with milk, fat added
56207095 Cream of wheat, instant, made with milk, no added fat
56207096 Cream of wheat, instant, made with milk, NS as to fat
56207101 Cream of wheat, instant, made with non-dairy milk, NS as to fat
56207102 Cream of wheat, instant, made with non-dairy milk, no added fat
56207103 Cream of wheat, instant, made with non-dairy milk, fat added
56207190 Whole wheat cereal, cooked, NS as to fat
56207200 Whole wheat cereal, cooked, no added fat
56207210 Whole wheat cereal, cooked, fat added
56207370 Wheat cereal, chocolate flavored, cooked
56208500 Oat bran cereal, cooked, no added fat
56208510 Oat bran cereal, cooked, fat added
56208520 Oat bran cereal, cooked, NS as to fat
56209000 Cream of rye
58174000 Upma, Indian breakfast dish

RTE, Puffed Cereals

[*L. rhamnosus* MP108] = 6.67 CFUx10^9/100 g

57124200 Cereal, chocolate flavored, frosted, puffed corn
57126000 Cereal (Kellogg's Cocoa Krispies)
57128000 Cereal (General Mills Cocoa Puffs)
57132000 Cereal (General Mills Chex Corn)
57137000 Cereal, corn puffs
57151000 Cereal, crispy rice
57216000 Cereal, frosted rice

57301500 Cereal (Kashi 7 Whole Grain Puffs)
57303100 Cereal (General Mills Kix)
57303105 Cereal (General Mills Honey Kix)
57306500 Cereal (Malt-O-Meal Golden Puffs)
57326000 Cereal (Barbara's Puffins)
57335550 Cereal (General Mills Reese's Puffs)
57336000 Cereal (General Mills Chex Rice)
57337000 Cereal, rice flakes
57339000 Cereal (Kellogg's Rice Krispies)
57339500 Cereal (Kellogg's Rice Krispies Treats Cereal)
57340000 Cereal, puffed rice
57347000 Cereal (Kellogg's Corn Pops)
57407100 Cereal (General Mills Trix)
57416000 Cereal, puffed wheat, plain
57416010 Cereal, puffed wheat, sweetened

RTE, High-Fiber Cereals
[*L. rhamnosus* MP108] = 2.50 CFUx10^9/100 g

57000100 Cereal, oat, NFS
57100100 Cereal, ready-to-eat, NFS
57101000 Cereal (Kellogg's All-Bran)
57103000 Cereal (Post Alpha-Bits)
57103100 Cereal (General Mills Cheerios Apple Cinnamon)
57104000 Cereal (Kellogg's Apple Jacks)
57106060 Cereal (General Mills Cheerios Banana Nut)
57106260 Cereal (General Mills Cheerios Berry Burst)
57117000 Cereal (Quaker Cap'n Crunch)
57117500 Cereal (Quaker Christmas Crunch)
57119000 Cereal (Quaker Cap'n Crunch's Crunchberries)
57120000 Cereal (Quaker Cap'n Crunch's Peanut Butter Crunch)
57123000 Cereal (General Mills Cheerios)
57124030 Cereal (General Mills Chex Chocolate)
57124050 Cereal (General Mills Chex Cinnamon)
57124100 Cereal (General Mills Cheerios Chocolate)
57124300 Cereal (General Mills Lucky Charms Chocolate)
57125000 Cereal (General Mills Cinnamon Toast Crunch)
57125010 Cereal (General Mills 25% Less Sugar Cinnamon Toast Crunch)
57125900 Cereal (General Mills Honey Nut Clusters)
57127000 Cereal (Post Cocoa Pebbles)
57130000 Cereal (General Mills Cookie Crisp)
57134000 Cereal, corn flakes
57135000 Cereal (Kellogg's Corn Flakes)
57139000 Cereal (General Mills Count Chocula)

57143500 Cereal (Post Great Grains, Cranberry Almond Crunch)
57148000 Cereal (Kellogg's Crispix)
57206700 Cereal (General Mills Fiber One)
57206710 Cereal (General Mills Fiber One Honey Clusters)
57206715 Cereal (General Mills Fiber One Raisin Bran Clusters)
57211000 Cereal (General Mills Frankenberry)
57213000 Cereal (Kellogg's Froot Loops)
57213010 Cereal (Kellogg's Froot Loops Marshmallow)
57213850 Cereal (General Mills Cheerios Frosted)
57214000 Cereal (Kellogg's Frosted Mini-Wheats)
57221700 Cereal, fruit rings
57221810 Cereal (General Mills Cheerios Fruity)
57223000 Cereal (Post Fruity Pebbles)
57230000 Cereal (Post Grape-Nuts)
57231200 Cereal (Post Great Grains Raisins, Dates, and Pecans)
57237100 Cereal (Post Honey Bunches of Oats Honey Roasted)
57237200 Cereal (Post Honey Bunches of Oats with Vanilla Bunches)
57237300 Cereal (Post Honey Bunches of Oats with Almonds)
57238000 Cereal (Post Honeycomb)
57240100 Cereal (General Mills Chex Honey Nut)
57241000 Cereal (General Mills Cheerios Honey Nut)
57241200 Cereal (Post Shredded Wheat Honey Nut)
57243000 Cereal (Kellogg's Honey Smacks)
57301505 Cereal (Kashi Autumn Wheat)
57301510 Cereal (Kashi GOLEAN)
57301511 Cereal (Kashi GOLEAN Crunch)
57301512 Cereal (Kashi GOLEAN Crunch Honey Almond Flax)
57301530 Cereal (Kashi Heart to Heart Honey Toasted Oat)
57303200 Cereal (Kellogg's Krave)
57304100 Cereal (Quaker Life)
57305100 Cereal (General Mills Lucky Charms)
57305150 Cereal, frosted oat cereal with marshmallows
57305160 Cereal (Malt-O-Meal Blueberry Muffin Tops)
57305165 Cereal (Malt-O-Meal Cinnamon Toasters)
57305170 Cereal (Malt-O-Meal Coco-Roos)
57305174 Cereal (Malt-O-Meal Colossal Crunch)
57305175 Cereal (Malt-O-Meal Cocoa Dyno-Bites)
57305180 Cereal (Malt-O-Meal Corn Bursts)
57305210 Cereal (Malt-O-Meal Frosted Flakes)
57305300 Cereal (Malt-O-Meal Fruity Dyno-Bites)
57305400 Cereal (Malt-O-Meal Honey Graham Squares)
57305500 Cereal (Malt-O-Meal Honey Nut Toasty O's)
57305600 Cereal (Malt-O-Meal Marshmallow Mateys)

57306700 Cereal (Malt-O-Meal Toasted Oat Cereal)
57306800 Cereal (Malt-O-Meal Tootie Fruities)
57308400 Cereal (General Mills Cheerios Multigrain)
57316380 Cereal (General Mills Cheerios Oat Cluster Crunch)
57316385 Cereal (General Mills Cheerios Protein)
57316710 Cereal (Quaker Honey Graham Oh's)
57327450 Cereal (Quaker Toasted Oat Bran)
57327500 Cereal (Quaker Oatmeal Squares)
57341200 Cereal (Kellogg's Smart Start Strong)
57341300 Cereal (Kellogg's Smorz)
57344000 Cereal (Kellogg's Special K)
57344001 Cereal (Kellogg's Special K Blueberry)
57344005 Cereal (Kellogg's Special K Chocolatey Delight)
57344010 Cereal (Kellogg's Special K Red Berries)
57344015 Cereal (Kellogg's Special K Fruit & Yogurt)
57344020 Cereal (Kellogg's Special K Vanilla Almond)
57344025 Cereal (Kellogg's Special K Cinnamon Pecan)
57348000 Cereal, frosted corn flakes
57349000 Cereal (Kellogg's Frosted Flakes)
57355000 Cereal (Post Golden Crisp)
57408100 Cereal (Uncle Sam)
57411000 Cereal (General Mills Chex Wheat)
57412000 Wheat germ, plain
57417000 Cereal (Post Shredded Wheat)
57418000 Cereal (General Mills Wheaties)

RTE, Biscuit-Type Cereals
[*L. rhamnosus* MP108] = 1.67 CFUx10^9/100 g

57106050 Cereal (Post Great Grains Banana Nut Crunch)
57143000 Cereal (Kellogg's Cracklin' Oat Bran)
57207000 Cereal, bran flakes
57208000 Cereal (Kellogg's All-Bran Complete Wheat Flakes)
57209000 Cereal (Post Bran Flakes)
57224000 Cereal (General Mills Golden Grahams)
57227000 Cereal, granola
57229000 Cereal (Kellogg's Low Fat Granola)
57308190 Cereal, muesli
57309100 Cereal (Nature Valley Granola)
57316450 Cereal (General Mills Oatmeal Crisp with Almonds)
57320500 Cereal (Quaker Granola with Oats, Honey, and Raisins)
57321900 Cereal (Nature's Path Organic Flax Plus)
57329000 Cereal, raisin bran
57330000 Cereal (Kellogg's Raisin Bran)

57330010	Cereal (Kellogg's Raisin Bran Crunch)
57331000	Cereal (Post Raisin Bran)
57332100	Cereal (General Mills Raisin Nut Bran)
57401100	Cereal, toasted oat

Cheeses

Cheeses

[*L. rhamnosus* MP108] = 3.33 CFUx10^9/100 g

14010000	Cheese, NFS
14101010	Cheese, Blue or Roquefort
14102010	Cheese, Brick
14103010	Cheese, Camembert
14103020	Cheese, Brie
14104100	Cheese, Cheddar
14104110	Cheese, Cheddar, reduced fat
14104115	Cheese, Cheddar, nonfat or fat free
14104200	Cheese, Colby
14104250	Cheese, Colby Jack
14104400	Cheese, Feta
14104600	Cheese, Fontina
14104700	Cheese, goat
14105010	Cheese, Gouda or Edam
14105200	Cheese, Gruyere
14106010	Cheese, Limburger
14106200	Cheese, Monterey
14106500	Cheese, Monterey, reduced fat
14107010	Cheese, Mozzarella, NFS
14107030	Cheese, Mozzarella, part skim
14107040	Cheese, Mozzarella, reduced sodium
14107060	Cheese, Mozzarella, nonfat or fat free
14107200	Cheese, Muenster
14107250	Cheese, Muenster, reduced fat
14108010	Cheese, Parmesan, dry grated
14108015	Cheese, Parmesan, dry grated, reduced fat
14108020	Cheese, Parmesan, hard
14108060	Cheese, Parmesan, dry grated, fat free
14108200	Cheese, Port du Salut
14108400	Cheese, Provolone
14108420	Cheese, provolone, reduced fat
14109010	Cheese, Swiss
14109020	Cheese, Swiss, reduced sodium

14109030	Cheese, Swiss, reduced fat
14109040	Cheese, Swiss, nonfat or fat free
14110010	Cheese, Cheddar, reduced sodium
14120010	Cheese, Mexican blend
14120020	Cheese, Mexican blend, reduced fat
14131000	Queso Anejo, aged Mexican cheese
14131500	Queso Asadero
14133000	Queso Fresco
14134000	Queso cotija
14200100	Cheese, cottage, NFS
14201010	Cheese, cottage, creamed, large or small curd
14201200	Cottage cheese, farmer's
14201500	Cheese, Ricotta
14203010	Cheese, cottage, dry curd
14203020	Cheese, cottage, salted, dry curd
14203510	Puerto Rican white cheese
14204010	Cheese, cottage, low fat
14206010	Cheese, cottage, lowfat, low sodium
14207010	Cheese, cottage, lowfat, lactose reduced
14301010	Cream cheese, regular, plain
14301100	Cream cheese, regular, flavored
14303010	Cream cheese, light
14410100	Cheese, American and Swiss blends
14410110	Cheese, American
14410120	Cheese, American, reduced fat
14410130	Cheese, American, nonfat or fat free
14410210	Cheese, American, reduced sodium
14410330	Cheese spread, American or Cheddar cheese base, reduced fat
14410380	Cream cheese spread, fat free
14410500	Cheese, processed cheese food
14410620	Cheese, with wine
14420100	Cheese spread, American or Cheddar cheese base
14420160	Cheese spread, Swiss cheese base
14420200	Cheese spread, cream cheese, regular
14420210	Cheese spread, cream cheese, light
14420300	Cheese spread, pressurized can
99991400	Cheese as ingredient in sandwiches

Mixed Foods Containing Cheeses

Adjusted for non-dairy milk content of 27.67 to 93.90%
[*L. rhamnosus* MP108] = 0.92 to 3.13 CFUx10^9/100 g

14202010	Cheese, cottage, with fruit
14202020	Cheese, cottage, with vegetables
14204020	Cheese, cottage, lowfat, with fruit
14410600	Cheese, processed, with vegetables
14610200	Cheese, cottage cheese, with gelatin dessert
14610210	Cheese, cottage cheese, with gelatin dessert and fruit
14610250	Cheese, cottage cheese, with gelatin dessert and vegetables
14610520	Cheese ball
14670000	Mozzarella cheese, tomato, and basil, with oil and vinegar dressing

Chewing Gum

Chewing Gum

[*L. rhamnosus* MP108] = 33.33 CFUx10^9/100 g

91800100	Chewing gum, NFS
91801000	Chewing gum, regular
91802000	Chewing gum, sugar free

Dairy Product Analogs

Non-Dairy Milk (Soy-Based Drinks)

[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

11300100	Non-dairy milk, NFS
11320000	Soy milk
11320100	Soy milk, light
11320200	Soy milk, nonfat
11321000	Soy milk, chocolate
11321100	Soy milk, light, chocolate
11321200	Soy milk, nonfat, chocolate

Mixed Foods Containing Non-Dairy Milk

Adjusted for non-dairy milk content of 77.47 to 81.33%
[*L. rhamnosus* MP108] = 0.33 to 0.34 CFUx10^9/100 g

11512030	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
11512120	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream
11513310	Chocolate milk, made from dry mix with non-dairy milk
11513375	Chocolate milk, made from reduced sugar mix with non-dairy milk
11513385	Chocolate milk, made from dry mix with non-dairy milk (Nesquik)
11513395	Chocolate milk, made from no sugar added dry mix with non-dairy milk (Nesquik)
11513750	Chocolate milk, made from syrup with non-dairy milk
11513805	Chocolate milk, made from light syrup with non-dairy milk
11513855	Chocolate milk, made from sugar free syrup with non-dairy milk
11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk
11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk
11519215	Strawberry milk, non-dairy

Gelatins, Puddings, and Fillings

Milk-Based Desserts

[*L. rhamnosus* MP108] = 0.77 CFUx10^9/100 g

13200110	Pudding, chocolate, NFS
13210110	Pudding, bread
13210280	Pudding, flavors other than chocolate, NFS
13210300	Custard
13210370	Creme brulee
13210410	Pudding, rice
13210450	Firni, Indian pudding
13210520	Pudding, tapioca, made from dry mix
13220110	Pudding, flavors other than chocolate, made from dry mix
13220120	Pudding, chocolate, made from dry mix
13220210	Pudding, flavors other than chocolate, made from dry mix, sugar free
13220220	Pudding, chocolate, made from dry mix, sugar free
13230110	Pudding, flavors other than chocolate, ready-to-eat
13230120	Pudding, flavors other than chocolate, ready-to-eat, sugar free
13230130	Pudding, chocolate, ready-to-eat
13230140	Pudding, chocolate, ready-to-eat, sugar free
13230500	Pudding, tapioca, ready-to-eat
13241000	Banana pudding
13250000	Mousse
13252200	Milk dessert or milk candy, Puerto Rican style
13252500	Barfi or Burfi, Indian dessert

Grain Products and Pastas

Cereal and Granola Bars

[*L. rhamnosus* MP108] = 2.50 CFUx10^9/100 g

53710400	Cereal or granola bar (General Mills Fiber One Chewy Bar)
53710500	Cereal or granola bar (Kellogg's Nutri-Grain Cereal Bar)
53710502	Cereal or granola bar (Kellogg's Nutri-Grain Yogurt Bar)
53710504	Cereal or granola bar (Kellogg's Nutri-Grain Fruit and Nut Bar)
53710600	Milk 'n Cereal bar
53710700	Cereal or granola bar (Kellogg's Special K bar)
53710800	Cereal or granola bar (Kashi Chewy)
53710802	Cereal or granola bar (Kashi Crunchy)
53710810	Cereal or granola bar (KIND Fruit and Nut Bar)
53710900	Cereal or granola bar (General Mills Nature Valley Chewy Trail Mix)
53710902	Cereal or granola bar, with yogurt coating (General Mills Nature Valley Chewy Granola Bar)
53710904	Cereal or granola bar (General Mills Nature Valley Sweet and Salty Granola Bar)
53710906	Cereal or granola bar (General Mills Nature Valley Crunchy Granola Bar)
53711000	Cereal or granola bar (Quaker Chewy Granola Bar)
53711002	Cereal or granola bar (Quaker Chewy 90 Calorie Granola Bar)
53711004	Cereal or granola bar (Quaker Chewy 25% Less Sugar Granola Bar)
53711006	Cereal or granola bar (Quaker Chewy Dipps Granola Bar)
53711100	Cereal or granola bar (Quaker Granola Bites)
53712000	Snack bar, oatmeal
53712100	Cereal or Granola bar, NFS
53712200	Cereal or granola bar, lowfat, NFS
53712210	Cereal or granola bar, nonfat
53713000	Cereal or granola bar, reduced sugar, NFS
53713010	Cereal or granola bar, fruit and nut
53713100	Cereal or granola bar, peanuts , oats, sugar, wheat germ
53714200	Cereal or granola bar, chocolate coated, NFS
53714210	Cereal or granola bar, with coconut, chocolate coated
53714220	Cereal or granola bar with nuts, chocolate coated
53714230	Cereal or granola bar, oats, nuts, coated with non-chocolate coating
53714250	Cereal or granola bar, coated with non-chocolate coating
53714300	Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating
53714400	Cereal or granola bar, with rice cereal

Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based Bars
[*L. rhamnosus* MP108] = 2.50 CFUx10^9/100 g

53714500	Breakfast bar, NFS
53714510	Breakfast bar, date, with yogurt coating
53714520	Breakfast bar, cereal crust with fruit filling, lowfat
53720100	Nutrition bar (Balance Original Bar)
53720200	Nutrition bar (Clif Bar)
53720210	Nutrition bar (Clif Kids Organic Zbar)
53720300	Nutrition bar (PowerBar)
53720400	Nutrition bar (Slim Fast Original Meal Bar)
53720500	Nutrition bar (Snickers Marathon Protein Bar)
53720600	Nutrition bar (South Beach Living Meal Bar)
53720610	Nutrition bar (South Beach Living High Protein Bar)
53720700	Nutrition bar (Tiger's Milk)
53720800	Nutrition bar (Zone Perfect Classic Crunch)
53729000	Nutrition bar or meal replacement bar, NFS

Hard Candy

Hard Candy
[*L. rhamnosus* MP108] = 6.67 CFUx10^9/100 g

91718000	Honey-combed hard candy with peanut butter
91718050	Honey-combed hard candy with peanut butter, chocolate covered
91745020	Hard candy
91745040	Butterscotch hard candy
91770020	Dietetic or low calorie hard candy

Milk Products

Buttermilk
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

11115000	Buttermilk, fat free (skim)
11115100	Buttermilk, low fat (1%)
11115200	Buttermilk, reduced fat (2%)
11115300	Buttermilk, whole

Evaporated, Condensed, and/or Dry Milks
[*L. rhamnosus* MP108] = 3.33 CFUx10^9/100 g

11120000	Milk, dry, reconstituted, NS as to fat content
11121100	Milk, dry, reconstituted, whole
11121210	Milk, dry, reconstituted, low fat (1%)
11121300	Milk, dry, reconstituted, fat free (skim)
11210050	Milk, evaporated, NS as to fat content
11211050	Milk, evaporated, whole
11211400	Milk, evaporated, reduced fat (2%)
11212050	Milk, evaporated, fat free (skim)
11220000	Milk, condensed, sweetened

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 11
[*L. rhamnosus* MP108] = 36.63%

11810000	Milk, dry, not reconstituted, NS as to fat content
11811000	Milk, dry, not reconstituted, whole
11812000	Milk, dry, not reconstituted, low fat (1%)
11813000	Milk, dry, not reconstituted, fat free (skim)

Fermented Milks, Plain
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

11112120	Milk, acidophilus, low fat (1%)
11112130	Milk, acidophilus, reduced fat (2%)
11115400	Kefir, NS as to fat content

Flavored Milks, Milk Drinks, and Mixes
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

11511000	Chocolate milk, NFS
11511100	Chocolate milk, ready to drink, whole
11511200	Chocolate milk, ready to drink, reduced fat
11511300	Chocolate milk, ready to drink, fat free
11511400	Chocolate milk, ready to drink, low fat
11511550	Chocolate milk, ready to drink, reduced sugar, NS as to milk
11511600	Chocolate milk, ready to drink, low fat (Nesquik)
11511610	Chocolate milk, ready to drink, fat free (Nesquik)
11511700	Chocolate milk, ready to drink, low fat, no sugar added (Nesquik)
11512010	Hot chocolate / Cocoa, ready to drink
11512020	Hot chocolate / Cocoa, ready to drink, made with nonfat milk
11512100	Hot chocolate / Cocoa, ready to drink, with whipped cream
11512110	Hot chocolate / Cocoa, ready to drink, made with nonfat milk and whipped cream

11513000 Chocolate milk, made from dry mix, NS as to type of milk
11513100 Chocolate milk, made from dry mix with whole milk
11513150 Chocolate milk, made from dry mix with reduced fat milk
11513200 Chocolate milk, made from dry mix with low fat milk
11513300 Chocolate milk, made from dry mix with fat free milk
11513350 Chocolate milk, made from reduced sugar mix, NS as to type of milk
11513355 Chocolate milk, made from reduced sugar mix with whole milk
11513360 Chocolate milk, made from reduced sugar mix with reduced fat milk
11513365 Chocolate milk, made from reduced sugar mix with low fat milk
11513370 Chocolate milk, made from reduced sugar mix with fat free milk
11513380 Chocolate milk, made from dry mix, NS as to type of milk (Nesquik)
11513381 Chocolate milk, made from dry mix with whole milk (Nesquik)
11513382 Chocolate milk, made from dry mix with reduced fat milk (Nesquik)
11513383 Chocolate milk, made from dry mix with low fat milk (Nesquik)
11513384 Chocolate milk, made from dry mix with fat free milk (Nesquik)
11513390 Chocolate milk, made from no sugar added dry mix, NS as to type of milk (Nesquik)
11513391 Chocolate milk, made from no sugar added dry mix with whole milk (Nesquik)
11513392 Chocolate milk, made from no sugar added dry mix with reduced fat milk (Nesquik)
11513393 Chocolate milk, made from no sugar added dry mix with low fat milk (Nesquik)
11513394 Chocolate milk, made from no sugar added dry mix with fat free milk (Nesquik)
11513400 Chocolate milk, made from syrup, NS as to type of milk
11513500 Chocolate milk, made from syrup with whole milk
11513550 Chocolate milk, made from syrup with reduced fat milk
11513600 Chocolate milk, made from syrup with low fat milk
11513700 Chocolate milk, made from syrup with fat free milk
11513800 Chocolate milk, made from light syrup, NS as to type of milk
11513801 Chocolate milk, made from light syrup with whole milk
11513802 Chocolate milk, made from light syrup with reduced fat milk
11513803 Chocolate milk, made from light syrup with low fat milk
11513804 Chocolate milk, made from light syrup with fat free milk
11513850 Chocolate milk, made from sugar free syrup, NS as to type of milk
11513851 Chocolate milk, made from sugar free syrup with whole milk
11513852 Chocolate milk, made from sugar free syrup with reduced fat milk
11513853 Chocolate milk, made from sugar free syrup with low fat milk
11513854 Chocolate milk, made from sugar free syrup with fat free milk
11514100 Hot chocolate / Cocoa, made with dry mix and water
11514110 Hot chocolate / Cocoa, made with dry mix and whole milk
11514120 Hot chocolate / Cocoa, made with dry mix and reduced fat milk
11514130 Hot chocolate / Cocoa, made with dry mix and low fat milk
11514140 Hot chocolate / Cocoa, made with dry mix and fat free milk
11514320 Hot chocolate / Cocoa, made with no sugar added dry mix and whole milk
11514330 Hot chocolate / Cocoa, made with no sugar added dry mix and reduced fat milk
11514340 Hot chocolate / Cocoa, made with no sugar added dry mix and low fat milk

11514350 Hot chocolate / Cocoa, made with no sugar added dry mix and fat free milk
11519040 Strawberry milk, NFS
11519050 Strawberry milk, whole
11519105 Strawberry milk, reduced fat
11519200 Strawberry milk, low fat
11519205 Strawberry milk, fat free
11519210 Strawberry milk, reduced sugar
11526000 Milk, malted
11531000 Eggnog
11551050 Licuado or Batido
11553100 Fruit smoothie, NFS
11553110 Fruit smoothie, with whole fruit and dairy
11553120 Fruit smoothie, with whole fruit and dairy, added protein
11553130 Fruit smoothie juice drink, with dairy
11560000 Chocolate milk drink
92610030 Horchata beverage, made with milk
92611100 Oatmeal beverage with milk
92613510 Cornmeal beverage with chocolate milk

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 10.6
[*L. rhamnosus* MP108] = 4.45 CFUx10^9/100 g

11830150 Cocoa powder, not reconstituted
11830160 Chocolate beverage powder, dry mix, not reconstituted
11830165 Chocolate beverage powder, light, dry mix, not reconstituted
11830260 Milk, malted, dry mix, not reconstituted
11830400 Strawberry beverage powder, dry mix, not reconstituted

Milk Shakes
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

11541400 Milk shake with malt
11542100 Milk shake, fast food, chocolate
11542200 Milk shake, fast food, flavors other than chocolate
11543000 Milk shake, bottled, chocolate
11543010 Milk shake, bottled, flavors other than chocolate

Milk-Based Meal Replacement, Nutrition, and Protein Beverages
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

95101000	Nutritional drink or shake, ready-to-drink (Boost)
95101010	Nutritional drink or shake, ready-to-drink (Boost Plus)
95102000	Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast)
95103000	Nutritional drink or shake, ready-to-drink (Ensure)
95103010	Nutritional drink or shake, ready-to-drink (Ensure Plus)
95104000	Nutritional drink or shake, ready-to-drink, sugar free (Glucerna)
95105000	Nutritional drink or shake, ready-to-drink (Kellogg's Special K Protein)
95106000	Nutritional drink or shake, ready-to-drink (Muscle Milk)
95106010	Nutritional drink or shake, ready-to-drink, light (Muscle Milk)
95110000	Nutritional drink or shake, ready-to-drink (Slim Fast)
95110010	Nutritional drink or shake, ready-to-drink, sugar free (Slim Fast)
95110020	Nutritional drink or shake, high protein, ready-to-drink (Slim Fast)
95120000	Nutritional drink or shake, ready-to-drink, NFS
95120010	Nutritional drink or shake, high protein, ready-to-drink, NFS
95120020	Nutritional drink or shake, high protein, light, ready-to-drink, NFS

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 6
[*L. rhamnosus* MP108] = 2.52 CFUx10^9/100 g

95220000	Nutritional powder mix, NFS
95220010	Nutritional powder mix, high protein, NFS

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 7
[*L. rhamnosus* MP108] = 2.94 CFUx10^9/100 g

95201200	Nutritional powder mix (EAS Whey Protein Powder)
95201500	Nutritional powder mix, high protein (Herbalife)
95201600	Nutritional powder mix (Isopure)
95201700	Nutritional powder mix (Kellogg's Special K20 Protein Water)
95230000	Nutritional powder mix, whey based, NFS
95230020	Nutritional powder mix, protein, light, NFS
95230030	Nutritional powder mix, protein, NFS

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 8
[*L. rhamnosus* MP108] = 3.36 CFUx10^9/100 g

95201000	Nutritional powder mix (Carnation Instant Breakfast)
95201010	Nutritional powder mix, sugar free (Carnation Instant Breakfast)
95202000	Nutritional powder mix (Muscle Milk)
95202010	Nutritional powder mix, light (Muscle Milk)

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 10
[*L. rhamnosus* MP108] = 4.20 CFUx10^9/100 g

95210000	Nutritional powder mix (Slim Fast)
95210010	Nutritional powder mix, sugar free (Slim Fast)
95210020	Nutritional powder mix, high protein (Slim Fast)

Plain or Flavored Yogurt
[*L. rhamnosus* MP108] = 0.59 CFUx10^9/100 g

11400000	Yogurt, NFS
11400010	Yogurt, Greek, NS as to type of milk or flavor
11410000	Yogurt, NS as to type of milk or flavor
11411010	Yogurt, NS as to type of milk, plain
11411100	Yogurt, whole milk, plain
11411200	Yogurt, low fat milk, plain
11411300	Yogurt, nonfat milk, plain
11411390	Yogurt, Greek, NS as to type of milk, plain
11411400	Yogurt, Greek, whole milk, plain
11411410	Yogurt, Greek, low fat milk, plain
11411420	Yogurt, Greek, nonfat milk, plain
11430000	Yogurt, NS as to type of milk, fruit
11431000	Yogurt, whole milk, fruit
11432000	Yogurt, low fat milk, fruit
11433000	Yogurt, nonfat milk, fruit
11433990	Yogurt, Greek, NS as to type of milk, fruit
11434000	Yogurt, Greek, whole milk, fruit
11434010	Yogurt, Greek, low fat milk, fruit
11434020	Yogurt, Greek, nonfat milk, fruit
11434090	Yogurt, NS as to type of milk, flavors other than fruit
11434100	Yogurt, whole milk, flavors other than fruit
11434200	Yogurt, low fat milk, flavors other than fruit
11434300	Yogurt, nonfat milk, flavors other than fruit
11435000	Yogurt, Greek, NS as to type of milk, flavors other than fruit

11435010 Yogurt, Greek, whole milk, flavors other than fruit
11435020 Yogurt, Greek, low fat milk, flavors other than fruit
11435030 Yogurt, Greek, nonfat milk, flavors other than fruit
11435100 Yogurt, Greek, with oats
11446000 Yogurt parfait, low fat, with fruit

Yogurt Drinks
[*L. rhamnosus* MP108] = 1.08 CFUx10^9/100 g

11436000 Yogurt, liquid

Plant Protein Products

Soy-Based Food
[*L. rhamnosus* MP108] = 1.18 CFUx10^9/100 g

41420010 Soybean curd
41420050 Soybean curd cheese
41421010 Soybean curd, deep fried
41421020 Soybean curd, breaded, fried
41425010 Vermicelli, made from soybeans
41810200 Bacon strip, meatless
41810250 Bacon bits
41810400 Breakfast link, pattie, or slice, meatless
41810600 Chicken, meatless, NFS
41810610 Chicken, meatless, breaded, fried
41811400 Frankfurter or hot dog, meatless
41811600 Luncheon slice, meatless-beef, chicken, salami or turkey
41811800 Meatball, meatless
41811890 Vegetarian burger or patty, meatless, no bun
41811950 Swiss steak, with gravy, meatless
41812000 Sandwich spread, meat substitute type
41812400 Vegetarian pot pie
41812450 Vegetarian chili, made with meat substitute
41812600 Vegetarian, fillet
41812800 Vegetarian stew
41812850 Vegetarian stroganoff
42203200 Soy nut butter

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 1.15
[*L. rhamnosus* MP108] = 1.36 CFUx10^9/100 g

41440000 Textured vegetable protein, dry

Mixed Foods Containing Soy-Based Food
Adjusted for non-dairy milk content of 6.49 to 48.72%
[*L. rhamnosus* MP108] = 0.08 to 0.57 CFUx10^9/100 g

27415120 Beef, tofu, and vegetables including carrots, broccoli, and/or dark-green leafy; no potatoes, soy-based sauce
27415220 Beef, tofu, and vegetables excluding carrots, broccoli, and dark-green leafy; no potatoes, soy-based sauce
27420100 Pork, tofu, and vegetables including carrots, broccoli, and/or dark-green leafy; no potatoes, soy-base sauce
27420370 Pork, tofu, and vegetables, excluding carrots, broccoli, and dark-green leafy; no potatoes, soy-based sauce
27450150 Fish, tofu, and vegetables, tempura
41812500 Tofu and vegetables including carrots, broccoli, and/or dark-green leafy; no potatoes, with soy-based sauce
41812510 Tofu and vegetables excluding carrots, broccoli, and dark-green leafy; no potatoes, with soy-based sauce
41901020 Soyburger, meatless, with cheese on bun
53390100 Pie, tofu with fruit

Processed Fruits and Fruit Juices

Fruit Drinks and Ades Including Smoothies
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

64134015 Fruit smoothie, with whole fruit, no dairy
64134020 Fruit smoothie, with whole fruit, no dairy, added protein
64134025 Fruit smoothie, with whole fruit, non-dairy
64134030 Fruit smoothie juice drink, no dairy
64134100 Fruit smoothie, light
64134200 Fruit smoothie, bottled
78101100 Fruit and vegetable smoothie, with dairy
78101110 Fruit and vegetable smoothie, added protein
78101115 Fruit and vegetable smoothie, non-dairy
78101118 Fruit and vegetable smoothie, non-dairy, added protein
78101120 Fruit and vegetable smoothie, bottled
78101125 Fruit and vegetable smoothie, no dairy
92510610 Fruit juice drink
92510650 Tamarind drink
92510720 Fruit punch, made with fruit juice and soda
92510730 Fruit punch, made with soda, fruit juice, and sherbet or ice cream
92510955 Lemonade, fruit juice drink
92510960 Lemonade, fruit flavored drink

92511015 Fruit flavored drink
92511250 Fruit juice beverage, 40-50% juice, citrus
92512050 Frozen daiquiri mix, from frozen concentrate, reconstituted
92512090 Pina Colada, nonalcoholic
92512110 Margarita mix, nonalcoholic
92513000 Slush frozen drink
92513010 Slush frozen drink, no sugar added
92530410 Fruit flavored drink, with high vitamin C
92530510 Cranberry juice drink, with high vitamin C
92530610 Fruit juice drink, with high vitamin C
92530950 Vegetable and fruit juice drink, with high vitamin C
92531030 Fruit juice drink (Sunny D)
92541010 Fruit flavored drink, powdered, reconstituted
92542000 Fruit flavored drink, with high vitamin C, powdered, reconstituted
92550030 Fruit juice drink, with high vitamin C, light
92550035 Fruit juice drink, light
92550040 Fruit juice drink, diet
92550110 Cranberry juice drink, with high vitamin C, light
92550200 Grape juice drink, light
92550350 Orange juice beverage, 40-50% juice, light
92550360 Apple juice beverage, 40-50% juice, light
92550370 Lemonade, fruit juice drink, light
92550380 Pomegranate juice beverage, 40-50% juice, light
92550400 Vegetable and fruit juice drink, with high vitamin C, diet
92550405 Vegetable and fruit juice drink, with high vitamin C, light
92550610 Fruit flavored drink, with high vitamin C, diet
92550620 Fruit flavored drink, diet
92552000 Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
92552010 Fruit flavored drink, powdered, reconstituted, diet
92552020 Fruit juice drink, reduced sugar (Sunny D)
92552030 Fruit juice drink (Capri Sun)
92582100 Fruit juice drink, with high vitamin C, plus added calcium
92582110 Fruit juice drink, added calcium (Sunny D)
95342000 Fruit juice, acai blend

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 4
[*L. rhamnosus* MP108] = 1.68 CFUx10^9/100 g

92511000 Lemonade, frozen concentrate, not reconstituted
92512040 Frozen daiquiri mix, frozen concentrate, not reconstituted

Foods Adjusted for Being Present in Dried Form

Reconstitution factor of 10.23

[*L. rhamnosus* MP108] = 4.30 CFUx10^9/100 g

92900100	Fruit flavored drink, with high vitamin C, powdered, not reconstituted
92900110	Fruit flavored drink, powdered, not reconstituted
92900200	Fruit flavored drink, powdered, not reconstituted, diet

Fruit Juices

[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

61201020	Grapefruit juice, 100%, NS as to form
61201220	Grapefruit juice, 100%, canned, bottled or in a carton
61201225	Grapefruit juice, 100%, with calcium added
61201620	Grapefruit juice,100%, frozen, reconstituted
61204000	Lemon juice, 100%, NS as to form
61204200	Lemon juice, 100%, canned or bottled
61207000	Lime juice, 100%, NS as to form
61207200	Lime juice, 100%, canned or bottled
61210000	Orange juice, 100%, NFS
61210220	Orange juice, 100%, canned, bottled or in a carton
61210250	Orange juice, 100%, with calcium added, canned, bottled or in a carton
61210620	Orange juice, 100%, frozen, reconstituted
61210820	Orange juice, 100%, with calcium added, frozen, reconstituted
61213220	Tangerine juice, 100%
61213800	Fruit juice blend, citrus, 100% juice
61213900	Fruit juice blend, citrus, 100% juice, with calcium added
64100100	Fruit juice, NFS
64100110	Fruit juice blend, 100% juice
64100200	Cranberry juice blend, 100% juice
64100220	Cranberry juice blend, 100% juice, with calcium added
64101010	Apple cider
64104010	Apple juice, 100%
64104030	Apple juice, 100%, with calcium added
64104600	Blackberry juice, 100%
64104610	Blueberry juice
64105400	Cranberry juice, 100%, not a blend
64116020	Grape juice, 100%
64116060	Grape juice, 100%, with calcium added
64120010	Papaya juice, 100%
64121000	Passion fruit juice, 100%
64124020	Pineapple juice, 100%
64126000	Pomegranate juice, 100%
64132010	Prune juice, 100%

64132500 Strawberry juice, 100%
64133100 Watermelon juice, 100%
78101000 Vegetable and fruit juice, 100% juice, with high vitamin C

Foods adjusted for being present in dried form
Reconstitution factor of 4
[*L. rhamnosus* MP108] = 1.68 CFUx10^9/100 g

61210720 Orange juice, 100%, frozen, not reconstituted

Fruit Nectars
[*L. rhamnosus* MP108] = 0.42 CFUx10^9/100 g

64200100 Fruit nectar, NFS
64201010 Apricot nectar
64201500 Banana nectar
64202010 Cantaloupe nectar
64203020 Guava nectar
64204010 Mango nectar
64205010 Peach nectar
64210010 Papaya nectar
64213010 Passion fruit nectar
64215010 Pear nectar
64221010 Soursop, nectar

Soft Candy

Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees
[*L. rhamnosus* MP108] = 3.33 CFUx10^9/100 g

44201000 Carob chips
91700010 Candy, NFS
91700500 M&M's Almond Chocolate Candies
91701010 Almonds, chocolate covered
91701020 Almonds, sugar-coated
91701030 Almonds, yogurt-covered
91702010 Butterscotch morsels
91703010 Caramel, chocolate-flavored roll
91703020 Caramel, flavor other than chocolate
91703030 Caramel, with nuts
91703040 Caramel candy, chocolate covered
91703050 Caramel with nuts and cereal, chocolate covered
91703060 Caramel with nuts, chocolate covered

91703070	Rolo
91703080	Caramel, all flavors, sugar free
91703150	Toblerone, milk chocolate with honey and almond nougat
91703200	TWIX Caramel Cookie Bars
91703250	TWIX Chocolate Fudge Cookie Bars
91703300	TWIX Peanut Butter Cookie Bars
91703400	Whatchamacallit
91703500	Nuts, carob-coated
91703600	Espresso coffee beans, chocolate-covered
91705010	Milk chocolate candy, plain
91705020	Milk chocolate candy, with cereal
91705030	Kit Kat
91705040	Chocolate, milk, with nuts, not almond or peanuts
91705050	Milk chocolate candy, with fruit and nuts
91705060	Milk chocolate candy, with almonds
91705070	Chocolate, milk, with peanuts
91705090	Chocolate candy with fondant and caramel
91705200	Chocolate, semi-sweet morsel
91705300	Chocolate, sweet or dark
91705310	Chocolate, sweet or dark, with almonds
91705400	Chocolate, white
91705410	Chocolate, white, with almonds
91705420	Chocolate, white, with cereal
91705430	Kit Kat White
91705500	Mexican chocolate, tablet
91706000	Coconut candy, chocolate covered
91706100	Coconut candy, no chocolate covering
91706400	Coconut candy, Puerto Rican style
91707000	Fondant
91707010	Fondant, chocolate covered
91708000	Fruit peel, candied
91708010	Date candy
91708020	Soft fruit confections
91708030	Fruit leather and fruit snacks candy
91708040	Fun Fruits Creme Supremes
91708070	Tamarind candy
91708100	Fruit snacks candy, with high vitamin C
91708150	Yogurt covered fruit snacks candy, with added vitamin C
91708160	Yogurt covered fruit snacks candy rolls, with high vitamin C
91709000	Gumdrops, chocolate covered
91713010	Fudge, chocolate, chocolate-coated
91713020	Fudge, chocolate, chocolate-coated, with nuts
91713030	Fudge, chocolate

91713040	Fudge, chocolate, with nuts
91713050	Fudge, peanut butter
91713060	Fudge, peanut butter, with nuts
91713070	Fudge, vanilla
91713080	Fudge, vanilla, with nuts
91713090	Fudge, divinity
91713100	Fudge, brown sugar, penuche
91715000	Fudge, caramel and nut, chocolate-coated candy
91715100	SNICKERS Bar
91715200	Baby Ruth
91715300	100 GRAND Bar
91716010	Halvah, plain
91716110	Halvah, chocolate covered
91718100	Butterfinger
91718110	Butterfinger Crisp
91718200	Chocolate-flavored sprinkles
91718300	Ladoo, round ball, Asian-Indian dessert
91721000	Licorice
91723000	Marshmallow
91723010	Marshmallow, chocolate covered
91723020	Marshmallow, candy-coated
91726000	Nougat, plain
91726110	Nougat, with caramel, chocolate covered
91726130	MILKY WAY Bar
91726140	MILKY WAY MIDNIGHT Bar
91726150	MARS Almond Bar
91726410	Nougat, chocolate covered
91726420	3 MUSKETEERS Bar
91726425	3 Musketeers Truffle Crisp Bar
91727010	Nuts, chocolate covered, not almonds or peanuts
91728000	Nut roll, fudge or nougat, caramel and nuts
91728500	Sugared pecans, sugar and egg white coating
91731000	Peanuts, chocolate covered
91731010	M&M's Peanut Chocolate Candies
91731060	M&M's Peanut Butter Chocolate Candies
91731100	Peanuts, sugar-coated
91731150	Peanuts, yogurt covered
91732000	Peanut bar
91732100	Planters Peanut Bar
91733000	Peanut brittle
91733200	Peanut Bar, chocolate covered candy
91734000	Peanut butter, chocolate covered
91734100	Reese's Peanut Butter Cup

91734200	Reese's Pieces
91734300	Reese's Sticks
91734400	Reese's Fast Break
91734450	Reese's Crispy Crunchy Bar
91734500	Peanut butter morsels
91735000	Pralines
91736000	Pineapple candy, Puerto Rican style
91739010	Raisins, chocolate covered
91739600	Raisins, yogurt covered
91742010	Sesame Crunch, Sahadi
91745010	Gumdrops
91745100	Skittles
91746010	Sugar-coated chocolate discs
91746100	M&M's Milk Chocolate Candies
91746120	Sixlets
91746150	Easter egg, candy coated chocolate
91746200	M&M's Pretzel Chocolate Candies
91750000	Taffy
91760000	Toffee, plain
91760100	Toffee, chocolate covered
91760200	Toffee, chocolate-coated, with nuts
91760500	Truffles
91760700	Wax candy, liquid filled
91770000	Dietetic or low calorie candy, NFS
91770010	Dietetic or low calorie gumdrops
91770030	Dietetic or low calorie candy, chocolate covered
91770050	Dietetic or low calorie mints

Other – Baby Food

Cereals, Dry Instant
[*L. rhamnosus* MP108] = 6.67 CFUx10^9/100 g

Foods Adjusted for Being Present in Dried Form
Reconstitution factor of 8.33
[*L. rhamnosus* MP108] = 55.56 CFUx10^9/100 g

57801000	Barley cereal, baby food, dry, instant
57803000	Mixed cereal, baby food, dry, instant
57804000	Oatmeal cereal, baby food, dry, instant
57805000	Rice cereal, baby food, dry, instant
57805080	Rice cereal with apples, baby food, dry, instant
57805090	Rice cereal with mixed fruits, baby food, dry, instant

57805100 Rice cereal with bananas, baby food, dry, instant
57805500 Brown rice cereal, baby food, dry, instant
57806000 Mixed cereal with bananas, baby food, dry, instant
57806050 Multigrain, whole grain cereal, baby food, dry, instant
57806100 Oatmeal cereal with bananas, baby food, dry, instant
57806200 Oatmeal cereal with fruit, baby food, dry, instant, toddler
57807010 Whole wheat cereal with apples, baby food, dry, instant

Cereals, Prepared, Ready-to-Serve

[*L. rhamnosus* MP108] = 0.91 CFUx10^9/100 g

56210000 Cereal, nestum
57820000 Cereal, baby food, jarred, NFS
57820100 Rice cereal, baby food, jarred, NFS
57822000 Mixed cereal with applesauce and bananas, baby food, jarred
57823000 Oatmeal with applesauce and bananas, baby food, jarred
57824000 Rice cereal with applesauce and bananas, baby food, jarred
57824500 Rice cereal with mixed fruit, baby food, jarred

RTE Cereals

[*L. rhamnosus* MP108] = 1.00 CFUx10^9/100 g

57830100 Gerber Graduates Finger Snacks Cereal, baby food

Fruits or Vegetables (strained)

[*L. rhamnosus* MP108] = 0.80 CFUx10^9/100 g

67100200 Tropical fruit medley, baby food, strained
67101000 Apple-raspberry, baby food, NS as to strained or junior
67101010 Apple-raspberry, baby food, strained
67102000 Applesauce, baby food, NS as to strained or junior
67102010 Applesauce, baby food, strained
67104000 Applesauce and apricots, baby food, NS as to strained or junior
67104010 Applesauce and apricots, baby food, strained
67104030 Applesauce with bananas, baby food, NS as to strained or junior
67104040 Applesauce with bananas, baby food, strained
67104070 Applesauce with cherries, baby food, strained
67104090 Applesauce with cherries, baby food, NS as to strained or junior
67105030 Bananas, baby food, strained
67106010 Bananas with apples and pears, baby food, strained
67106030 Bananas with orange, baby food, strained
67106050 Banana with mixed berries, baby food, strained
67108000 Peaches, baby food, NS as to strained or junior
67108010 Peaches, baby food, strained

67109000 Pears, baby food, NS as to strained or junior
67109010 Pears, baby food, strained
67110000 Prunes, baby food, strained
67113000 Apples and pears, baby food, NS as to strained or junior
67113010 Apples and pears, baby food, strained
67114000 Pears and pineapple, baby food, NS as to strained or junior
67114010 Pears and pineapple, baby food, strained
67304000 Plums, baby food, NS as to strained or junior
67304010 Plums, baby food, strained
67304030 Plums, bananas, and rice, baby food strained
67304500 Prunes with oatmeal, baby food, strained
67307000 Apricots, baby food, NS as to strained or junior
67307010 Apricots, baby food, strained
67308000 Bananas, baby food, NS as to strained or junior
67309000 Bananas and pineapple, baby food, NS as to strained or junior
67309010 Bananas and pineapple, baby food, strained
67600100 Apples and sweet potatoes, baby food, strained
76102010 Spinach, creamed, baby food, strained
76201000 Carrots, baby food, NS as to strained or junior
76201010 Carrots, baby food, strained
76202000 Carrots and peas, baby food, strained
76205010 Squash, baby food, strained
76205030 Squash and corn, baby food, strained
76205060 Corn and sweet potatoes, baby food, strained
76209010 Sweet potatoes, baby food, strained
76401000 Beans, green string, baby food, NS as to strained or junior
76401010 Beans, green string, baby food, strained
76402000 Green beans and potatoes, baby food, strained
76403010 Beets, baby food, strained
76405000 Corn, creamed, baby food, NS as to strained or junior
76405010 Corn, creamed, baby food, strained
76407000 Mixed vegetables, garden vegetables, baby food, NS as to strained or junior
76407010 Mixed vegetables, garden vegetables, baby food, strained
76409000 Peas, baby food, NS as to strained or junior
76409010 Peas, baby food, strained
76501000 Vegetables and rice, baby food, strained
76602000 Carrots and beef, baby food, strained
76603000 Vegetable and beef, baby food, NS as to strained or junior
76603010 Vegetable and beef, baby food, strained
76604000 Broccoli and chicken, baby food, strained
76604500 Sweet potatoes and chicken, baby food, strained
76605000 Vegetable and chicken, baby food, NS as to strained or junior
76605010 Vegetable and chicken, baby food, strained

76611000 Vegetable and turkey, baby food, NS as to strained or junior
76611010 Vegetable and turkey, baby food, strained

Fruit Juice

[*L. rhamnosus* MP108] = 0.83 CFUx10^9/100 g

67202000 Apple juice, baby food
67202010 Apple juice, with added calcium, baby food
67203000 Apple-fruit juice blend, baby food
67203200 Apple-banana juice, baby food
67203400 Apple-cherry juice, baby food
67203500 Apple-grape juice, baby food
67203600 Apple-peach juice, baby food
67203700 Apple-prune juice, baby food
67203800 Grape juice, baby food
67204000 Mixed fruit juice, not citrus, baby food
67204100 Mixed fruit juice, not citrus, with added calcium, baby food
67205000 Orange juice, baby food
67211000 Orange-apple-banana juice, baby food
67212000 Pear juice, baby food
67230000 Apple-sweet potato juice, baby food
67230500 Orange-carrot juice, baby food
67250100 Banana juice with lowfat yogurt, baby food
67250150 Mixed fruit juice with lowfat yogurt, baby food
67260000 Fruit juice and water drink, with high vitamin C and added calcium, baby food

APPENDIX B
Strain Characterization, Antibiotic Resistance, and Virulence Factor Data

Bacteria Genome Sequencing Report (Complete Genome)

2019/9/3

Table of Contents

Results

1 Project Summary

Project Info

Table 1 Project Info (Download)

Itmes	Description
Project Code	F19FTSCCKF0831_GERethD
Project Type	CompleteGenome
Species	Bacteria
Sample Number	1

Analytic Statistics

Table 2 Analytic Statistics A (Download)

Sample Name (#)	Illumina Data (Mb)	PacBio Data (Mb)	Contig Number (#)	Genome Size (bp)	Species Name (#)
MP108	888(303X)	2,061(704X)	1	2,925,062	Lactobacillus rhamnosus

Table 3 Analytic Statistics B (Download)

Sample Name (#)	Gene Number (#)	ncRNA Number (#)	Repeat Number (#)	Annotation Number (#)
MP108	2,884	80	145	2,870 (99.51%)

Note:X represents the sequencing multiplier,the amount of sequencing reads divided by the size of the genome;Species Name indicates the species of the sequenced strain, which is obtained through the Nt database comparison.

2 Technology Introduction

2.1 Product Description

Bacterial genome de novo is a de novo assembly of the bacterial genome after sequencing, genome components Analysis,functional annotation and genome comparison are included as well. The final assembly level according to the needs of the study and the characteristics of the bacteria itself. This product can be divided into primary assembly, advanced assembly and complete assembly map. One of the highest indicators is complete assembly map, which assembled the complete genome of the bacterial genome sequence (including chromosome and plasmid sequence information). Bacterial *De novo sequencing* has replaced traditional methods as an important tool for studying the genetic mechanisms of bacterial evolution, key functional genes. It can be used to identify the pathogenicity-related genes of pathogenic bacteria, study on the evolutionary relationships within species, engineering bacteria transformation, genetics theory and model studies.

2.2 Experiment Introduction

Illumina Platform

Genomic DNA is extracted and fragmented randomly and then required length DNA fragments are retained by electrophoresis. And after this, we ligate adapters to DNA fragments then conduct cluster preparation, sequencing finally. The library preparation

method and sequencing pipeline is shown below.

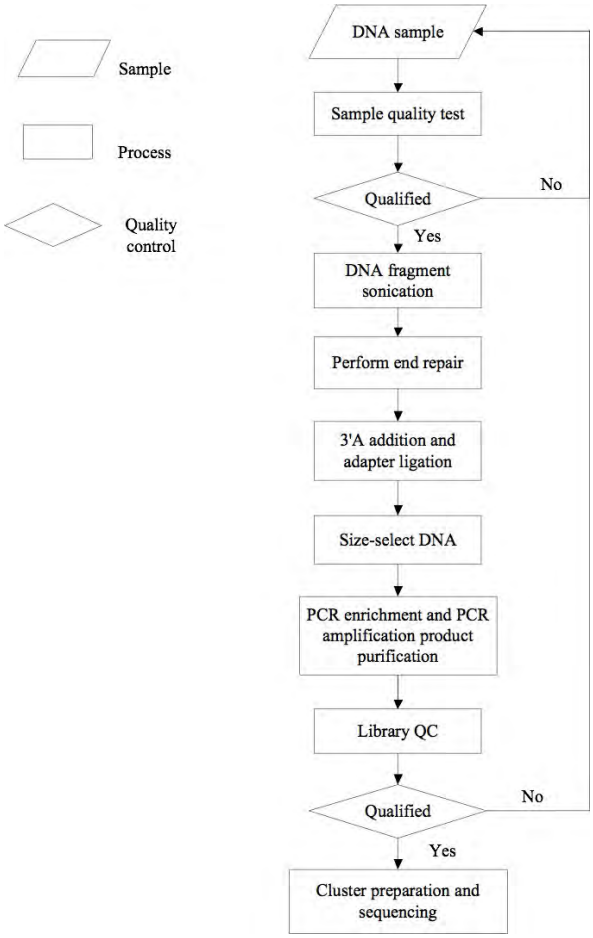


Figure 1 Pipeline of Experiment.

After the DNA sample(s) was(were) delivered, we did a sample quality test first. Then we used this(those) qualified DNA sample(s) to construct BS library： Purified DNA sample, such as genomic DNA, Bacterial Artificial Chromosome or long-length PCR productions, is sheared into smaller fragments with a desired size by Covaris S/E210 or Bioruptor firstly. Then the overhangs resulting from fragmentation are converted into blunt ends by using T4 DNA polymerase, Klenow Fragment and T4 Polynucleotide Kinase. After adding an 'A' base to the 3' end of the blunt phosphorylated DNA fragments, adapters are ligated to the ends of the DNA fragments. The desired fragments can be purified though gel-electrophoresis, then selectively enriched and amplified by PCR. The index tag could be introduced into the adapter at the PCR stage as appropriate and we did a library quality test. At last, the qualified BS library would be used for sequencing.

PacBio Platform

Each step of the experiment (such as sample testing, library construction, sequencing, etc.) may affect the quality and quantity of the data, and thus directly affect the information analysis results. In order to get highly reliable sequencing data, we conducted rigorous quality control at each step of the experiment. Library preparation methods and sequencing process as shown below:

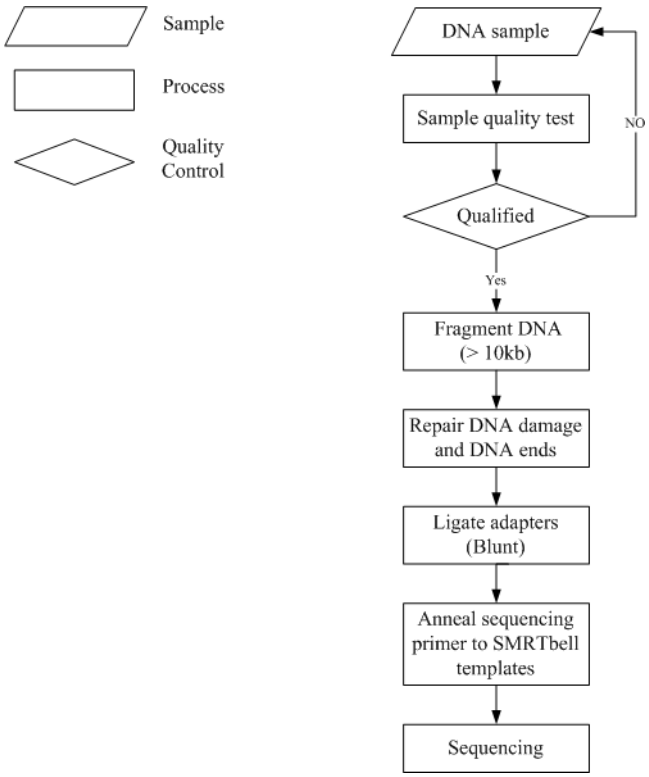


Figure 2 Pipeline of Experiment.

The DNA fragments were first treated with g-TUBE to the appropriate size, then the fragment ends was repaired, and both ends of the DNA fragment were ligated to the connector of the hairpin structure to form a dumbbell structure called SMRTbell. The annealed smrtbell is mixed with the polymerase on the bottom of the ZWM, which will be used for the final sequencing.

2.3 Pipeline of Bioinformatics Analysis

Bioinformatics analysis will be proceeding after data filtering. The content of bioinformatics analysis pipeline is shown below.

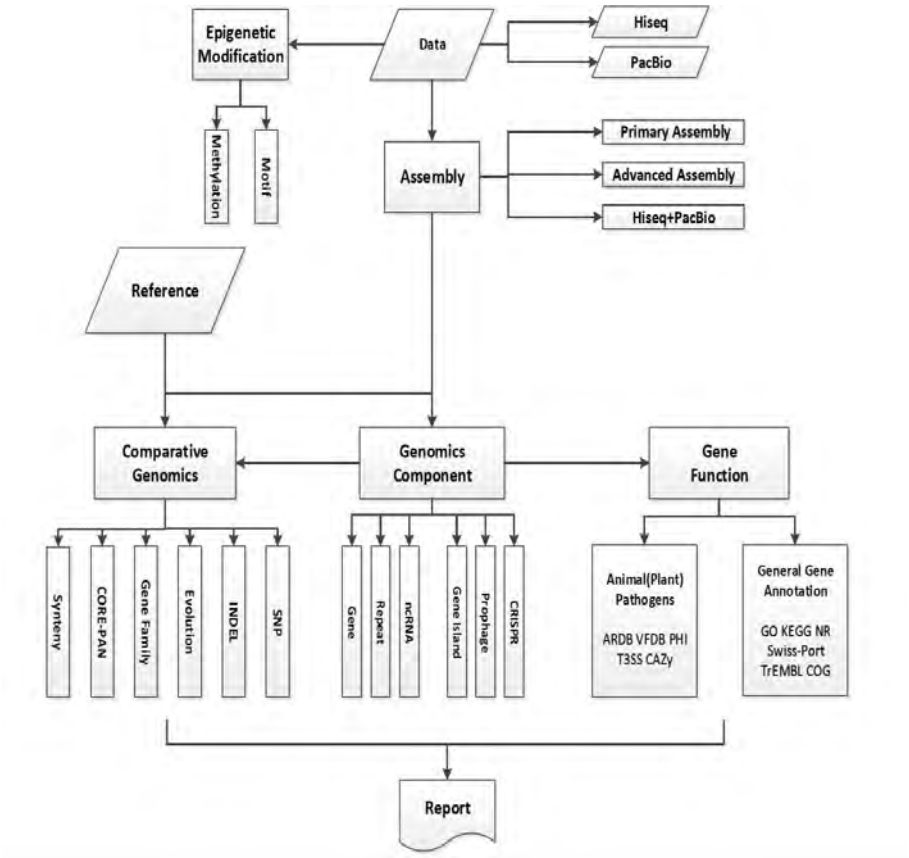


Figure 3 Bioinformatics analysis flow.

The overall analysis can be divided into seven modules:(1) Data filtering: The raw data is filtered and generate clean data; (2) Assembly: Assemble the reads after filtering into genome and assess the assembly; (3) Genomic component analysis, including: (a) Analysis on repeat sequences which includes tandem repeats sequence, minisatellite DNA and microsatellite DNA; (b) CRISPER prediction; (c) Non-coding RNA prediction. Non-coding RNA includes rRNA, tRNA and sRNA; (d) Genomic islands (GIs) prediction; (e) Prophage prediction; (f) Gene prediction; (4) Analysis on gene function, including: (a) Gene annotation: The predicted ORFs are annotated by GO, KEGG, Swiss-Prot(default), NR database and COG database respectively; (b) Analysis on animal pathogens including: T3SS effector protein, PHI, VFDB, ARDB database annotation; (c) Pathogenicity analysis on plant pathogens, including: T3SS effector protein, CAZy, PHI; (5) Comparative genomic, including: (a) detecte SNP and annotation; (b) detecte InDel and annotation; (c) Structural Variation (Synteny); (d) Core-pan gene analysis; (e) Evolution analysis: construction of phylogenetic tree and ka/ks analysis; (f) Gene family analysis; (6) DNA methylation: Based on the three generations of sequencing data, the methylation modification sites and motif sequences in the sequencing genome were analyzed to explore the epigenetic phenomenon of bacterial genes.(7)Report accomplishment.

3 Data Summary

3.1 Illumina Data

There exists a certain amount of low quality data in raw data. In order to obtain more accurate and reliable results in subsequent bioinformatics analysis, the raw data will be treated. Statistics results as follow:

Table 4 Illumina Statistics (Download)

Sample Name (#)	Insert Size (bp)	Reads Length (bp)	Raw Data (Mb)	Adapter (%)	Duplication (%)	Total Reads (#)	Filtered Reads (%)	Low Quality Filtered Reads (%)	Clean Data (Mb)
MP108	500	(125:125)	1,211	0.56	3.53	8,078,910	11.97	7.83	888

Note: Insert Size,the length of inserted fragment;Reads Length，length of reads;Raw Data，the size of raw data;Adapter，The proportion of Adapter;Duplicaiton，The proportion of same reads;Total Reads，total reads number;Filtered Reads，The proportion of filtered reads;Low Quality Filtered Reads,The proportion of Low quality filtered reads;Clean Data，the size of reads we delivered.

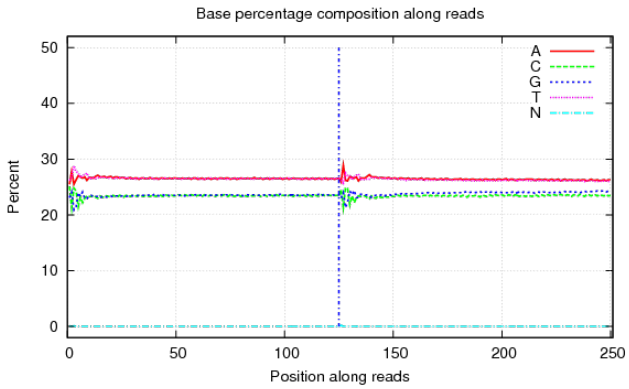


Figure 4 Base distribution.

The X-axis shows the positions of bases in read1 and read2. When the base composition is balanced, the A and T curves overlap and the G and C curves overlap.

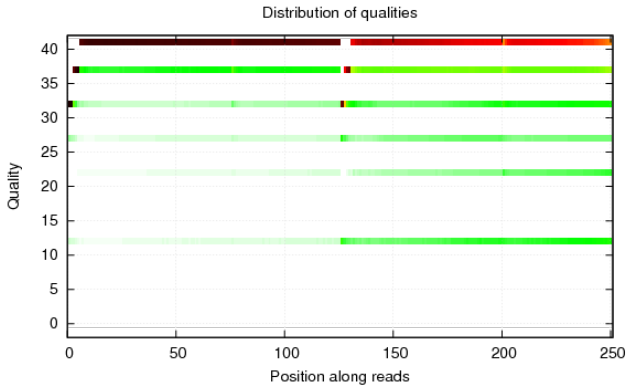


Figure 5 Quality distribution.

The X-axis shows the positions of bases in read1 and read2,the Y-axis shows the quality value of each base.Each point in the graph represents the base quality value of the corresponding position in a certain read.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/1.Cleandata:

|-- SampleName.Illumina_Cleandata.xls [Statistics of Illumina filtering]

|-- SampleName.ISInserSize_Clean.1.fq.gz [Illumina reads1 compressed file in fastq format]

|-- SampleName.ISInserSize_Clean.2.fq.gz [Illumina reads2 compressed file in fastq format]

|-- SampleName.ISInserSize_Clean.base.png [Filtered Illumina reads GC distribution]

|-- SampleName.ISInserSize_Clean.qual.png [Filtered Illumina reads quality distribution]

|-- SampleName.ISInserSize_Raw.base.png [Raw Illumina reads GC distribution]

|-- SampleName.ISInserSize_Raw.qual.png [Raw Illumina reads quality distribution]

Note: InsertSize represents the size of the library. The Illumina data saved as fastq format generally,see fastq decription at "Help->Data Format".Please don't view the fastq file directly under Windows.If you want to update your reads to the NCBI,just submit the two file *Clean.*.fq.gz.

3.2 PacBio Data

There exists a certain amount of low quality data and adapter sequence in ***Polymerase Reads*** when sequencing on Pacbio plantform.In order to obtain more accurate and reliable results in subsequent bioinformatics analysis, the raw data will be treated. Statistics results as follow:

Table 5 PacBio Reads Statistics (Download)

Sample Name (#)	Valid ZWM Number (#)	Subreads Number (#)	Subreads Total Bases (bp)	Subreads Mean Length (bp)	Subreads N50 (bp)	Subreads N90 (bp)	Subreads Max Length (bp)	Subreads Min Length (bp)
MP108	35,853	287,466	2,061,327,683	7,170	8,646	4,588	106,960	1,000

Note: Valid ZWM Number,the number of valid ZMWs; Subreads Number,the number of Subreads after filtering; Subreads Total Bases, data size of all Subreads; Subreads Mean Length,The average length of Subreads.

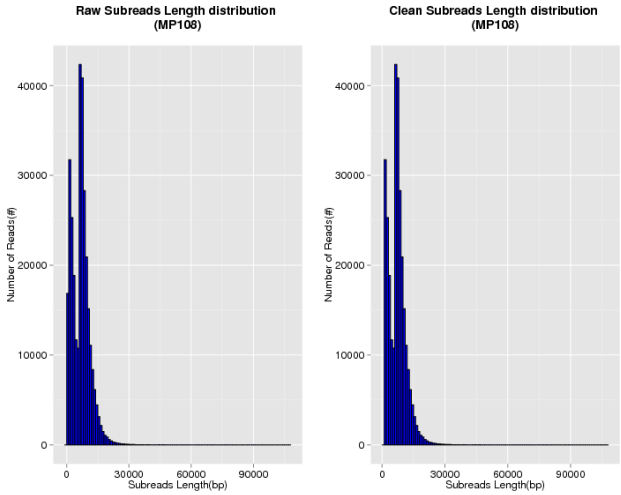


Figure 6 Subreads Length Distribution.

The left figure shows the length distribution of Subreads before filtering,the right figure shows the length distribution of Subreads after filtering. The X-axis shows the length of Subreads,and the Y-axis shows the number of Subreads.

4 Assembly Summary

4.1 Genome Estimation

Before assembling, we used ***K-mer*** analysis to estimate the size of genome (the assemble result was the real genome size), the degree of heterozygosis and the degree of duplication. The detail information was shown in figure below.

Table 6 k-mer Statistics (Download)

Sample_name	K mer(#)	Kmer Num(Mbp)	Pk_Depth(#)	Genome Size(Mbp)	Genome Depth(#)
MP108	15	147.80	45	3.27	50.85

Note: K-mer,the kmer value set; Kmer_Num,number of all kmer; PK_Depth,the depth of kmer peak; Genome_Size,the estimated genome size.

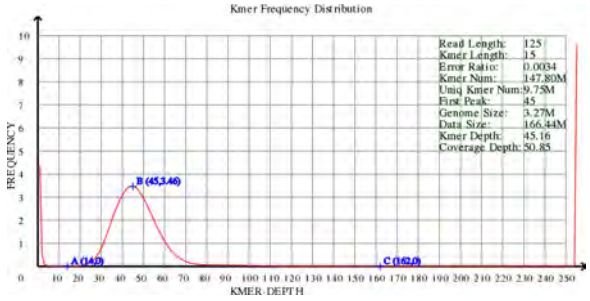


Figure 7 15-mer analysis on sample.

X-coordinate is depth, and Y-coordinate is proportion. Regardless of the sequencing error, genome heterozygosis and duplication, 15-mer distribution should follow the Poisson distribution. However, low-depth k-mer takes up high proportion due to sequencing error actually. Sometimes due to heterozygosis, other peak may appear at the 1/2 of the main peak, while due to duplication, repeating peaks may appear near the integer times of the main peak.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/2.Assembly:

|-- SampleName.kmer.png [Figure of kmer analysis]

|-- SampleName.kmer.stat.xls [Statistics of kmer results]

4.2 Assembly

Based on the valid data from the previous sequencing platform, the CleanData was assembled for each sample, and the optimal assembly results were obtained after multiple adjustments. Then, the assembly sequence was analyzed by correcting single base, circular judgment and plasmid comparison. The results of genome assembly statistics of each sample in the table below:

Table 7 AssemblyStat (Download)

Sample Name	ID Name	Sequence Type(#)	Sequence Topology	Sequence Number(#)	Total Length (bp)	GC Content (%)
MP108	Chromosome1	Chromosome	circular	1	2,925,062	46.76
	All	All	-	1	2,925,062	46.76

Note: Sequence Type, chromosome or plasmid;Sequence Topology, circular or linear.

Result DIR:

BGI_result/Separate/SampleName/2.Assembly:

|-- SampleName.Complete.Assembly.stat.xls [Statistics of assemble results]

|-- SampleName.Complete.genome.fasta [Assembly result]

|-- SampleName.genome.gb [Genome infomation in genebank format]

|-- SampleName.genome.tbl [Genome infomation in tbl format]

|-- SampleName.CorrectRate.stat.xls [Statistics of CorrectSingleBase]

Note: Please view the files with TextEditer such as NotePad++,UEditor except file end with xls.

4.3 GC-Depth

Based on the NGS data, GC-Depth analysis was performed on the assembly results to show the GC content and depth distribution of the samples, so as to roughly determine whether the samples are contaminated, whether the sequencing is random, and so on. The analysis results are as follows.

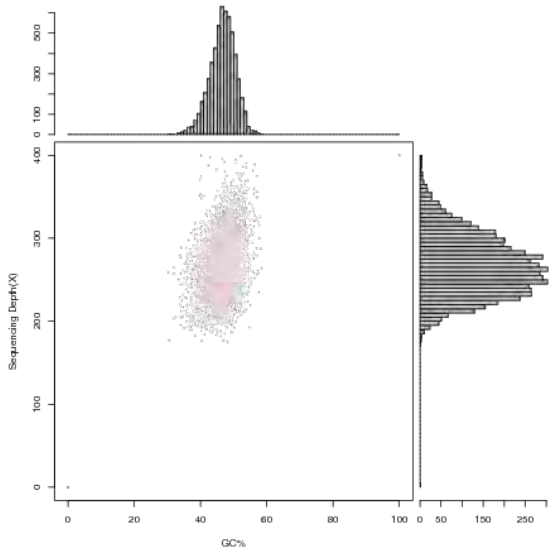


Figure 8 GC content and Depth correlative analysis.

X-coordinate is GC content, and Y-coordinate is average depth. Through calculating GC content and average depth with 500 bp as a window, we can analyze whether GC bias exists. If not seriously biased, this scatter diagram takes on the shape similar to Poisson distribution, there will be a peak near the GC content of the genome, and the more deviation from it, the lower the depth is.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/2.Assembly:

|-- SampleName.coverage_depth.table.xls [Statistics of coverage rate]

|-- SampleName.GC-depth.png [Figure of GC and depth]

4.4 Nt Database Comparison

The alignment of the assembly results with the Nt database can roughly infer the species of the sequenced strain. The accuracy of the inference depends on the integrity of the Nt database. The more complete the Nt database, the more accurate and detailed it is. The comparison results are as follows.

Table 8 Nt Statistics (Download)

Sample Name	TaxID	Organism	Cover_Len(bp)	Scaffolds_Len	Coverage(%)	Genomics(%)	Scaffold_Num
MP108	47715	Lactobacillus rhamnosus	2918774	2925062	99.79	100.00	1

Note:TaxID,taxonomy id; Organism,species name; Cover_Len,total covered length of alignment; Scaffolds_Len,the length of scaffolds which covered with Nt database; Coverage,the percentage of covered length in scaffold; In Genomics,the percentage of length of covered scaffold in total assembly result; Scaffold_Num,the number of scaffolds which covered with Nt database.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/NtBlast:

|-- SampleName.max_tax_organism [The best matched species]

|-- SampleName.nt_blast. *Scaffold* .cover.xls [Statistics of each macthed scaffolds]

|-- SampleName.organism.cover.xls [Statistics of each macthed species]

|-- SampleName.tax_organism.cover.xls [Statistics of each macthed taxon]

Note: Please view the files with TextEditer such as NotePad++,UEditor except file end with xls.

5 Genome Component

After getting a genomic sequence, analysis for the distribution of functional elements is necessary to study the characteristics of the strain, functional areas, mutation, strain evolution and so on. Although the microbial genome is relatively small, its various functional elements are abundant and can occupy more than 90% of the genome sequence which is coding regions for coding functional genes. Besides, there are also various non-coding regions that participate in expression regulation and apparent modification.

5.1 Gene

For finding out gene composition, gene prediction was applied. The statistics is in the table below :

Table 9 Gene Stat (Download)

Sample Name (#)	Genome Size (#)	Total Number (#)	Total Length (bp)	Average Length (#)	Length / Genome Length (%)	GC Content (%)
MP108	2,925,062	2,884	2,501,868	867.50	85.53	47.47

Note: Total Number,the count of genes; Total Length,total length of all genes; Average Length,average length of all genes; GC Content,the content of G and C in gene; Length/Genome Length, The proportion of gene length in genome.

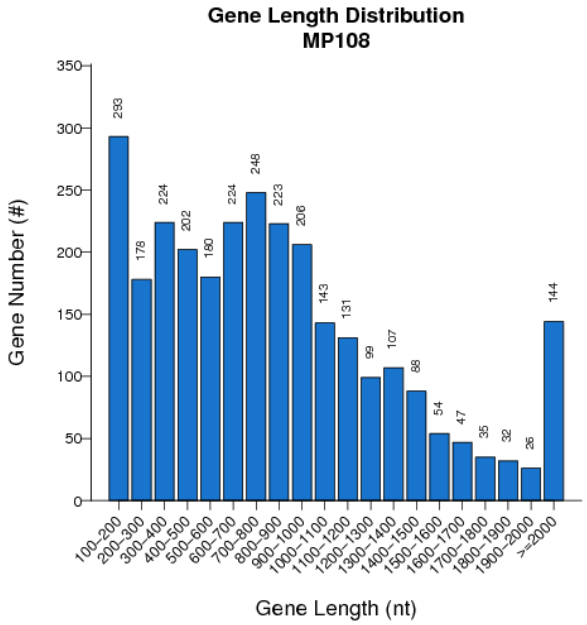


Figure 9 Gene Length Distribution.

The X-axias shows the length of gene,and the Y-axias shows the number of gene.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/3.Genome_Component/Gene_Predict:

|-- SampleName.Gene.cds.fasta [Predicted genes in CDS format]

|-- SampleName.Gene.pep.fasta [Protein sequences of predicted genes

|-- SampleName.Gene.gff [Predicted genes in GFF3format]

|-- SampleName.Gene.cds.png [Figure of Gene length distribution]

|-- SampleName.Gene.stat.xls [Statistics of predict genes]

Note: Please view the files with TextEditer such as NotePad++,UEditor except file end with xls.

5.2 Non-coding RNA

Non-coding RNA (ncRNA) widely exists in bacteria, archaea and eukaryote. They carry out many biological functions but not code proteins. NcRNA contains sRNA, rRNA, tRNA, snRNA and miRNA etc.

sRNA:sRNA exist in bacteria, archaea and eukaryote but it was mainly discovered in bacteria. We generally call those ncRNA with length between 50 nt and 500 nt small RNA(sRNA).

rRNA : Ribosome RNA. In eukaryote, rRNA contains 5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA.

miRNA: MicroRNA (miRNA) is a kind of endogenous non-coding RNA with regulatory function found in eukaryotes. Its precursor is about 90bp and the size of the mature miRNA is about 20 to 25 nucleotides. miRNA exists in eukaryotes and it may have a regulatory role in gene expression.

snRNA:(small nuclearRNA). It is the main component of eukaryotic RNA splicing produced in post-transcriptional processing.

The statistics of non-coding RNA in the under table:

Table 10 ncRNA Stat (Download)

Sample Name (#)	Type (#)	Copy Number (#)	Average Length (bp)	Total Length (#)	% in Genome
MP108	tRNA	59	75.67	4,465	0.1526
	5s_rRNA (Denovo)	5	116	580	0.0198
	16s_rRNA (Denovo)	5	1,558	7790	0.2663
	23s_rRNA (Denovo)	5	2,916	14580	0.4984
	sRNA	6	154	924	0.0316

Note: Type,the type of ncRNA; % in genome,the proportion of length of ncRNA in genome.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/3.Genome_Component/ncRNA_finding:

|-- SampleName.ncRNA.stat.xls [Statistics of ncRNA prediction]

|-- SampleName.denovo.rRNA.fasta [rRNAmmer prediction result]

|-- SampleName.denovo.rRNA.gff [rRNAmmer prediction result in GFF3format]

|-- SampleName.sRNA.cmsearch.confident.gff [Filtered result of sRNA prediction]

|-- SampleName.sRNA.cmsearch.confident.nr.gff [Final result of sRNA after duplication removal]

|-- SampleName.tRNA.gff [tRNA prediction result in GFF3format]

|-- SampleName.tRNA.structure [tRNA secondary structure file]

|-- SampleName.tRNA.xls [tRNA prediction result]

Note: Please view the files with TextEditer such as NotePad++,UEditor except file end with xls.

5.3 Repeat

Tandem repeat (TR) was the sequence which contains more than two neighbored repeat units. The length of repeat unit ranges from 1 bp to 500 bp, and it exhibited the specificity of species which contribute to the researches of evolution. Minisatellite DNA was also named as tandem repeat sequences with variable number which was a kind of small repeated DNA sequence, and the length of repeat unit was 15-65 bp. Microsatellite DNA was also named as short tandem repeat sequences or simple tandem repeat sequences, and the length of its repeat unit was 2-10 bp. The repeat unit and repeat frequency of microsatellite DNA between different species was different, so it can be

used as molecular marker.

The repeat prediction result is as follow:

Table 11 Repeat statistic (Download)

Sample Name (#)	Type (#)	Number (#)	Repeat Size (bp)	Total Length (bp)	In Genome (%)
MP108	TRF	120	8-723	33,336	1.1397
	Minisatellite DNA	23	15-63	1,252	0.0428
	Microsatellite DNA	2	8-9	84	0.0029

Note: Total Length,total length of all repeat; % in genome, The proportion of the length of repeat in Genome.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/3.Genome_Component/Repeat_finding:

|-- SampleName.TRF.stat.xls [Statistics of tandem repeat analysis]

|-- SampleName.Microsatellite.DNA.dat.gff [Microsatellite DNA file in GFF3format]

|-- SampleName.Minisatellite.DNA.dat.gff [Minisatellite DNA file in GFF3format]

|-- SampleName.trf.dat [Primary results of TRF analysis]

|-- SampleName.trf.dat.gff [*.trf.dat file in GFF3format]

Note: Please view the files with TextEditer such as NotePad++,UEditor except file end with xls.

6 Gene Annotation

The function annotation is accomplished by analysis of protein sequences. We align genes with databases to obtain their corresponding annotations. To ensure the biological meaning, the highest quality alignment result is chosen as gene annotation. Function annotation is completed by blasting genes with different databases.

In this project we have finished VFDB , CAZY , PHI , IPR , SWISSPROT , COG , CARD , GO , KEGG , NR , T3SS...11databases annotation,each result in the fllowing table:

Table 12 Annotation Statistics A (Download)

Sample Name (#)	Total (#)	VFDB (#)	CAZY (#)	PHI (#)	IPR (#)	SWISSPROT (#)	COG (#)
MP108	2,884	94 (3.25%)	48 (1.66%)	145 (5.02%)	2,275 (78.88%)	1,138 (39.45%)	1,794 (62.2%)

Table 13 Annotation Statistics B (Download)

Sample Name (#)	CARD (#)	GO (#)	KEGG (#)	NR (#)	T3SS (#)	OverAll (#)
MP108	35 (1.21%)	1,731 (60.02%)	1,659 (57.52%)	2,869 (99.47%)	258 (8.94%)	2,870 (99.51%)

VFDB Database Annotation

Virulence factors of pathogenic bacteria (VFDB) database mainly focus on the infectious agents of bacteria, mycoplasma and Chlamydia. It contains 24 species, 425 infectious agents, 24 pathogenicity islands, and 2,359 genes which related to virulence factor.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/4.Genome_Function/Pathogen_Analysis/Animal:

|-- SampleName.vfdb.list.anno.xls [VFDB annotation result]

|-- SampleName.vfdb.list.filter.xls [VFDB blast result]

CAZy Database Annotation

CAZy:Carbohydrate-Active enZYmes Database.It contains enzyme families which

specify for carbohydrate degradation, decoration and synthesis. The database can be divided into four types: glycoside hydrolases (GHs), glycosyl transferase (GTs), polysaccharide lyases(PLs) and carbohydrate esterases (CEs). Besides these, the database contains carbohydrate-binding modules (CBMs).

Table 14 CAZy statistics (Download)

Sample Name (#)	AAs Number (#)	CBMs Number (#)	CEs Number (#)	GHs Number (#)	GTs Number (#)	PLs Number (#)
MP108	1	4	4	29	8	2

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/4.Genome_Function/Pathogen_Analysis/Plant:

|-- SampleName.cazy.catalog.xls [Statistics of CAZy catalog]

|-- SampleName.cazy.list.anno.xls [CAZy annotation result]

|-- SampleName.cazy.list.filter.xls [CAZY blast result]

|-- SampleName.cazy.statis_5class.stat.xls [Statistics of 5 CAZy classes]

|-- SampleName.cazy.statis_allclass.stat.xls [Statistics of all CAZy subclass]

PHI Database Annotation

PHI:Pathogen Host Interactions. A database which contains the relationship between pathogens and hosts. The database is verified by experiments. The pathogen contains fungus, oomycetes and bacterial pathogens, and the hosts contain animals, plants, fungus and insects.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/4.Genome_Function/Pathogen_Analysis:

|-- SampleName.phi.list.anno.xls [PHI annotation result]

|-- SampleName.phi.list.filter.xls [PHI blast result]

Swiss-Prot Database Annotation

Swiss-Port is a database created by UniProt consortium in 2002. Because the annotation results are verified by experiments, the database is credible and it can be used as reference for other kinds of annotations.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SampleName/4.Genome_Function/General_Gene_Annotation:

|-- SampleName.swissprot.list.anno.xls [Swissprot annotation result]

|-- SampleName.swissprot.list.filter.xls [Swissprot blast result]

COG Database Annotation

COG:Cluster of Orthologous Groups of proteins. It is a protein database which is created and maintained by NCBI. The database is based on the evolution relation of protein system among bacteria, algae and eukaryotes. Protein sequence can be classified into one kind of COG parts and each kind of COG part is composed by homologues sequences which can be used to deduce the function of protein. COG database is divided into twenty parts by their functions. The statistics was list below.

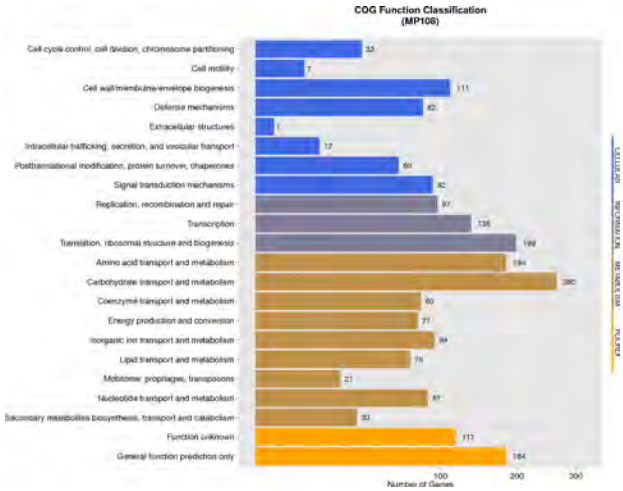


Figure 10 COG annotation.

y:Annotation result,x:Gene number.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/4.Genome_Function/General_Gene_Annotation:

|-- SampleName.cog.list.anno.xls [COG annotation result]

|-- SampleName.cog.list.class.catalog.xls [COG classification result]

|-- SampleName.cog.list.cogclass.pdf [Figure of COG classification in PDF format]

|-- SampleName.cog.list.cogclass.png [Figure of COG classification in PNG format]

|-- SampleName.cog.list.filter.xls [COG BLAST result in M8 format]

CARD Database Annotation

CARD:The Comprehensive Antibiotic Resistance Database.The CARD is a rigorously curated collection of known resistance determinants and associated antibiotics, organized by the Antibiotic Resistance Ontology (ARO) and AMR gene detection models.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/4.Genome_Function/Pathogen_Analysis/Animal:

|-- FA.card.list.anno.xls [CARD annotation result]

|-- FA.card.list.filter.xls [CARD blast result]

GO Database Annotation

GO:Gene Ontology. It is an database which is created by The Gene Ontology Consortium in 1988, and it is divided into three main parts: 1) Cellular component: It is used to describe the subcellular structure, position and large molecular complex including nucleolus, telomere, initial-site reorganization complex and etc. 2) Molecular function: It is used to describe the functions of genes and gene productions, for example the combination of carbohydrate, the activity of ATP hydrolase and etc. 3) Biological process: It is used to describe the combination of functional molecular and the acquisition of broader biological function, for example mitosis, purine metabolism and etc.Genes were classified into one or several parts of GO by their functions. Relying on the GO annotation results, we could detect gene functions. The statistics of GO

annotation is list in the following figure.

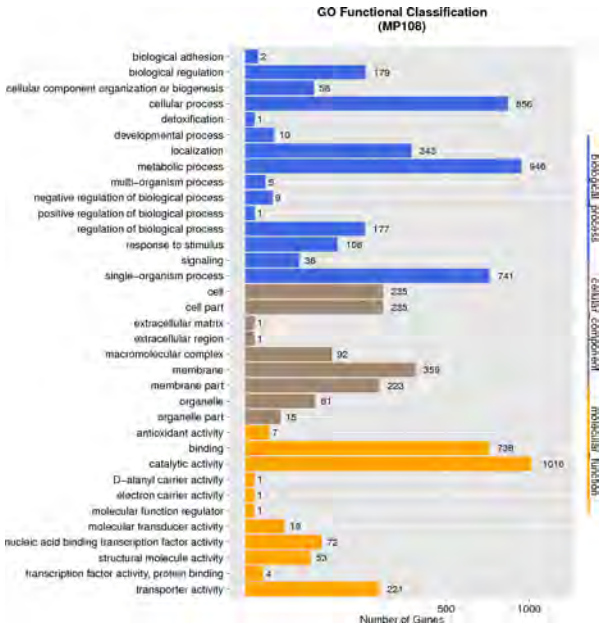


Figure 11 GO annotation.

y:Annotation result,x:Gene number.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/4.Genome_Function/General_Gene_Annotation:

|-- SampleName.go.pdf [A secondary classification histogram with the gene corresponding to GO in PDF format]

|-- SampleName.go.png [A secondary classification histogram with the gene corresponding to GO in PNG format]

|-- SampleName.iprscan.gene.GO.xls [Relationship between gene and GO database]

|-- SampleName.iprscan.gene.ipr.xls [Relationship between gene and wego]

|-- SampleName.iprscan.gene.wego.xls [Relationship between gene and IPR]

|-- SampleName.iprscan.xls [GO annotation result]

KEGG Database Annotation

KEGG:Kyoto Encyclopedia of Genes and Genomes.KEGG version 0.1 is published by Kanehisa Laboratories in 1995, and it is developed into an integrity database now. Its core database is KEGG PATHWAY database. KEGG PATHWAY divides the biological pathways into eight main parts, and each part is combined by several subparts. Each part is annotated by related genes and exhibited in the figure. Using KEGG annotation, we could find genes that related to the annotated gene conveniently.The following figure was obtained from the statistics of KEGG annotation, and it can be used to overview KEGG analysis results.

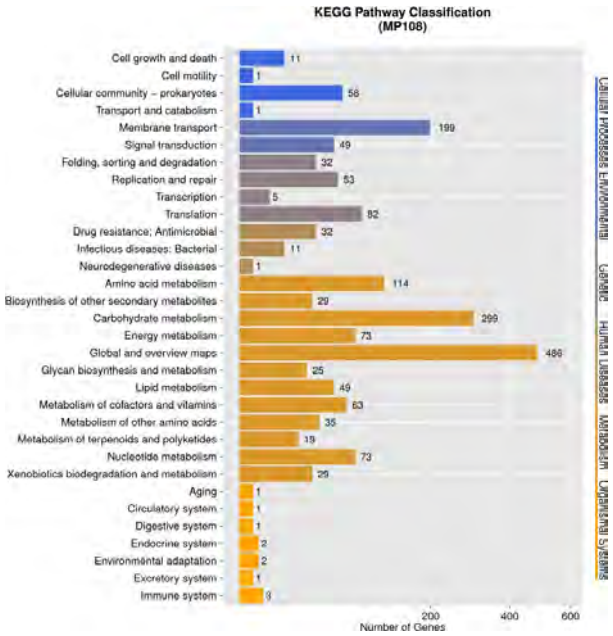


Figure 12 KEGG annotation.

y:Annotation result,x:Gene number.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/4.Genome_Function/General_Gene_Annotation:

|-- SampleName.kegg.functional_classification_2.pdf [KEGG functional classification figure in PDF format]

|-- SampleName.kegg.functional_classification_2.png [KEGG functional classification figure in PNG format]

|-- SampleName.kegg.list.anno.xls [KEGG annotation result]

|-- SampleName.kegg.list.filter.xls [KEGG blast result]

|-- SampleName.kegg.list.Gene2KEGG.xls [Statistic of KEGG genes and corresponding KoNumber]

|-- SampleName.kegg.list.KEGG2Gene.xls [Statistic of KEGG classifications and corresponding genes]

|-- SampleName.kegg.list.ko.htm [Relative URL of ko]

|-- SampleName.kegg.list.ko.path.xls [Statistic of KEGG pathways and corresponding genes]

|-- SampleName.kegg.list.ko.xls [Description of each ko]

|-- KEGG_MAP.tar.gz [Maps packed file]

NR Database Annotation

NR:Non-Redundant Protein Database. It is protein database without duplications which is created and maintained by NCGI. The database is more complete and the annotation results contain specie information which can be used for specie classification. But most of the annotation results are not verified.

FILE STUCTURE OF RESULTES：

BGI_result/Separate/SampleName/4.Genome_Function/General_Gene_Annotation:

|-- SampleName.nr.list.anno.xls [NR annotation result]

|-- SampleName.nr.list.filter.xls [NR BLAST result]

Type III Secretion System Effector Protein Prediction

Type III secretion system Effector protein (T3SS) have close relationship with gram-

negative pathogens. Toxin protein is secreted to extracellular fluid or hostcell by type X secretion system (TXSS which can be divided into seven types, from type I to type VII), and cause immunological reaction or cell death. Most of researches are focus on T3SS which helps to detect the infection mechanism and toxicity at molecular level.

FILE STUCTURE OF RESULTES:

|-- SampleName.effectiveT3.plant.anno.xls [T3SS annotation result]

|-- SampleName.effectiveT3.plant.stat.xls [Statistics of T3SS annotation]

|-- SampleName.effectiveT3.plant.xls [T3SS raw result]

\end%Results

7 Circular

According to the analysis of the sequencing samples, Circos software was used to display the genome, ncRNA, repetitive sequences, annotation information, methylation, GC content, GC skew and other information on the genome of sequencing strains.If the assembly reaches the level of the completed genome, a separate circular map will be drawn for each genomic sequence and plasmid sequence.

7.1 Genome Circular

GC skew analysis was performed using (G-C) / (G + C) calculations based on Genomic sequences of sequenced strains, the results of gene distribution, ncRNA distribution and gene annotation are also shown on this figure at the same time.

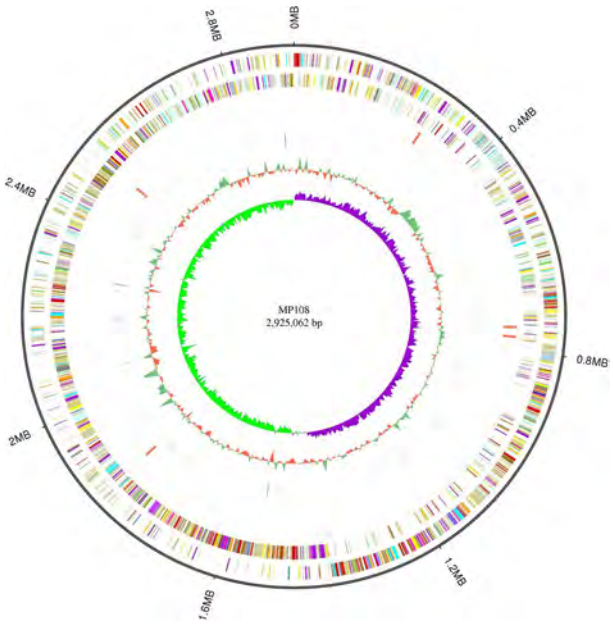


Figure 13 Circular representation of genome.

Circular representation of the genome, see the appropriate readme file for details.

FILE STUCTURE OF RESULTES:

BGI_result/Separate/SA/6.Circles_Graphs:

|-- illustration.jpg [Legend image]

|-- *.Circos.png [Circular representation of the genome/plasmid in png format]

|-- *.Circos.svg [Circular representation of the genome/plasmid in svg format]

|-- *.Circos.readme.xls [Documentation of the circular map]

8 Comparative Genomics

Compare sequencing strain with reference strains by using their genome sequence and gene sequence.The result shows the structural differences, mutation and evolution relationship between them.

8.1 Structural Variation (Synteny)

Divided into nucleotide level and amino acid level, structure variations could detect the location variations of genes that caused by recombination and transportation when comparing sequenced genome with reference genome. Compared with amino acid level, analysis on the nucleotide level could detect the information of insertion and deletion. Structure variation analysis could detect the evolution of homology genomes, for example, the location variations of gene clusters with similar function.

5 plans were used in amino acid and nucleotide two different level:

Plan 1:MP108 , Lactobacillus.rhamnosus.GG synteny：

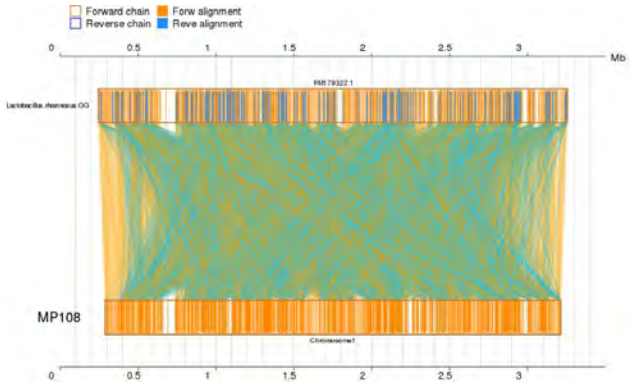


Figure 14 MP108 , Lactobacillus.rhamnosus.GG nucleic acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

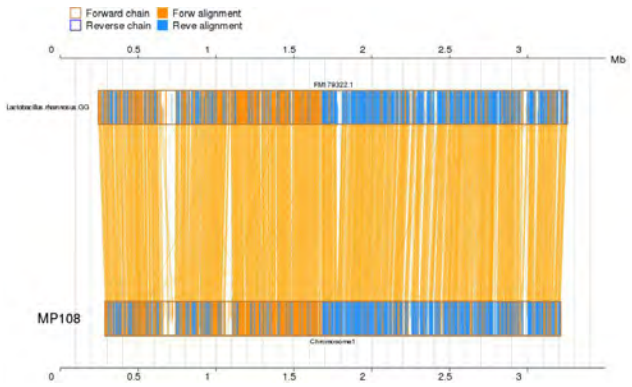


Figure 15 MP108 , Lactobacillus.rhamnosus.GG animo acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the animo acid sequence in the forward chain of this genome sequence and the blue region stands for the animo acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment

Plan 10:MP108 , Lactobacillus.rhamnosus.ATCC.8530 synteny:

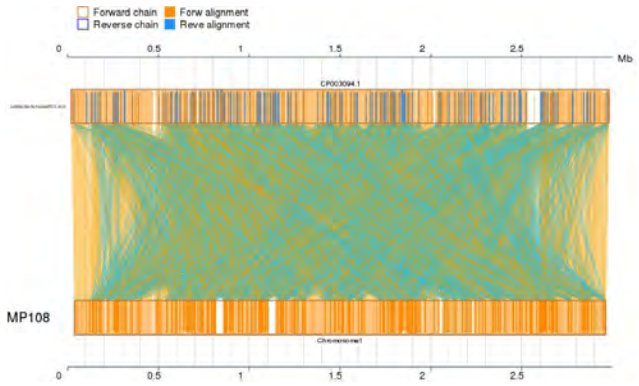


Figure 16 MP108 , Lactobacillus.rhamnosus.ATCC.8530 nucleic acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

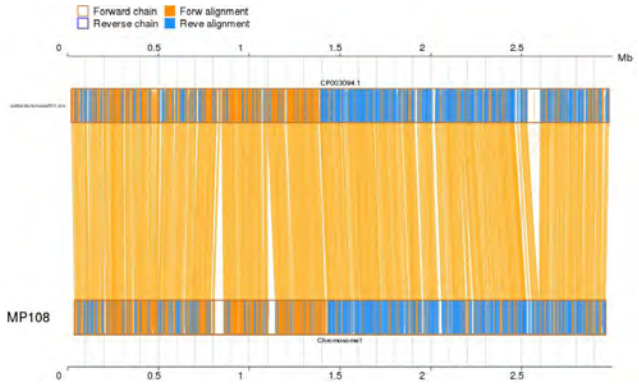


Figure 17 MP108 , Lactobacillus.rhamnosus.ATCC.8530 animo acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the animo acid sequence in the forward chain of this genome sequence and the blue region stands for the animo acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment

Plan 2:MP108 , Lactobacillus.rhamnosus.LOCK900 synteny:

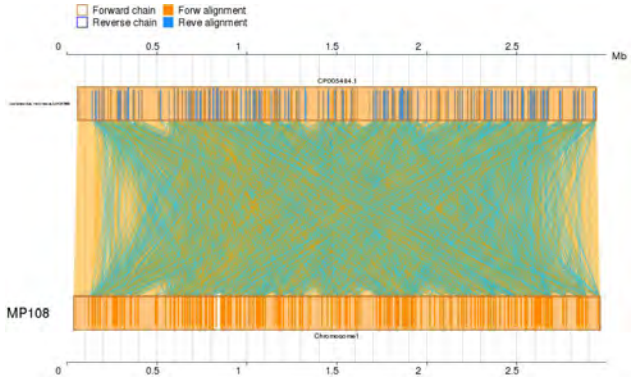


Figure 18 MP108 , Lactobacillus.rhamnosus.LOCK900 nucleic acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

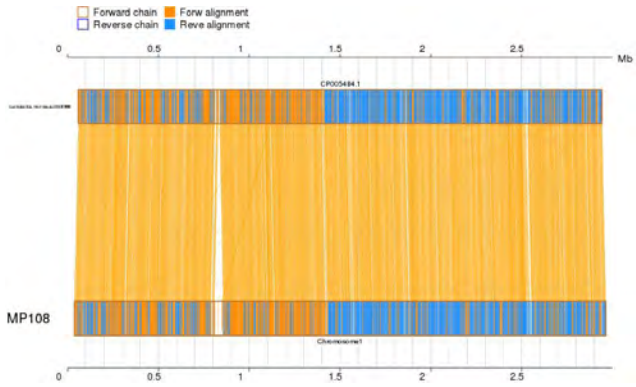


Figure 19 MP108 , Lactobacillus.rhamnosus.LOCK900 animo acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the animo acid sequence in the forward chain of this genome sequence and the blue region stands for the animo acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment

Plan 8:MP108 , Lactobacillus.rhamnosus.NCTC13710 synteny：

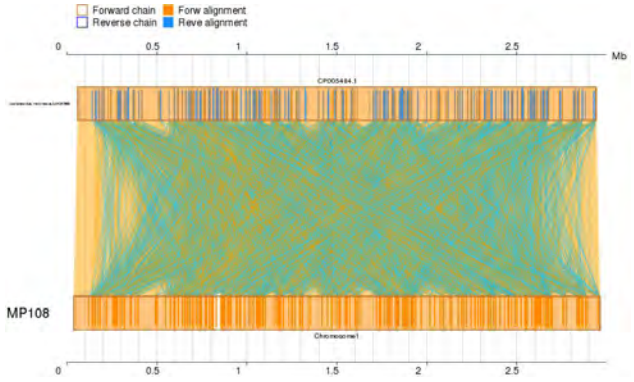


Figure 20 MP108 , Lactobacillus.rhamnosus.NCTC13710 nucleic acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

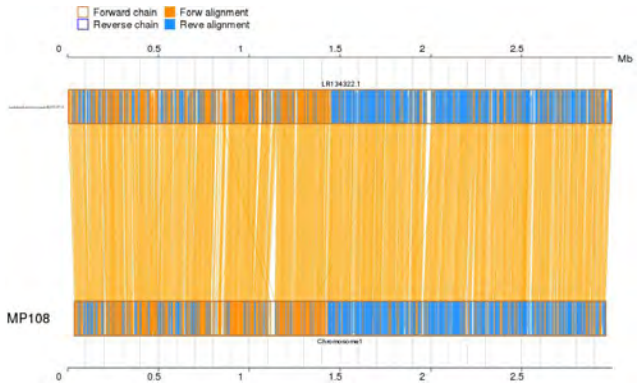


Figure 21 MP108 , Lactobacillus.rhamnosus.NCTC13710 animo acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the animo acid sequence in the forward chain of this genome sequence and the blue region stands for the animo acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment

Plan 9:MP108 , Lactobacillus.rhamnosus.4B15 synteny：

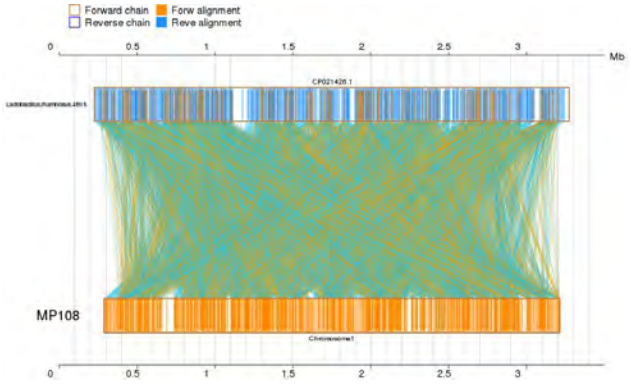


Figure 22 MP108 , Lactobacillus.rhamnosus.4B15 nucleic acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the nucleic acid sequence in the forward chain of this genome sequence and the blue region stands for the nucleic acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment.

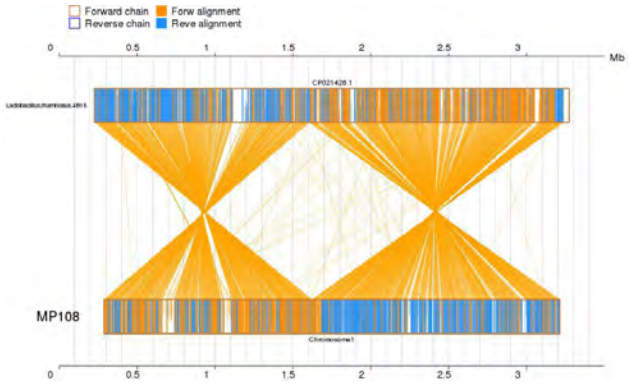


Figure 23 MP108 , Lactobacillus.rhamnosus.4B15 animo acid level synteny.

Yellow box stands for forward chain and blue box stands for reverse chain within the upper and following sequence region. In the box of sequence, the yellow region stands for the animo acid sequence in the forward chain of this genome sequence and the blue region stands for the animo acid sequence in the reverse chain of this genome sequence. In the middle region of two sequences, the yellow line stands for forward alignment and the blue line stands for reverse complementary alignment

FILE STUCTURE OF RESULTES:

BGI_result/Combination/Synteny/Synteny_Number:

|-- SampleName-RefName.amino_acid.png [Amino acid synteny figure of comparison between sample and reference in PNG format]

|-- SampleName-RefName.amino_acid.svg [Amino acid synteny figure of comparison between sample and reference in SVG format]

|-- SampleName-RefName.amino_acid.stat.xls [Statistic of amino acid coverage rate]

|-- SampleName-RefName.synteny.list.xls [Amino acid blast result]

|-- SampleName-RefName.identity.png [Distribution of protein identity figure in PNG format]

|-- SampleName-RefName.identity.svg [Distribution of protein identity figure in SVG format]

|-- SampleName-RefName.m8.xls [Aucleic acid blast result]

|-- SampleName-RefName.nucleic_acid.png [Aucleic acid synteny figure of comparison between sample and reference in PNG format]

|-- SampleName-RefName.nucleic_acid.svg [Aucleic acid synteny figure of comparison between sample and reference in SVG format]

|-- SampleName-RefName.nucleic_acid.stat.xls [Statistic of aucleic acid coverage rate]

P.S. Synteny_Number represent different plan.

8.2 Core-Pan Gene

The genomes of different strains (4 samples or more) are compared. The genes shared by all of the bacteria are core genes (most of the genes are genes necessary for growth), and the genes are special genes when they are contained only by one of the bacteria. The research on special gene and core gene are important for the detection of the functional differences and similarities between samples, and provide molecular evidences for the phenotype differences and similarities.

Core-Pan gene analysis in 1 plan(s).Result is shown below.

Plan 2:Lactobacillus.rhamnosus.4B15 , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.NCTC13710 , MP108 the Core gene and Pan gene in each strain:

Table 15 Gene number in strains (Download)

SampleName	TotalGeneNum	FilteredGeneNum	FinalGeneNum
Lactobacillus.rhamnosus.4B15	2,901	0	2,901
Lactobacillus.rhamnosus.ATCC.8530	2,887	0	2,887
Lactobacillus.rhamnosus.GG	2,944	0	2,944
Lactobacillus.rhamnosus.LOCK900	2,827	0	2,827
Lactobacillus.rhamnosus.NCTC13710	2,746	0	2,746
MP108	2,884	0	2,884

Note:TotalGeneNum,the gene number in all strains; FilteredGeneNum,filter the gene which contain N;FinalGeneNum,the gene used in analyze.

Table 16 CorePanGeneStat (Download)

CoreGene Num (#)	CoreGene Size (bp)	PanGene Num (#)	PanGene Size {bp}	Dispensable Num (#)	Dispensable Size {bp}
2,052	651,847	3,724	1,017,738	1,104	286,028

Note: CoreGene Num;CoreGene Size;PanGene Num;PanGene Size;Dispensable Num;Dispensable Size.

Dilution curve of strain's genes :

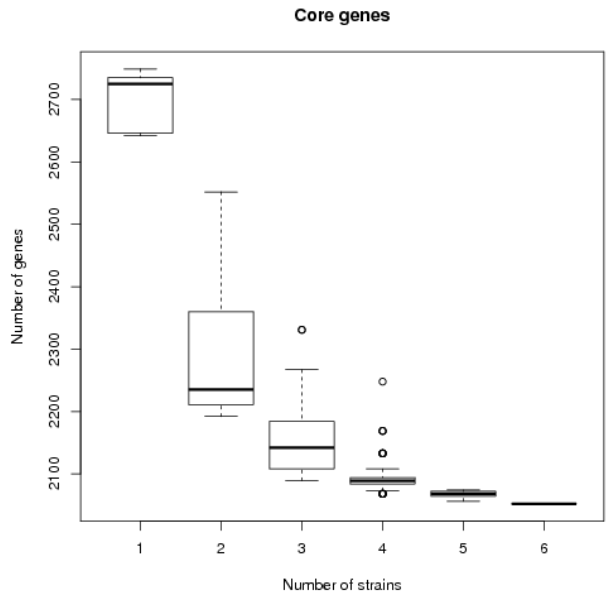


Figure 24 Core gene dilution curve .

x:the strains number we selected in each turn. y:gene number.（from up to down：min,Q1,median,Q3,max,and Q3,median,Q1 were made a box）

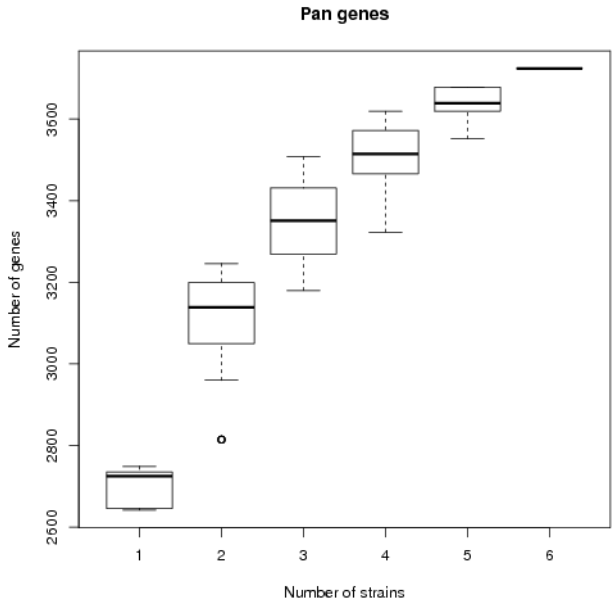


Figure 25 Pan gene dilution curve .

x:the strains number we selected in each turn. y:gene number.（from up to down：min,Q1,median,Q3,max,and Q3,median,Q1 were made a box）

Dispensable Gene heat map in each strain to show the cluster :

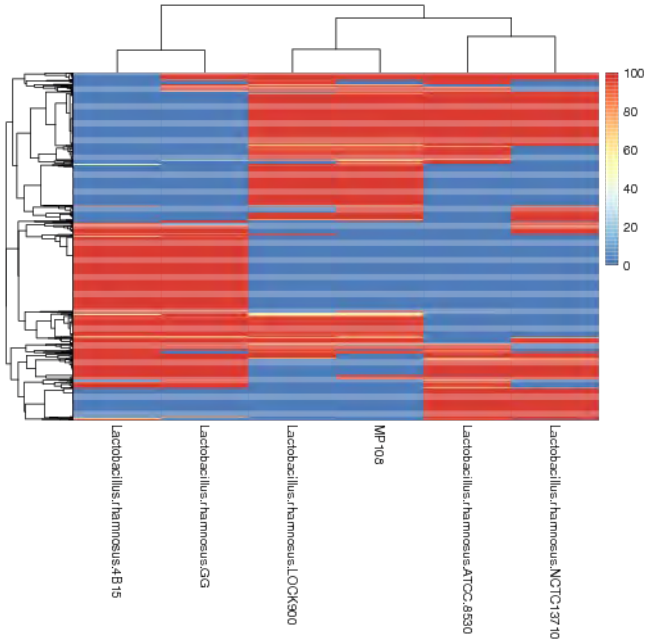


Figure 26 Dispensable gene heat map.

below are each strain name,left are Dispensable gene cluster,top are strain cluster,the similarities of gene are shown in the middle with different color represent different coverage by heat map. color/depth in top right pic.

Lactobacillus.rhamnosus.4B15 , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.NCTC13710 , MP108Pan Gene venn graph：

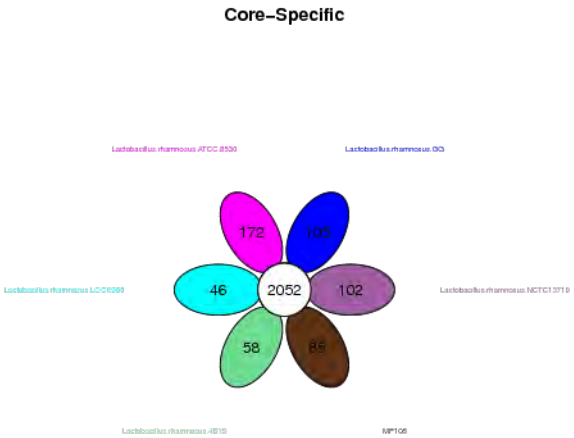


Figure 27 Pan gene Venn graph.

Each ellipse represent one strain,the number in the ellipse means the only cluster number.One cluster have the genes that more than 50 percent identity and less than 0.3 length diversity.

FILE STUCTURE OF RESULTES:

BGI_result/Combination/Core_Pan/Core_Pan_Number:

|-- CorePanGene.1.Stat.xls [Statistics of CorePanGene]

|-- CorePanGene.2.Stat.xls [Statistics of CorePanGene]

|-- cluster.stat.xls [Statistic of gene clustering]

|-- Core.cog.list.anno.xls [COG annotation result of core gene]

|-- Core.cog.list.class.catalog.xls [COG catalog description of core gene]

|-- Core.cog.list.cogclass.pdf [Figure of COG annotation for core gene in PDF format]

|-- Core.cog.list.cogclass.png [Figure of COG annotation for core gene in PNG format]

|-- Core.cog.list.filter.xls [Core gene BLAST result for COG annotation]

|-- Core_Dilution_Curve.pdf [Dilution curve of core gene]

|-- Core_Dilution_Curve.png [Dilution curve of core gene]

|-- CoreGene.fa [File of core gene]

|-- CoreGene.matrix [Distribution array of core gene]

|-- Dispensable.cog.list.anno.xls [COG annotation result of dispensable gene]

|-- Dispensable.cog.list.class.catalog.xls [COG catalog description of dispensable gene]

|-- Dispensable.cog.list.cogclass.pdf [Figure of COG annotation for dispensable gene in PDF format]

|-- Dispensable.cog.list.cogclass.png [Figure of COG annotation for dispensable gene in PNG format]

|-- Dispensable.cog.list.filter.xls [COG BLAST result of dispensable gene]

|-- Dispensable.fa [File of dispensable gene]

|-- Dispensable_heatmap.pdf [Heatmap of Dispensable gene in PDF format]

|-- Dispensable_heatmap.png [Heatmap of Dispensable gene in PNG format]

|-- Dispensable.matrix [Distribution array of dispensable gene]

|-- Pan_Dilution_Curve.pdf [Dilution curve of pan gene]

|-- Pan_Dilution_Curve.png [Dilution curve of pan gene]

|-- PanGene.annotation.xls [Statistics of pan gene annotation]

|-- Pangene.cluster.xls [Statistics of pan gene clustering]

|-- PanGene.fa [File of pan gene]

|-- PanGene.featrue.xls [Statistics of pan gene]

|-- PanGene.matrix [Distribution array of pan gene]

|-- Venn-*D.svg [Venn graph,strains≤5]

|-- Venn-*D.png [Venn graph,strains≤5]

|-- All.Flower.pdf [Gene graph,strains numer > 5]

|-- All.Flower.png [Gene graph,strains numer > 5]

|-- Strain_specific.list [Specific genes in each strains]

|-- StrainSpecific

| |-- *_specific.ffn [Specific genes]

| |-- *_specific.cog.list.anno.xls [COG annotation result of specific genes]

| |-- *_specific.cog.list.class.catalog.xls [COG catalog description of specific genes]

| |-- *_specific.cog.list.cogclass.pdf [Figure of COG annotation for specific genes in PDF format]

| |-- *_specific.cog.list.cogclass.png [Figure of COG annotation for specific genes in PNG format]

| |-- *_specific.cog.list.filter.xls [Specific genes annotation result]

P.S. Core_Pan_Number represent different plan. Besides *.xls file,we recommand NotePad++,UEditor to open the other file.

8.3 Gene Family

Gene family is a group of genes who have the same ancestor and formed by more than two gene copies. The members of gene family have similarity on structure and function, and the produced protein is also similar. Gene family could be used to detect the

evolution history and gene differentiation. At the same time, the function of unknown protein can be predicted when it is a member of gene family.

Ortholog:Descended from the same ancestral sequence separated by a speciation event: when a species diverges into two separate species, the copies of a single gene in the two resulting species are said to be orthologous.

Paralog:Created by a duplication event within the genome.

Single-copy gene:Has one physical location in the genome and can have orthologs in different species.

Multiple-copy gene:In the process of evolution, the genomic DNA sequence of microorganisms can be duplicated, these repeated some continue to evolve into new gene differences, different from the original series; and some to the structure and function are still basically the same form retained a multi copy gene.

1 plan(s) in genefamily analysis:

Plan 1:The genefamily in Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.4B15 , MP108 , Lactobacillus.rhamnosus.NCTC13710:

Table 17 The statistic in Genefamily (Download)

SampleID	Gene Number	Clustered Gene	UnClustered Gene	Family Num	Unique Family
Lactobacillus.rhamnosus.4B15	2,901	2,860	41	1,647	3
Lactobacillus.rhamnosus.ATCC.8530	2,887	2,767	120	1,673	2
Lactobacillus.rhamnosus.GG	2,944	2,898	46	1,693	8
Lactobacillus.rhamnosus.LOCK900	2,827	2,797	30	1,708	1
Lactobacillus.rhamnosus.NCTC13710	2,746	2,685	61	1,625	3
MP108	2,884	2,828	56	1,721	0

Note:Gene Number,the gene number in each strain;Clustered Gene,the gene number that can be clutered in gene family;UnClustered Gene,the gene number that can not be clutered in gene family;Family Num,the gene family number in strains;Unique Family,the unique gene family number in strain.

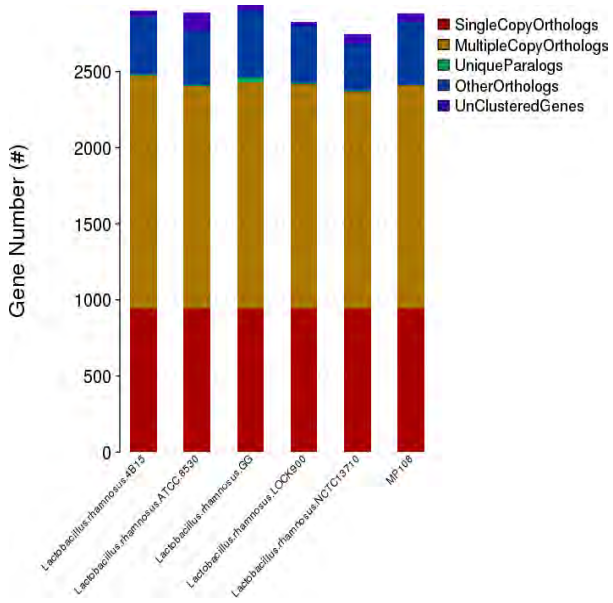


Figure 28 Orthologs number.

Single-copy orthologs,Multiple-copy orthologs,Unique paralogs,Other orthologs,Unclustered genes.

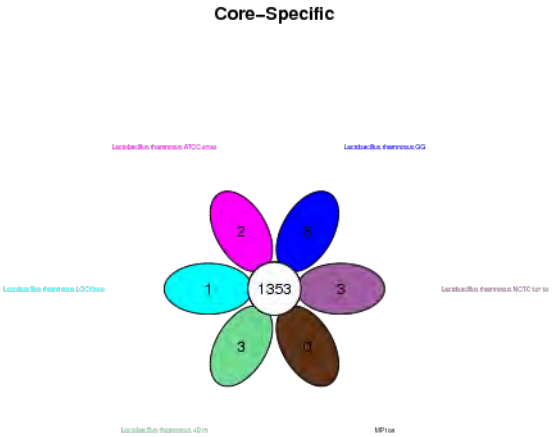


Figure 29 "Orthologs in different species gene family Venn graph.

Each ellipse represent one strain,the number in the ellipse means the family number in this speices.

FILE STUCTURE OF RESULTES:

BGI_result/Combination/Gene_Family/Gene_Family_Number:

|-- All.Flower.pdf [Gene graph,strains numer > 5]

|-- All.Flower.png [Gene graph,strains numer > 5]

|-- Venn-*D.svg [Gene Venn,strains numer ≤ 5]

|-- Venn-*D.png [Gene Venn,strains numer ≤ 5]

|-- all.KaKs.xls [KaKs result]

|-- gene_families.tar.gz [Gene family fasta, blast result, tree information.]

|-- GeneFamily.Annotation.xls [Annotation of gene family]

|-- GeneFamily.BarPlot.pdf [Type of gene family graph in PDF format]

|-- GeneFamily.BarPlot.png [Type of gene family graph in PNG format]

|-- GeneFamily.BarPlot.Table.xls [Type statistics of gene family]

|-- GeneFamily.single-copy.xls [List of single copy gene family]

|-- GeneFamily.stat.single-copy.xls [Statistics of single copy gene family]

|-- GeneFamily.stat.xls [Statistics of gene family cluster analysis results]

|-- GeneFamily.xls [Results of gene family cluster analysis]

|-- GeneFamily.Stat.Table.xls [Statistics of gene family cluster]

|-- distance_data

| |-- all.4dtv.xls [Ka/Ks result]

| |-- all.identity.xls [Ka/Ks results of every two genes in each gene family]

| |-- all.KaKs [Ka/Ks results of every two genes in each gene family]

| |-- all.ortho.xls [The ortho result in gene family]

P.S. Gene_Family_Number represent different plan. Besides *.xls file,we recommand NotePad++,UEditor to open the other file.

8.4 Evolution

The phylogenetic tree which based on the similarity and difference of genotype and phenotype between species could reflect the evolution relationship of the species. The researches of specie evolution play an important role in taxonomy. In phylogenetic tree, each node stands for the ancestor of the branches, and the distance of nodes respond to the evolution distance. Phylogenetic trees are divided into two types: the tree with root and the tree without root. The method of phylogenetic tree construction contains distance-based method (including UPGMA and N-J), maximum parsimony method (MP), and maximum likelihood method (ML). The used software are including PAUP, Mega, TreeBeST, PHYLIP and etc. TreeBeST was applied in the analysis.

In this project CorePan2,GeneFamily2,2 methods to built 2 trees.

Plan 2:Lactobacillus.rhamnosus.4B15 , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.NCTC13710 , MP108 the tree based on CorePan2 result,the figure is in the following:

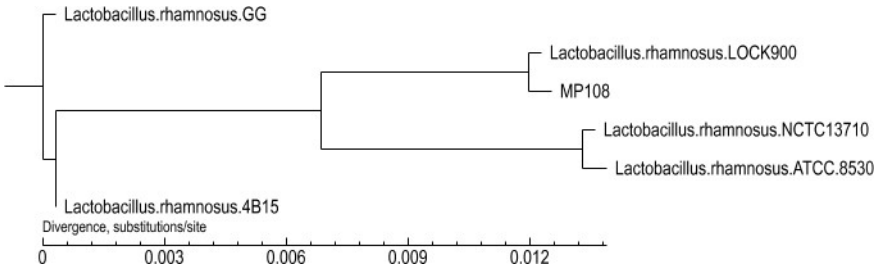


Figure 30 PhylogeneticTree.

Lactobacillus.rhamnosus.4B15 , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.NCTC13710 , MP108 the tree based on CorePan2 result

Plan 4:Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.4B15 , MP108 , Lactobacillus.rhamnosus.NCTC13710 the tree based on GeneFamily2 result,the figure is in the following:

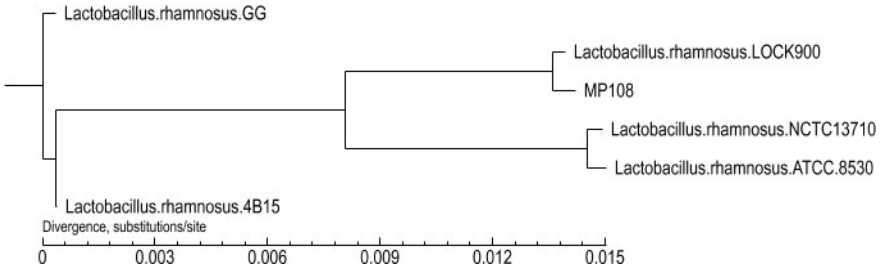


Figure 31 PhylogeneticTree.

Lactobacillus.rhamnosus.GG , Lactobacillus.rhamnosus.ATCC.8530 , Lactobacillus.rhamnosus.LOCK900 , Lactobacillus.rhamnosus.4B15 , MP108 , Lactobacillus.rhamnosus.NCTC13710 the tree based on GeneFamily2 result

FILE STUCTURE OF RESULTES:

BGI_result/Combination/Phylogenetic_Tree/Type_Number:

|-- *.mfa [Original fasta file]

|-- *.readme [Methods and parameter]

|-- *.tree [Original Tree file]

|-- *.tree.png [Phylogenetic Tree in PNG format]

|-- *.tree.svg [Phylogenetic Tree in SVG format]

P.S. Type_Number represent different plan.Besides *.xls file,we recommand NotePad++,UEditor to open the other file.

Methods

1 Data Filter

1.1 Illumina Data

There exists a certain amount of low quality data in raw data. In order to obtain more accurate and reliable results in subsequent bioinformatics analysis, the raw data will be treated.

1) Remove reads with a certain proportion of low quality(≤20) bases(40% as default).

2) Remove reads with a certain proportion of Ns (40% as default).

3) Remove adapter contamination.

4) Remove duplication contamination.

The above processes are applied to read1 and read2. After that, 10%-20% of the data is eliminated generally (For small *Insert Size* reads). Because large *Insert Size* reads have higher duplication and sometimes there are short *Insert Size* reads contaminations due to library construction problem, the data eliminated is more but there is no certain proportion.

1.2 PacBio 数据

PacBio Sequle平台上单个**SMRT cell**中有**100**万个**Zero-Mode Waveguides (ZMWs)**孔，当测序时**DNA**模板随机分配到每个**ZMW**小孔会存在三种情况：单个**ZMW**小孔中没有**DNA**模板（**P0**）;单个**ZMW**小孔中有一条**DNA**模块**(P1)**；单个**ZMW**小孔中有两条及以上**DNA**模板**(P2);**能够用于后续分析的有效数据为**P1**中的***Polymerase Reads***。 **PacBio Sequel**测序得到原始数据为 ***Polymerase Reads***，过滤掉测序接头及低质量等数据最终得到可用**Subreads**并以**bam**格式保存[1][2][3]。 ***Polymerase Reads*** 包含测序接头序列以及模板序列**(Subreads)**，**Subreads**可以用于后续组装、比对等等分析。但是**Subreads**自身存在**15%Indel**错误。对单个**ZMW**小孔中的**Subreads**求一致性序列得到高精确的**Circular Consensus Sequencing(CCS)**数据（也叫**Reads Of Insert**）。**CCS**数据数据高精确度**reads**，可以直接用于后的组装、对比、**16S**物种分类等等，一般只对小文库**(1~2k、5-6Kb)**才做**CCS**数据分析。

Polymerase Reads 、**Subreads**，**CCS(Reads of Insert),**三者之间的关系可以理解为在**SMRT cell**上有**100**万个**ZMW**孔，只选取单个孔中只有一条**DNA**模板序列的**ZMW**，每个孔产生一条 ***Polymerase Reads***， ***Polymerase Reads*** 去掉接头得到多条**Subreads**，单个孔中的多条**Subreads**求一致性得到一条**CCS**（**Reads of insert**）数据，如下图：

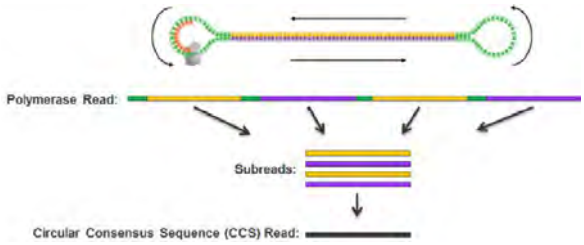


Figure 1 PacBio Polymerase reads、Subreads、CCS 示意图.

PacBio 数据**Polymerase reads**、**Subreads**、**CCS** 示意图。

PacBio平台原始测序数据中存在大量的接头序列、低质量序列、测序错误序列等，为了得到更精确的组装结果，同样需要对原始的测序数据进行如下处理：

3） 从***Polymerase Reads*** 中提取**Subreads**，过滤掉**adapter**序列**; 4**

） 过滤掉长度小于**1000bp**的**Subreads;**

2 Assembly

2.1 Kmer Analysis

Regardless of the sequencing error, genome heterozygosis and duplication, 15-mer distribution should follow the Poisson distribution. However, low-depth *K-mer* takes up high proportion due to sequencing error actually. Sometimes due to heterozygosis, other peak may appear at the 1/2 of the main peak, while due to duplication, repeating peaks may appear near the integer times of the main peak.

2.2 Assembly

We assemble the reads using variety of software,and it can be roughly divided into four parts: 1)Subreads correct; 2)Corrected Reads Assembly; 3)Correct single base; 4)Sequence loop judgment and chromosome, plasmid sequence discrimination[4][5][6][7][8].

1) Subreads correct: Using software(Pbdagcon、FalconConsensus) to correct Subreads itself,or mix to correct Subreads with Proovread,the corrected Subreads is more accurate and reliable.

2) Corrected Reads Assembly: Assemble based on Corrected Reads using several software(Celera、Falcon) respectively,and then choose the best assembly result.

3) Correct single base: Correct the single base error in assembly result with NGS data,software such as Quiver,GATK,SOAPsnp/SOAPindel were used.

4) Sequence loop judgment and chromosome, plasmid sequencediscrimination: Judge whether the assembly sequences are circular or not,chromosome or plasmid should be distinguished too.

Software:Falcon;Version: v0.3.0.

Parameters:-v -dal8 -t32 -h60 -e.96 -l500 -s100 -H3000

Website:https://github.com/PacificBiosciences/falco

Software:proovread;Version: 2.12

Parameters:-t 4 --coverage 60 --mode sr

Website:https://github.com/BioInf-Wuerzburg/proovread

Software:Celera Assembler;Version:8.3

Parameters:doTrim_initialQualityBased=1，doTrim_finalEvidenceBased=1，doRemoveSpd properties -U

Website:http://sourceforge.net/projects/wgs-assembler/files/wgs-assembler/wgs-8.3/

Software:SMRT Analysis;Version:v2.3.0 Parameters:estn=24，

nproc=8，cov=6

Website:https://github.com/PacificBiosciences/SMRT-Analysis/wiki/SMRT-Pipe-Reference-Guide-v2.3.0

Software:GATK;Version:v1.6-13

Parameters:-cluster 2 -window 5 -stand_call_conf 50 -stand_emit_conf 10.0 -dcov 200 MQ0 >= 4

Website:http://www.broadinstitute.org/gatk/

3 Genome Component

3.1 Gene

Using Glimmer software[9][10][11] to predict genes of assembly. The Glimmer is develop for bacteria, archaebacteria, viruses and other microorganisms in speciality,comparing to previous version,it's more valid for prediction of start site and CDS and it's more accurate for prediction of high-GC sequence.

Software:Glimmer:Version:3.02`

Website:http://www.cbcb.umd.edu/software/glimmer/

Parameters：-o*-g*-t*-l linear

3.2 Non-coding RNA

Finding rRNAs by comparing with rRNA database or predicting with RNAmmer software; Using tRNAscan to predict the area of tRNA and it's secondary structure; Using Infernal to compare with Rfam database and get sRNAs.

Software:RNAmmer[12]:Version:1.2

Parameters:–s Species –m Type –gff *. rRNA.gff –f *.rRNA.fq

Website:http://www.cbs.dtu.dk/services/RNAmmer/

Software:tRNAscan-SE[13]:Version:1.3.1

Parameters:–Spec_tag(BAOG) –o *. tRNA –f * .tRNA.structure

Website:http://gtrnadb.ucsc.edu/

Database:Rfam[14]:Version:9.1

Parameters:–p blastn –W 7 –e 1 –v 10000 –b 10000 –m 8 –i subfile –o *.blast.m8

Website:http://rfam.sanger.ac.uk/

3.3 Repeat

Using the Tandem Repeat Finder software to predict tandem repeat sequence(TR) and screening out the minisatellite sequence and minisatellite sequence in TRs according to

repeat length and number.

Software:Tandem Repeats Finder:Version:4.04

Website:http://tandem.bu.edu/trf/trf.html

Parameters: 2 7 7 80 10 50 2000 -d -h

4 Gene Function Analysis

Annotation method

The function annotation is accomplished by analysis of protein sequences. We align genes with databases to obtain their corresponding annotations. To ensure the biological meaning, the highest quality alignment result is chosen as gene annotation. Function annotation is completed by blasting genes with different databases. We provide BLAST results in M8 format and collect the annotation results with different databases.Currently, we provide the following databases:

Gene Ontology (GO)[15][16]：releases_2017-09-08

Kyoto Encyclopedia of Genes and Genomes(KEGG)[17]；version：81

Cluster of Orthologous Groups of proteins(COG)[18][19]；version：2014-11-10

Swiss-Prot[20]；version：release-2017-07

Trembl；version：release-2017-09

NR；version：2017-10-10

EggNOG[21]；version：4.5

Antibiotic Resistance Genes Database(ARDB)[22]；version：1.1

Pathogen Host Interactions (PHI)[23]；version：4.3

Fungal Cytochrome P450 Database[24]；version：1.1

Carbohydrate-Active enZYmes Database (CAZy)[25]；version：2017-09

virulence factor database (VFDB)[26]；version：2017-09

Type III secretion system Effector protein(T3SS)[27]；version：1.0

TransportDB；version2.0[28]

5 Comparative Genomics

5.1 Structural Variation (Synteny)

Amino acid level:

(1) The sequence of the target bacterium is ordered according to that of the reference bacterium based on Mummer. Then the upper and following axes of linear synteny graph

are constructed after the same proportion of size reduction in length of both sequences.

(2) The protein set P1 of target bacterium is aligned with the protein set P2 of reference bacterium. Firstly P1 is aligned with P2 in BLASTP m8 by taking P2 as database, e-value <= 1e-5, identity>=85% and the best hit of each protein is selected; Secondly the same alignment is carried out by taking P1 as database; Finally the results with best hit value for both alignments are reserved and the consistent value is the average of two consistent values.

(3) Each pair of best hits for two alignments is marked in the coordinate diagram according to its position information after the same proportion of size reduction.

Nucleic acid level:

(1) The sequence of the target bacterium is ordered according to that of the reference bacterium based on Mummer. Then upper and following axes of linear synteny graph are constructed after the same proportion of size reduction in length of both sequences.

(2) According to BLAST each pair nucleic acid sequence of two alignments is marked in the coordinate diagram according to its position information after the same proportion of size reduction.

software：MUMmer，version：3.22

website：http://mummer.sourceforge.net/

parameter：-b 200 -c 65 --extend -l 20

5.2 Core-Pan Gene

The genes are taken from reference genome as gene pool. Then the genes predicted by Query samples are BLAST with the gene pool, and the blast results are filtered by their length and identity. The BLAST coverage ratios (BCR) of genes from gene pool and Query samples are calculated separately. If the BCR values from reference and Query sample are smaller than the setting value, the gene from reference is not homology with Query's, and the gene from Query genome is added to the gene pool. Query samples are repeated by the upper steps one by one, and the final gene pool is called the pan gene pool.

software：CD-HIT[29]；version：v4.6.6

parameter：-c 0.5 -n 3 -p 1 -g 1 -d 0 -s 0.7 -aL 0.7 -aS 0.7

website：http://weizhongli-lab.org/cd-hit/

5.3 Gene Family

Gene family is constructed by the gene of the reference and the target bacterium, and then the gene family is analyzed. At present the analysis is aimed at single copy gene family.

(1) We align the protein sequence in BLAST and eliminate the redundancy by solar.

(2) We carry out gene family TreeFam clustering treatment for the alignment results with

Hcluster_sg software.

(3) We convert the alignment results of protein into those of the multiple sequence amino acids in CDS area, after multiple sequences alignment with the clustered gene family by using Muscle[30][31]software.

(4) We carry out the gene family tree constructing analysis for multiple sequences alignment results based on Muscle through NJ method with Treebest[32]software.

software：Muscleversion：3.8.31 website：

http://www.drive5.com/muscle； parameter

：-in -out -maxiters 16 software：TreeBeST，

version：treebest-1.9.2

website：http://treesoft.svn.sourceforge.net/viewvc/treesoft/trunk/treebest/ parameter：

treebest nj -b 1000

5.4 Evolution

The phylogenetic tree is constructed by the array of SNPs getting from sample and reference. As for each bacterium, all of the SNPs are connected with the same order and the sequences with the same length are obtained as input file in the format of fasta. Then the phylogenetic tree is constructed by the TreeBeST[33] using the method of PHYML, and the setting of bootstraps is 1,000.

software：TreeBeST，version：treebest-1.9.2 website：

http://treesoft.svn.sourceforge.net/viewvc/treesoft/trunk/treebest/ parameter：treebest phyml

-b 1000

Help

1 Data Mining

1.1 Basic applications of annotation

GO Database

GO annotation use the quick GO database which is part of Interpro database, so the annotation results contain the information of Interpro database and the result file is ended by x.iprscan.gene.ipr. The annotation results of quick GO database are ended by x.iprscan.go. Because three different types of GO database have overlaps, the functions of genes that annotated to several types can be confirmed by summarizing its annotation information. For example,

Gene001 2 GO:0003677; DNA binding; Molecular Function GO:0006306; DNA methylation; Biological Process

We find that gene001 can be annotated by two GO pathways: one is molecular function,

and the other is biological process. So the gene is related to DNA combination on the level of molecular function and it is also related to DNA methylation on the level of biological process at the same time. So the gene is related to DNA combination in the process of DNA methylation.

KEGG Database

KEGG database has advantage on the figures of metabolic pathways. For example, if we want to know the genes that participating alanine metabolic pathway, we could search "Alanine" in the annotation result (x.kegg.list.anno). The searching result is list below.

Gene0002197 64.32 4e-126 tbi:Tbis_0822 K00259 ald alanine

dehydrogenase 1.4.1.1 Metabolism; Amino Acid Metabolism; Alanine, aspartate

and glutamate metabolism [PATH:ko00250] Metabolism; Metabolism of Other Amino

Acids; Taurine and hypotaurine metabolism [PATH:ko00430]

Gene0002983 53.47 6e-93 mau:Micau_2216 K00135 E1.2.1.16, gabD

succinate-semialdehyde dehydrogenase (NADP+) 1.2.1.16 Metabolism;

Carbohydrate Metabolism; Butanoate metabolism [PATH:ko00650] Metabolism; Amino

Acid Metabolism; Alanine, aspartate and glutamate metabolism [PATH:ko00250]

Metabolism; Amino Acid Metabolism; Tyrosine metabolism [PATH:ko00350]

From the result above, we find that alanine participating pathway ko00250. And the pathway belongs to alanine, aspartate and glutamate metabolism, so the pathway is what we search for. Because map number is corresponded to PATH: ko number, we could search "map00250" in the file of *.kegg.list.catalog.map.gene and detect the genes that can be annotated the pathway map00250. The result is shown below.

map00250 13 Gene002983,K00135,1.2.1.16

Gene003337,K00135,1.2.1.16 Gene002197,K00259,1.4.1.1

Gene001641,K00278,1.4.3.16 Gene002422,K00609,2.1.3.

Gene000926,K00820,2.6.1.16 Gene003451,K01755,4.3.2.1

Gene000233,K01756,4.3.2.2 Gene002830,K01915,6.3.1.2

Gene003449,K01940,6.3.4.5 Gene002419,K01955,6.3.5.5

Gene002420,K01956,6.3.5.5 Gene001368,K13821,1.5.99.8 1.5.1.12

Until now we find the genes that participating alanine metabolic pathway from the sequenced genome. If you want to detect the detail of the pathway, you could check the file of map00250.png under the directory of KEGG_MAP.

Swiss-Prot Database

The advantage of Swiss-Prot database is that all of its annotation results are verified by experiment, so the database has high credibility. For example, gene 1 is annotated by GO database, KEGG database and Swiss-Prot database separately, and the result is shown below.

GO:{GO:0016020; membrane; Cellular Component}

KEGG:{K09771 K09771 hypothetical protein -- Unclassified; Poorly Characterized; Function unknown}

Swiss-Prot:{Y6609_RHOSR UPF0060 membrane protein RHA1_ro06609 OS=Rhodococcus sp. (strain RHA1) GN=RHA1_ro06609 PE=3 SV=1}

From the result, we can find that the annotation result of Swiss-Prot is complete. The result not only exhibit the function of the gene, but also show the organism specie (OS) used for function variation, gene name (GN), protein existence (PE) and sequence version (SV). PE has 5 statuses including:

1: Evidence at protein level

2: Evidence at transcript level

3: Inferred from homology

4: Predicted

5: Uncertain

As comparison, GO database only provide the annotation information, and KEGG database does not contain such information.

COG Database

Among KEGG database, GO database and COG database, the functional classification of COG database is more detail than other databases except KEGG database. And this could help us to detect the function of gene by using COG database. For example,

NR:{UspA domain-containing protein [Jonesia denitrificans DSM 20603]}

Swiss-Prot:{NHAX_BACSU Stress response protein nhaX OS=Bacillus subtilis GN=nhaX PE=2 SV=2}

COG:{COG0589 Universal stress protein UspA and related nucleotide-binding proteins T Signal transduction mechanisms ;}

KEGG:{NA} GO:IPR006016; UspA

From the results above, we find that the gene is not annotated by KEGG database; it is annotated to the protein related to stress reaction and nucleotide binding by COG database; it is verified to be related to stress reaction by Swiss-Port database; NR

database and GO database only exhibit the gene name. So we can conclude that functional classification of COG could provide direction when an uncertain situation happened in KEGG annotation and GO annotation.

NR Database

As the main database of NCBI, NR database is large and sequences can be annotated by many genes. But many information of annotated gene is not verified and some of the gene functions are illustrated unclearly which would affect gene function detecting, so combining with other database annotation results is needed for gene function detecting. Besides these, NR database contains information to ensure which specie the sequenced bacterium is.

Using the example list in Swiss-Prot database, the annotation result of NR database is {hypothetical protein Bcav_0666 [Beutenbergia cavernae DSM 12333]}. From the result, we can know nothing except it is a hypothetical protein from Beutenbergia cavernae DSM 12333. But the gene is annotated to Rhodococcus sp. (strain RHA1) by Swiss-Prot database. So we can deduce that the same gene can be annotated to different species by using different database, and the annotation result of NR database can be only used as reference.

PHI Database

The database is special for the interaction between pathogens and hosts, and it contains many information including: gene name (PHI: XXX), EMBL accession (AAXXXXXX), NCBI taxonomy number (TX: XXX), pathogens and diseases. Because the database is designed for professional field and the database emphasis on applications, the form of annotation results is different from other database.

CAZy Database

The database specifies for carbohydrate enzymes, and the gene function is annotated by the classification information. For example, if two genes annotated to the classification of GH55, the functions of the two genes are same. Because the research subject of CAZy database is enzyme, most of the annotation result contains EC number. But functions of some enzyme are taken from paper, so the annotation result contains PMID information of NCBI.

1.2 Recommended analysis on different fileds of bacteria

Pathogenicity and Drug Resistance Analysis of Animal Pathogens

For the procedure of animal pathogen infection, many stages have been processed from the start to the end, including adsorption and colonization stage, immune evasion stage, and local pathopoiesis stage.

Adsorption and colonization stage: Most of the animal pathogen infections are start from host cell membrane. Using the reaction between adhesion factors of pathogens and receptors of host cell, the adsorption stage is complete. Some of the adhesion factors, including flagella, pili, and outer membrane protein which can be searched from

annotation results by using key words, play an important role during the reaction.

Immune evasion stage: Since get into the host, the pathogens need to avoid the natural immune clearance and antibody mediated immune defense. At this stage, the virulence factors mainly include lipopolysaccharide, capsular polysaccharide, protein enzymes (related genes can be searched from annotation results by using key words) and pathogens hosts interaction factors (PHI annotation results).

Local pathopoiesis stage: At this stage, the virulence factors mainly include iron ion acquisition system (related genes can be searched from annotation results by using key words) and toxin secretion system. Iron ion is the cofactors for many metabolic systems, for example, the synthesis of nucleotides, the transformation of oxygen and the production of energy. The acquisition ability of pathogens on iron ion is important for its virulence factor. Till now, we know that pathogens contain seven toxin secretion systems from T1SS to T7SS. Because T3SS secretion system is necessary for virulence and disease introduction, T3SS secretion system may become the target of many antibiotic-drugs in the future.

Besides these, the prediction results of genome islands and prophage can also be used for pathogenicity analysis. For example, pathogenicity related genome islands, which characterized by repeat sequences and insertion elements, could produce secretary protein and membrane protein. Some of the genome islands could produce toxin secretion systems (e.g. T3SS), information transfer system and regulatory system. Many pathogens contains more than one genome island, and the analysis is meaningful for pethogenicity detecting at the gene level. Prophage is the carrier of horizon gene transferring, which could be used to detect the new features of pathogens. And the sequences which carried by prophage are related to environment adaption and the diversity of virulence factors. So the results of prophage prediction can be used to detect the genome diversity during host evolution.

Pathogenicity Analysis of Plant Pathogens

Generally, the infection procedure of plant pathogens contains one or several following stages: cell wall degradation and colonization stage, pathogen host interaction stage, virulence secretion and transformation stage, and pathopoiesis stage.

At colonization stage, the pathogens destroy the polysaccharide in the cell wall by using carbohydrate enzyme and obtain the nutrient substance from the host cell.

At pathogen host interaction stage, the pathogens need to survive from the immune reaction of host (the pathway of jasmonic acid or salicylic acid). At the stage, the virulence factors mainly include lipopolysaccharide, capsular polysaccharide, protein enzymes (related genes can be searched from annotation results by using key words) and pathogens hosts interaction factors (PHI annotation results).

At virulence secretion and transformation stage, T3SS is the main virulence factor which could secrete effector protein, and metal ion acquisition system is another import virulence factor (related genes can be searched from annotation results by using key words). Metal ion is the cofactors for many metabolic systems, for example, the synthesis of nucleotides, the transformation of oxygen and the production of energy. The

acquisition ability of pathogens on iron ion is important for its virulence factor. The virulence transformation is also important for pathogenicity. The transformation systems mainly include ABC-transporter system and MFS-transporter system (related genes can be searched from annotation results by using key words).

Besides these, the prediction results of genome islands and prophage can also be used for pathogenicity analysis. For example, pathogenicity related genome islands, which characterized by repeat sequences and insertion elements, could produce secretary protein and membrane protein. Some of the genome islands could produce toxin secretion systems (e.g. T3SS), information transfer system and regulatory system. Many pathogens contains more than one genome island, and the analysis is meaningful for detecting the pethogenicity at the gene level. Prophage is the carrier for horizon gene transferring, which could be used to detect the new features of pathogens. And the sequences which carried by prophage are related to environment adaption and the diversity of virulence factors. So the results of prophage prediction can be used to detect the genome diversity during host evolution.

Research on Industrial Bacteria

Industrial bacteria applications mainly includes the research of probiotic mechanism of lactic acid bacteria, the research of antibiotic synthesis mechanism of actinomycetes, and the research of enzyme production mechanism of bacillus.

As for lactic acid bacteria, which are mainly used by food industry, their safety is important and need to be noticed. The following points should be considered when we select a lactic acid bacteria: the ability of antibiotic transferring and acquisition, which can be known by the result of gene annotation and gene prediction, should not be contained; the activity of harmful enzyme, including N- acetyl β -glucosaminidase, β -glucosidase and β-glucuronidase which can be predicted by gene annotation, should be checked; the biological functions, including acid resistance, adhesion ability on intestinal epithelial cells, and regulation ability on intestinal flora, should also be checked; the heritable characteristics, which contains CRISPR, should be stable.

As for actinomycetes, which are mainly used to produce antibiotic, their ability on secondary metabolic production is an important point that we focus on. The following points should be considered when we select an actinomycete: the ability to produce new antibiotics, which can be acquired by comparing with known bacteria; the transferable ability, which can be know by searching ABC-transporter and MFS-transporter (elated genes can be searched from annotation results by using key words); the ability of antibiotic resistance, which could be tested by experiments.

As for bacilli, which are mainly used to produce industrial enzyme including amylase and protease, their yield is an important point that we care about. The following points should be considered when we select a bacillus: the metabolic pathway, which can be searched by KEGG annotation; the number of genes related to enzyme production, which can also be confirmed by KEGG annotation; the expression extent of enzyme, which could be checked by qRT-PCR; the fermentation conditions, which could explain the high yields; the stability, which could be judged by CRISPR.

2 Data Format

FASTQ

Fastq output format:

The first read in x1.fq:

```
@FC4290FAAXX:4:1:3:84#CAGATC/1
CCAACTATGATAGCCAANAAGGGAAAGCCATAGAG
+
abb_aab_aa`a^aba^D[`a_`aaaa`_a_`__a
```

The first read in x2.fq:

```
@FC4290FAAXX:4:1:3:84#CAGATC/2
CGAAAGCTAGTGCTAAAGAAAACAATTTATATTTCATAAAATTG
+
ab`baa``aa_ba`aaa`aa`b_a^aa`a_aa`a`aa`a_aa_^
```

Format explanation:

Table 1 Fastq format explanation （Download)

Row	Description
1	@Reads ID
2	Base
3	+ Reads ID
4	Base Qulity

FASTA

FASTA format(also called Pearson format),which based on the format of text is used to record the sequences of DNA and protein. In the format, the sequence of DNA and protein are coded by single characters, and the name is allowed to be added as annotation at the beginning of each sequence. The first line of the sequence file is start by the symbol of ">" or ";" which is followed by annotation. The sequence is started from the second line, and only allowed characters can be used for coding. For DNA sequences, capitals or lowercases can be used for coding; for protein sequence, only capitals can be used. For example::

Fasta:

```
>scaffold1 35.9
AACTCCAAATGTTTTACATCCTTTTTTTATCCATAATATATAATCAACTGATATACA
```

Format explanation:

Table 2 Fasta format explanation （Download)

Row	Description
1	Sequence ID
2	Sequence Base

AGP

AGP file illustrates how ***Contig*** turn into ***Scaffold*** .Further info http://www.ncbi.nlm.nih.gov/projects/genome/assembly/agp/AGP_Specification.shtml .

AGP format:

scaffold5 1 83615 1 W scaffold5_1 1 83615 +
scaffold5 83616 83616 2 N 1 *Scaffold* yes paired-ends

Format explanation:

Table 3 AGP format explanation (Download)

Row	Description
1	Sequence ID
2	Start position of target sequence
3	End position of target sequence
4	Contig or gap Id for contig
5	Type of sequence (W-contig or N-gap)
6	Sequence ID or Length of gap
7	Sequence start position or type of gap
8	Sequence end position or the relationship between two gaps
9	Relatively direction or spaces

GFF

Gff format is defined by Sanger Institute. It is a simple and convenient format for features description of DNA, RNA and protein sequence. For example, we could detect the location of genes according to gff file. It has become a universal format, for example, many gene prediction software are compatible to it. Currently the version is gff (3).

gff:

Scaffold1 glimmer gene 113 2818 . + .
ID=CellulomonasGL000001;Name=CellulomonasGL000001;
Scaffold1 glimmer mRNA 113 2818 . + .
ID=CellulomonasGL000001;Parent=CellulomonasGL000001;
Scaffold1 glimmer CDS 113 2818 13.49 + 0 Parent=CellulomonasGL000001;

gffFormat explanation:

Table 4 gff format explanation (Download)

Row	Description
1	The ID of the landmark is used to establish the coordinate system for the current feature. IDs may contain any characters, but must escape any characters not in the set [a-zA-Z0-9.:^*$@!+_?-|].
2	The source is a free text qualifier intended to describe the algorithm or operating procedure that generated this feature.
3	The type of the feature (previously called the "method").
4	Start of feature
5	End of feature
6	The score of the feature, a floating point number.
7	The strand of the feature
8	For features of type "CDS", the phase indicates where the feature begins with reference to the reading frame.
9	A list of feature attributes in the format tag=value.

BLAST

BLAST m8 format is a list of blast results.

m8 format:

GL000017	98490	47.73	176	80	4	18	185	8	179	2e-28	124
GL000048	50873	62.31	650	234	3	267	913	5	646	0.0	795
GL000073	54575	43.20	125	64	4	420	540	61	182	1e-14	82.0

Format explanation:

Table 5 Blast m8 format explanation (Download)

Row	Description
1	Query ID
2	Subject ID
3	Identity value
4	Alignment length
5	Miss match
6	Gaps
7	Query start
8	Query end
9	Subject start
10	Subject end
11	E-value
12	Score

Synteny *.best.hit

13 columns are contained in the linear amino acid analysis results (*.best.hit), and the format is list below.

VDG2_01553	Scaffold_263	236249	238164	+	VDG1_00002	Scaffold_960	171	380	+	+	1.6e-35	100
VDG2_03579	Scaffold_438	383474	384874	-	VDG1_00004	Scaffold_358	32	430	+	+	2.3e-56	100
VDG2_06607	Scaffold_48	625429	626505	-	VDG1_00006	Scaffold_563	241	498	+	+	4.545e-34	95.24
VDG2_00894	Scaffold_160	109150	110319	+	VDG1_00008	Scaffold_405	665	1015	-	+	3e-37	94.67

*.best.hit format:

Table 6 *best.hit format explanation (Download)

ow	Description
1	Query Gene ID
2	Query ID
3	Query gene start position
4	Query gene end position
5	Query gene direction
6	Subject gene ID
7	Subject ID
8	Subject gene start position
9	Subject gene end position
10	Subject gene direction
11	Alignment direction of amino acid

12	E-value
13	Score

Core-Pan Gene *.matrix Format

*.matrix format:

VC1191 VC1215 VC1232 VC1242 VC1374 VC1447
VC1191GL000019: 100 100 100 100 100 100
VC1191GL000035: 100 100 100 100 100 100

Format explanation:

*.matrix is a two-dimension array combined the information of specie and gene. The first line of the file is sample name, and the number in the middle stand for the coverage of protein who correspond to its homology protein.

Core-Pan gene_cluster.list Format

gene_cluster.list format:

gi|116515320|ref|YP_816946.1| [4] gi|182684619|ref|YP_001836366.1| gi|221232414|ref|YP_002511567.1| gi|225859432|ref|YP_002740942.1| gi|307068302|ref|YP_003877268.1|

gene_cluster.list format:

Table 7 gene_cluster.list format explanation (Download)

Row	File Description
1	The first column of PanGene.matrix
2	The number of genes that homology with the gene in the first column
3	Name of homology gene

Gene Family KaKs results format

KaKs:

PGTG_06377T0_puccinia_graminis&PGTG_19280T0_puccinia_graminis YN 0.0266059 0.0431371 0.616775 0.187645 1140 336.24 803.76 NA 35 1 4 21 NA NA 0.0314817 3.10323:3.10323:1:1:1:1 0.432796(0.47235:0.327189:0.498848) NA NA NA NA

PGTG_06377T0_puccinia_graminis&PTTG_03193T0_puccinia_triticina YN 0.63951 0.986994 0.647938 0.0176614 1266 334.438 931.562 NA 568 168.397 3 99.603 NA NA 0.731305 2.18172:2.18172:1:1:1:1 0.484255(0.516129:0.369816:0.56682) NA NA NA NA

PGTG_06377T0_puccinia_graminis&PTTG_03194T0_puccinia_triticina YN 0.552949 0.867029 0.637752 0.0100534 1206 323.665 882.335 NA 498 1 53.254 344.746 NA NA 0.637242 2.14442:2.14442:1:1:1:1 0.460829(0.5:0.351382:0.531106) NA NA NA NA

KaKs format:

Table 8 KaKs format explanation (See all)

Row	Description
1	sequence identification
2	Ka, Ks algorithm name
3	Ka: non-synonymous substitution rate
4	Ks: synonymous substitution rate

5	Ka/Ks: selection pressure
6	P-Value(Fisher): Fisher accurate test
7	Length: sequence length (after filtration of gap and stop codon)
8	S-Sites: synonymous site number
9	S-Sites: synonymous site number
10	Fold-Sites(0:2:4): 0、2、4 substitution site number
11	Substitutions: substitution number
12	S-Substitutions: synonymous substitution number
13	N-Substitutions: non-synonymous substitution number
14	Fold-S-Substitutions(0:2:4): 0、2、4 synonymous substitution site number
15	Fold-N-Substitutions(0:2:4): 0、2、4 non-synonymous substitution site number
16	Divergence-Time: divergence time
17	Substitution-Rate-Ratio(rTC:rAG:rTA:rCG:rTG:rCA/rCA): ratio of substitution rate and rCA
18	GC(1:2:3): GC content of three sites in one codon and that of whole sequence
19	ML-Score: maximal likely score
20	AICc: AICc value

Detail illustration on *.tree.

***.tree:**

((spec1:0.28000,spec5:0.28000):0.08034[&&NHX:B=100],
(spec2:0.42025,spec3:0.37387):0.05261[&&NHX:B=100],spec4:0.41966);

***.tree format explanation:**

The two units included by a bracket stand for two branches at the same node, and the colon followed number stands for the degree of ramification (the average replacement frequency of each base). The number after 'B=' stands for the credibility of branch, and the branch is more dependable when the number close to 100.

3 Article Methods Described

3.1 Genome sequencing and assembly

The(species name) strain (sample name) genome was sequenced using a PacBio RS II platform and Illumina HiSeq 4000 platform at the Beijing Genomics Institute (BGI, Shenzhen, China). Four SMRT cells *Zero-Mode Waveguide* arrays of sequencing, were used by the PacBio platform to generate the subreads set. PacBio subreads (length < 1 kb) were removed. The program Pbdagcon (https://github.com/PacificBiosciences/pbdagcon) was used for selfcorrection. Draft genomic unitigs, which are uncontested groups of fragments, were assembled using the Celera Assembler against a highquality corrected circular consensus sequence subreads set. To improve the accuracy of the genome sequences, GATK (https://www.broadinstitute.org/gatk/) and SOAP tool packages (SOAP2, SOAPsnp, SOAPindel)were used to make single-base corrections.To trace the presence of any plasmid, the filtered Illumina reads were mapped using SOAP to the bacterial plasmid database (http://www.ebi.ac.uk/genomes/plasmid.html, last accessed July 8, 2016).

3.2 Genome Component prediction

Gene prediction was performed on the (sample name)genome assembly by glimmer3 (http://www.cbcb.umd.edu/software/glimmer/)with Hidden Markov models.tRNA, rRNA and sRNAs recognition made use of tRNAscan-SE (Lowe and Eddy, 1997), RNAmmer,

and the Rfam database.The tandem repeats annotation was obtained using the Tandem Repeat Finder (http://tandem.bu.edu/trf/trf.html) , and the minisatellite DNA and microsatellite DNA selected based on the number and length of repeat units .The Genomic Island Suite of Tools (GIST) used for genomicis lands analysis(http://www5.esu.edu/cpsc/bioinfo/software/GIST/) with IslandPath-DIOMB, SIGI-HMM, IslandPicker method . Prophage regions were predicted using the PHAge Search Tool (PHAST) web server (http://phast.wishartlab.com/) and CRISPR identification using CRISPRFinder.

3.3 Gene annotation and protein classification

The best hit abstracted using Blast alignment tool for function annotation.Seven databases which are KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), NR(Non-Redundant Protein Database databases), Swiss-Prot[18],and GO (Gene Ontology), TrEMBL, EggNOG are used for general function annotation . Four databases for pathogenicity and drug resistance analysis. Virulence factors and resistance gene were identified based on the core dataset in VFDB (Virulence Factors of Pathogenic Bacteria) and ARDB (Antibiotic Resistance Genes Database) database, other two are PHI (Pathogen Host Interactions) and (Carbohydrate-Active enZYmes Database). Type III secretion system effector proteins were detected by EffectiveT3.

3.4 Comparative genomics and phylogenetic analysis

The synteny of *** and **** was performed using MUMmer and BLAST Core/Pan genes of ***,*** and ****were clustered by the CD-HIT rapid clustering of similar proteins software with a threshold of 50% pairwise identity and 0.7 length difference cutoff in amino acid. Gene family is constructed by the gene of ***, ***and ****, integrating multi software: align the protein sequence in BLAST and eliminate the redundancy by solar and carry out gene family clustering treatment for the alignment results with Hcluster_sg software. The phylogenetic tree is constructed by the TreeBeST using the method of NJ.

4 NCBI Upload Help

This document provides methods for bacterial complete genome data upload;

4.1 Introduction to NCBI data terms

1) Data types that can be uploaded to NCBI

For a detailed list, see: https://www.ncbi.nlm.nih.gov/guide/howto/submit-sequence-data/

This document gives the upload process of the following data:

Table 9 Data type （Download)

Starting with...	NOTES	SUBMISSION TOOLS & HELP DOCUMENTS	DATABASE
Large complete genomes	ncludes paired chromosome and plasmids, as well as bacterial or eukaryotic chromosomes Questions regarding a specific submission that are not answered in the documented instructions can be sent to genomes@ncbi.nlm.nih.gov.	Prokaryotic Genomes submission Eukaryotic Genomes submission	GenBank

Incomplete genomes	These can be whole genome shotgun (WGS) sequences. WGS submissions should be prepared using the tbl2asn or Sequin tools. For assistance contact genomes@ncbi.nlm.nih.gov	Assembly submission information / Examples WGS submissions	GenBank
High throughput sequences	The Sequence Read Archive (SRA) accepts reads from high throughput sequencing instruments. Some submissions include sets of SRA reads as part of a comprehensive package. For the specific datasets described below, please initiate submissions with the appropriate archive:Human sequence or metagenome sequence data derived from clinical isolates or from sources with privacy concerns should be submitted to dbGaP. Functional genomics studies that examine gene expression, regulation or epigenomics (using methods such as RNA-Seq, miRNA-Seq, ChIP-Seq or methyl-Seq) should be submitted to GEO. Transcript survey sequence assemblies should go to the Transcriptome Shotgun Assembly (TSA) archive. Non-human and environmental metagenomics data should go to the Metagenome archive. Whole genome sequence assemblies should be submitted to WGS. Capillary traces should be deposited in the Trace Archive. Sequences from the Barcode of Life project should be submitted to Barcode. Curators of these resources will assist submitters in sending the data to SRA during the submission process.	For data types not mentioned to the left, submit directly to SRA: SRA submit page SRA submission guidance	SRA

2) Introduction of uploading tools

BankIt

URL: http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank.

Use BankIt if:

you have a single sequence, a simple set of sequences (for example:16S rRNA,matK, ITS/rRNA, amoE, tefB, cytb, or COI sets), or a small batch of different sequences

you prefer to use a web-based submission tool the feature annotation for your sequences is notcomplicated you do not require advanced sequence analysis tools

the following types of submissions are NOT acceptable:

sequences less than 200 nucleotides long, unless they represent complete exons, non-coding RNAs (ncRNAs),microsatellites or ancient DNA non-contiguous sequences that have been artificially joined; for example, multiple exons without their intervening introns or without a 'gap' of internal NNNs representing any missing sequence

protein-only sequences

single sequencesthat are a mix of molecule types, such as mix ofgenomic and mRNA sequence data

Expressed Sequence Tags (ESTs; these should be submitted through the dbEST system)

Genome Survey Sequences (GSSs; these should be submitted through the dbGSSc system)

Sequence Tagged Sites (STSs; these should be submitted through the dbSTS system)

Bankit is an online upload tool. Using bankit uploads, you need to provide these information: http://www.ncbi.nlm.nih.gov/WebSub/html/requirements.html.

Sequin

URL: http://www.ncbi.nlm.nih.gov/Sequin/index.html.

Use Sequin if:

you prefer to work on your submission off-line

you have a sequence or sequences that are complex

you would like graphical viewing andediting options, including an alignment editor

you would like the option to have network access to related analytical tools

Sequin is a native software that can be used after installation.The result of annotation can be generated by tbl2asn software as the input to sequin.

tbl2asn(Data conversion tool)

URL: http://www.ncbi.nlm.nih.gov/genbank/tbl2asn2.

tTbl2asn is command-line software. It converts sequence, annotation information into *.Sqn files.For the use of methods and detailed parameters, see the software home page.

Download Address: ftp://ftp.ncbi.nih.gov/toolbox/ncbi_tools/converters/by_program/tbl2asn/

3) Introduction of the databases

GenBank

URL: http://www.ncbi.nlm.nih.gov/genbank/

GenBank is the National Institutes of Health (NIH) gene sequence database, Contains all of the public DNA sequence and annotation information, it exchanges data with DDBJ and EMBL every day. Most of the sequence information is provided by submitter.

SRA

URL: http://www.ncbi.nlm.nih.gov/sra/

It stores sequenced data from the Next Generation Sequencing (NGS) platform include Roche 454 GS System®, Illumina Genome Analyzer®, Applied Biosystems SOLiD® System, Helicos Heliscope®, Complete Genomics®, and Pacific Biosciences SMRT®.

Transcriptome Shotgun Assembly (TSA)

URL: http://www.ncbi.nlm.nih.gov/genbank/tsa/.

It stores transcriptome assembly sequence of the Next Generation Sequencing.

RefSeq

URL: http://www.ncbi.nlm.nih.gov/RefSeq/

A comprehensive , comprehensive , non - redundant DNA sequence database based on the GeneBank database.Contains genomic DNA sequence, transcriptional sequence, protein sequence.

4) Interpretation of various NCBI numbers

My NCBI account: NCBI website account, You need to use this account to log in to NCBI before you can upload data. You can also use an external account to log in, look at https://www.ncbi.nlm.nih.gov/account/?back_url=https%3A%2F%2Fsubmit.ncbi.nlm.nih.gov%2Fsubs%2Fbioproject%2F.

BioProject number: total id of the data upload for each project.

accession number: id numbers allocated by NCBI after each sequence data is uploaded successfully.Even if the sequence updates, this id will not change.

VERSION: Version number of each sequence, The initial version is 1, updated to 2, and so on.The format is expressed as "accession.version".

GI id: Another id for each sequence, In the same genome, the genome sequence and its protein sequence have a unique GI number.

"Accession.version" and GI number can both uniquely identify a sequence, at the same time use these two id because:

-Some data sources processed by NCBI for incorporation into its Entrez sequence retrieval system do not version their own sequences.

-GIs provide a uniform, integer identifier system for every sequence NCBI has processed. Some products and systems derived from (or reliant upon) NCBI products and services prefer to use these integer identifiersbecause they can all be processed in the same manner.

For the concept of accession number, VERSION, GI number, please see: http://www.ncbi.nlm.nih.gov/Sitemap/samplerecord#AccessionB.

5) NCBI account application and login

All upload operations need to be carried out in a personal account, so first register My NCBI account, access to account and password. Log in directly If you already have a NCBIPDA account or third-party account of NCBI.

See How to create My NCBI account for details: http://www.ncbi.nlm.nih.gov/books/NBK3842/#MyNCBI.Registering_with_My_NCBI;

Step1: Open the account creation page: Click on the login link on the upper right corner of NCBI, As shown below:

Figure 1 .

See login screen as shown below, click "Register for an account" to create an account:

Figure 2 .

Step2: Fill in the information

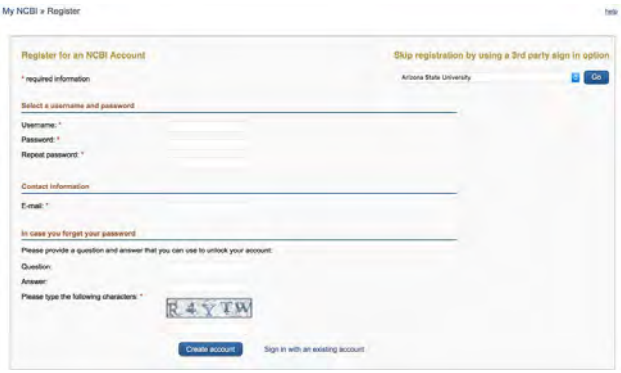


Figure 3 .

After you finish saving will receive a login name and password mail, Return to Step1 and login.

4.2 BioProject number, bioSample number application

To upload data, BioProject Number must be applied to describe the research project, And BioSample Number to describe the sample source, collection and other information of the research project;

1) BioProject Number application

URL: https://submit.ncbi.nlm.nih.gov/subs/bioproject/

Step1: Create a new submission

Figure 4 .

Step2: Fill in the submitter's information (the asterisk is marked as required, below)

Figure 5 .

Step3: Fill in the project type and sample source

Figure 6 .

Step4: Fill in the name of the species

Figure 7 .

Step5: Fill in the usual necessary information

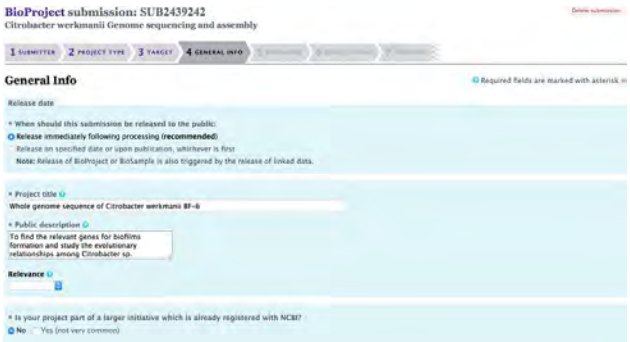


Figure 8 .

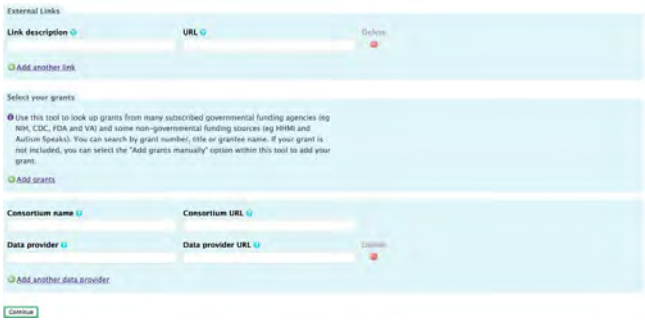


Figure 9 .

Step6: Fill in the BioSample number corresponding to the sample, If there is no such number, click "register at BioSample" to jump to the BioSample application page to apply.BioSample shape such as "SAMN02469977".

Figure 10 .

Step7: Publications information, without these information you don`t need to fill in

Figure 11 .

Step8: Check information

Figure 12 .

Check the information is correct, click Submit, wait a moment to refresh the page, you will get BioProject number: PRJNA376618,At the same time, your email will also receive the relevant information.

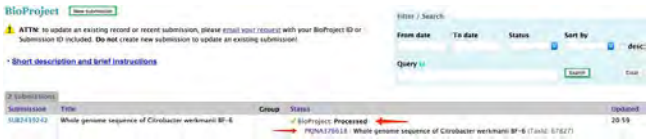


Figure 13 .

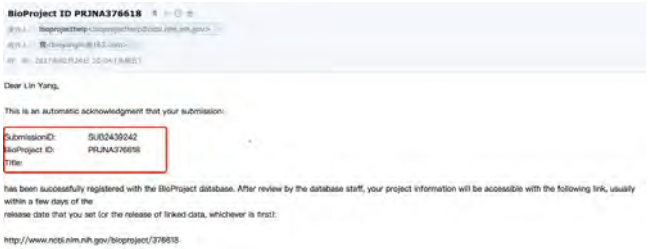


Figure 14 .

2) BioSample Number application

URL: https://submit.ncbi.nlm.nih.gov/subs/biosample/

Step1: Create a new submission

Figure 15 .

Step2: Select the data release date, preferably the same as the release date of BioProject

Figure 16 .

Step3: Choose the type of sample

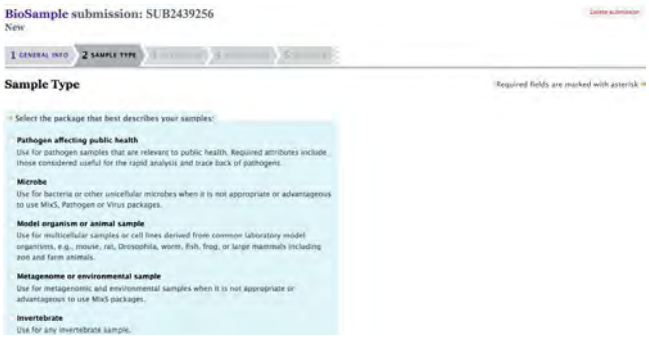


Figure 17 .

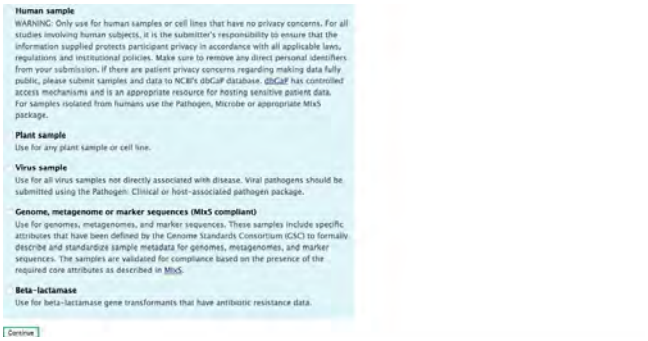


Figure 18 .

Step4: Fill in the strain related information

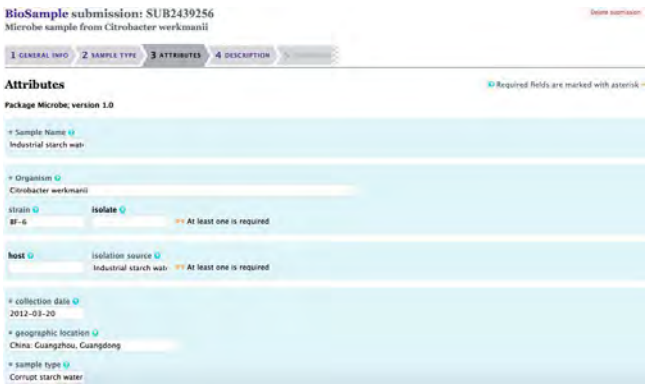


Figure 19 .

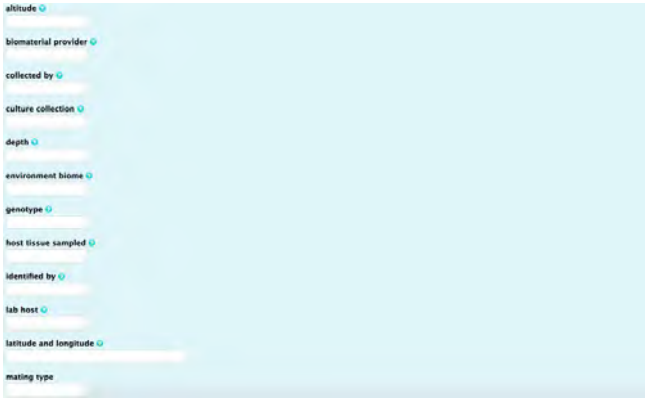


Figure 20 .

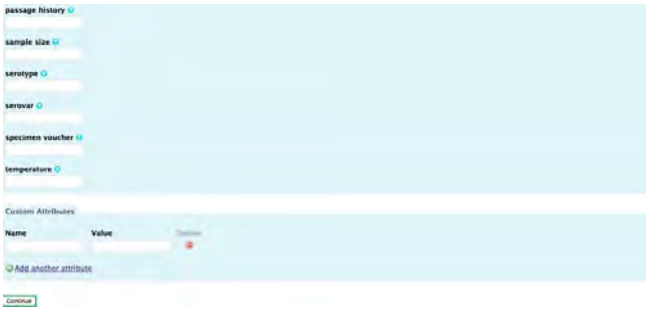


Figure 21 .

Step5: Fill in the sample title and description

Figure 22 .

Step6: Check information

Figure 23 .

Check the information is correct, click Submit, wait a moment to refresh the page, you will get BioSample number: SAMN06444903,At the same time, your email will also receive the relevant information.

Figure 24 .

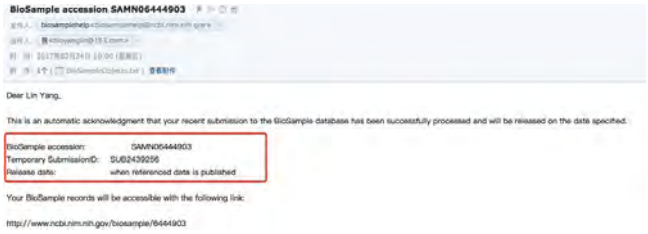


Figure 25 .

4.3 Methods for uploading bacterial complete genome data to GenomesMacroSend

uploading Bacterial complete genome data to GenomesMacroSend require three steps:

1) Apply BioSample Number, BioProject Number;

2) Prepare data format for upload;

4)Submit data to WGS and postal mail communication;

Complete Genome Submission Guide:

https://www.ncbi.nlm.nih.gov/genbank/genomesubmit/

GenomesMacroSend:

https://www.ncbi.nlm.nih.gov/projects/GenomeSubmit/genome_submit.cgi

1) Apply for BioSample Number, BioProject Number

Refer to the above document for application.

2) Prepare the data format to upload

Create a submission template file

URL: https://submit.ncbi.nlm.nih.gov/genbank/template/submission/

step1: Fill in the contact information

Figure 26 .

step2: Fill in the sequencing author, Reference tile and reference author, the sequencing author and Reference author can be the same, you can add more.

Figure 27 .

step3: Fill in Bioproject and BioSample

Figure 28 .

Finally click "Create Template" to save the file as Template.sbt.

Prepare the Contig file

Contigs sequence files with no gap, that is, the sequence does not contain N. No more than 10,000 sequences per file. Format: *Contig* file as standard fasta format, The first line is descriptive information, beginning with ">"; The second line is sequence information, each line length of not more than 80 characters. As shown below:

```
>contig0001
AAAACCTTCCCGTTGGCCTTCACCGTCTACTTAACGAGCCACGCCCCTCCTAGGACACCGCAAGAGAAAT
GCTGGGGTCACCCCTGGCCGAGGCCTCCCTCCTGCTGGCCACACGTAAGAAAGGACTTCACAAGGGAGAC
CTCCGTGGCTGCCACACACATTCACCCCAAATGCTTCCTGGAGAAAGCACCTGCCCTCACACTGTGAGCT
CGTGAGTTTGCCAAAAAGGAGATGCAGGAGCCTGAGATCACCTCCTGTCTTGCTGCTAAAATATCCCAGC
CGTGGAAAAGCAAGGCTGGCCTCAAATTGGGGAATCTGGTCTTGCCAGCCCAGCTGTGCTCCAGGGACTC
CGGTTTGCATTGGGAATGAGAGAGTGTTGGCCGGGTAAGATGGCAAGACAGACACAGTCCTCCTACAGAC
TTGTAGAAGGGCTTTCTGCCCGCCCCCACCCAGGGCAGAAAGAGGAGGCACAGGGGAAAACAACAAGAGC
CCTGGCCAAGAATGAGCCCTCTGCCGTCGTCCTGTGTGTGGCCTTGTGGCCCAGCACCAGCGCTGGGGGG
NCACTTTGCCCTGCCTGACAGGAGGAAGGGATGCCCTAGTGAGGTGGGAAACAGAGGGAGAGGTTGAGAC
CACCTTGGACAAGAAGGGCCAGGGAAGGCCCTNCCNTCACCTGTCACTACAGCCCGACACTTAGAAGGTA
```

Figure 29 .

File suffix can be * .fsa, * .fa, * .ctg, *. *Contig* (only need to fasta format), If the source of *Contig* is known (eg, from a plasmid), it should be indicated in the *Contig* file.As shown below: Plasmid name is unknown, then marked "unnamed".

```
>contig_seqid1 [organelle=mitochondrian]
>contig_seqid100 [plasmid=unnamed]
>contig_seqid200 [plasmid=pBB1]
```

Figure 30 .

Use tbl2asn software to generate * .sqn file

Two files are required to generate this file 1) Step 3.1 Generated: *. Sbt; 2) Step 3.2 Generated *Contig* file: *. Fsa.

put the above two files in tbl2asn software directory, Enter command prompt mode (Start - Run - cmd), into the tbl2asn software directory. Enter the following command, press Enter to run (note the space and ""): tbl2asn.exe -i *.fsa-t *.sbt-a s -V v -Z log -j "[organism=*][strain=*]"

-i: This parameter is the location of the ***Contig*** file, Such as d: /E.coli/ ***Contig*** .fsa, the sequence format should be fasta;

-t: This parameter is used to set the location of the template file, such as d: /E.coli/submit.sbt;

-a: Whether there is more than one ***Contig*** in the ***Contig*** file, set to s that there are multiple ***Contig*** , Non-complete genome sequences are composed of multiple ***Contig*** ;

-V: Output * .val file, used to detect whether there will be error exists to impact on the upload;

-Z: Output log files, easy to view the conversion process;

-j: Parameters that must be used to add sequence source information;

*.fsa: ***Contig*** file, only requires fasta format, no matter what file extension;

*.sbt: sequence information template file generated in Step 3.2;

[organism=*][strain=*]: You need to add * part of the content yourself;

After running smoothly, tbl2asn will generate three files named by * .fsa: *. Sqn, *. Val, log (same file name, different suffixes):

*.sqn file for the final submission job;

*.val file is used to see whether there is a problem with the conversion process;

The log file is used to monitor the entire conversion process.

In general, there is no problem with the entire conversion process if the * .val file size is 0 k. About -j parameters: source information, Selectivity is relatively large, including species, strains and other information can choose, Customers can choose according to the actual project situation, a detailed description can be viewed (https://www.ncbi.nlm.nih.gov/Sequin/modifiers.html): For the microbial genome, commonly used are: organism, strain, isolate, serovar, pathovar, the parameters used in the following format:

-j "[organism=Pseudomonas][pathovar=syringae][strain=A2]"

Other parameters of tbl2asn, please refer to: http://www.ncbi.nlm.nih.gov/genbank/tbl2asn2.html

3) Submit data to GenomesMacroSend and postal mail communication

Submit complete genome data to NCBI via GenomesMacroSend;

GenomesMacroSend:

https://www.ncbi.nlm.nih.gov/projects/GenomeSubmit/genome_submit.cgi

The submission page is shown below:

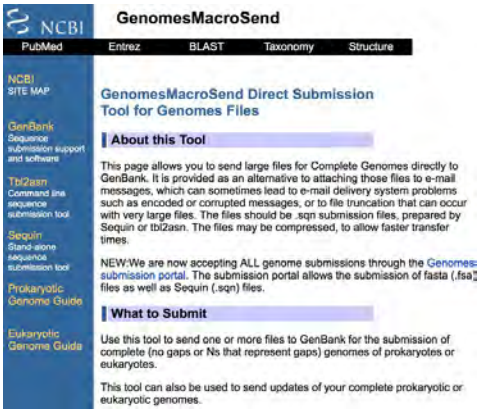


Figure 31 .

The asterisk is required, submit the sequence by selecting the file, If there is more than one file click the Add more file button.Confirm the information and click the Submit button.

Figure 32 .

It will jump to the following interface after successful submission, you will receive e-mail notification at the same time, NCBI staff will email you a few working days later.

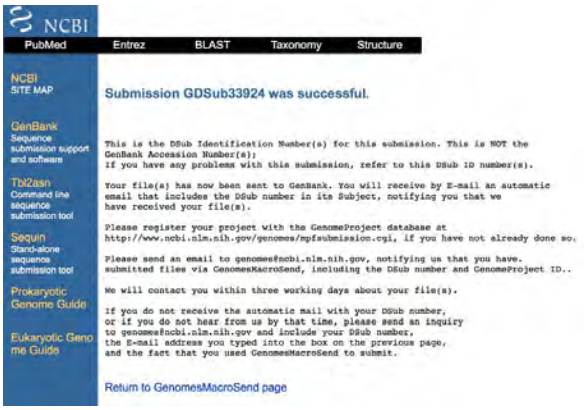
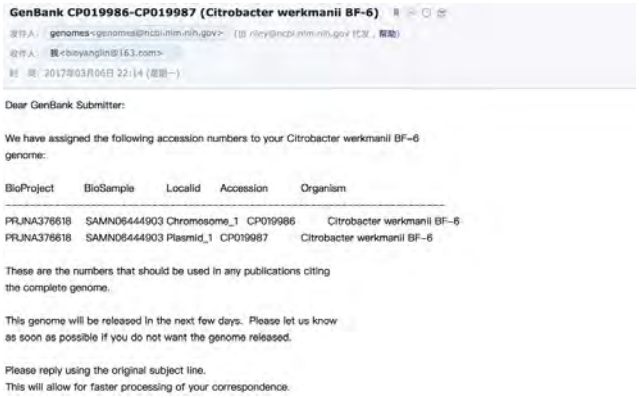


Figure 33 .

After the data is finally accepted, you will receive the following e-mail



GenBank CP019986-CP019987 (Citrobacter werkmanii BF-6)

发件人： genomes<genomes@ncbi.nlm.nih.gov>

收件人： 我<bioyanglin@163.com>

时 间： 2017年03月06日 22:14 (星期一)

Dear GenBank Submitter:

We have assigned the following accession numbers to your Citrobacter werkmanii BF-6 genome:

BioProject	BioSample	Locallid	Accession	Organism
PRJNA376618	SAMN06444903	Chromosome_1	CP019986	Citrobacter werkmanii BF-6
PRJNA376618	SAMN06444903	Plasmid_1	CP019987	Citrobacter werkmanii BF-6

These are the numbers that should be used in any publications citing the complete genome.

This genome will be released in the next few days. Please let us know as soon as possible if you do not want the genome released.

Please reply using the original subject line.
This will allow for faster processing of your correspondence.

Figure 34 .

FAQs

How to deal with the data if there have plasmid?

3 situations镑欹)if the plasmid(s) are very important to the customers or have highly request to the assembly result.We adivce to seperate the plasmid from genome and take Sanger sequencing镑沚)if the customer concerns in the genome, and genomic reference, we can go to the reference sequence of the plasmid is removed by comparison, but if in the presence of plasmid sequence sequence similarity with genomic sequence, it may remove not clean, and are assembled into the bacterial genome;c)if customers want to extract genomic DNA (containing plasmid DNA) were sequenced, if the plasmid reference sequence, will be map to reference read with separate assembly, but if some plasmids in HGT, may lose the new sequence information, if there is no plasmid reference sequence is difficult, according to the related plasmid replication the gene, locate the plasmid scaffold where, according to the pair-end, scaffold is able to look at both ends of the cyclization, or directly on the assembled scaffold whether there is any ring, but may have missed more plasmid sequence.

Why is the GC exceptions to build a PCR-free library?

PCR-free library is a library of small fragments of 200bp or 500bp, there is no PCR amplified library this step, because the general library in the process of constructing the PCR amplification step, while for high GC or low GC area, not easy to do in PCR amplification, sequencing result in the process of the regional coverage to reduce the degree of increase, PCR-free library can reduce the deviation, so as to improve the coverage of the region, improve the assembly results. The total amount of the PCR-free library is not less than 15ug (minimum 10ug), the concentration is not less than 30ng/ul, OD280/260 is 1.8-2.0, and there is no RNA pollution. The PacBio platform is the synthesis of "natural chain", and the abnormal GC has no effect on its sequencing, so the construction of PacBio sequenced library has no special requirements for the content of GC.

Do not know the reference sequence of the bacteria can do the completion of the map? What about the sample size?

In this case, the condition of GC content and repeat sequence ratio is unknown. It is difficult to achieve 1contig standard at a time. It is necessary to clarify strategies and indicators after negotiation. The sample amount of DNA is above 40ug.

What is the current strategy to bacterial completed genome?

At present, we have completed the joint assembly process of the bacterial completed genome (Illumina+PacBio). Because of the repeat base in the strains, the number of plasmids and plasmid sequences and genomic similarity degree of diversity, can not promise that all strains can definitely assembled into 1 contig, so now for free plasmid and simple bacterial sequence, repeat base <10% can promise, the other strains or according to the actual assembly evaluation results which belongs to the complex bacteria, according to the situation of negotiation assemble index, please contact the customers this point in the project before signing.

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Bioflag
Office Address : 4F., No. 17, Guoji Rd., Xinshi Dist., Tainan City 744, Taiwan (R.O.C.)
TEL: + 886 - 6 - 5891876 FAX: +886 - 6 - 5894810

L. rhamnosus, MP108, Antibiotic Resistance Determination

Antibiotic resistance testing of probiotic organism is advisable to ensure that antibiotic resistance determinants are not introduced into a context where these genes are at risk of being transferred to pathogenic organisms. The minimum inhibitory concentrations (MIC) of various antibiotics against *L. rhamnosus*, MP108, were determined using the microdilution method in accordance with ISO 10932 guidelines (ISO 10932: 2012 Milk and milk products- Determination of the minimal inhibitory concentration of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria). Assessment of the antimicrobial resistance pattern of *L. rhamnosus*, MP108, was determined by comparing the observed MIC's with the most recent European Food Safety Agency (EFSA) breakpoint values (EFSA Journal 2012). *L. rhamnosus*, MP108, was shown to be susceptible to most of antibiotics tested below the cut-off MIC established by EFSA, except for erythromycin and chloramphenicol. Further investigation is required to determine the nature of the resistance to these two antibiotics.

Antibiotic resistance profile of *L. rhamnosus* MP108			
Antibiotic	*L. rhamnosus* MP108*	EFSA Breakpoint Values *L. rhamnosus***	Susceptible (S) Resistant (R)
	µg/mL	µg/mL	
Gentamicin	8	16	S
Kanamycin	64	64	S
Streptomycin	16	32	S
Tetracycline	4	8	S
Erythromycin	2	1	R
Clindamycin	1	1	S
Chloramphenicol	8	4	R
Ampicillin	1	4	S
Vancomycin		n.r.	

n.r. not required.

*Report NO.: 110SN00744 tested by Food Industry Research & Development Institute, Hsinchu City 30062, Taiwan

**EFSA Journal 2012: 10(6): 2740

Result interpreter: Jui-Fen Chen

Date: 2021/03/15

APPENDIX C
GRAS Panel Consensus Statement

GRAS Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Use of *Lactobacillus rhamnosus* MP108 as an Ingredient in Conventional Food and Beverage Products

11 FEBRUARY 2022

INTRODUCTION

At the request of Glac Biotech Co., Ltd. (Glac Biotech), a panel of independent scientists, qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients (the GRAS Panel), was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information on *Lactobacillus rhamnosus* MP108 and to determine whether the intended uses of *L. rhamnosus* MP108 in various conventional food and beverage products, as described in Table A-1, are Generally Recognized as Safe (GRAS) based on scientific procedures. For purposes of the GRAS Panel's evaluation, "safe" or "safety" means there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use, as defined by the United States (U.S.) Food and Drug Administration (FDA) in 21 CFR §170.3(i) (U.S. FDA, 2021a). The GRAS Panel consisted of the below-signed qualified scientific experts: Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine); Michael W. Pariza, Ph.D. (University of Wisconsin-Madison), and I. Glenn Sipes, Ph.D. (University of Arizona College of Medicine).

The GRAS Panel was selected and convened in accordance with the U.S. FDA's draft guidance for industry on *Best Practices for Convening a GRAS Panel* (U.S. FDA, 2017). Prior to convening the GRAS Panel, all reasonable efforts were made to identify and select a balanced GRAS Panel with expertise in appropriate scientific disciplines deemed necessary for the safety evaluation of *L. rhamnosus* MP108, and efforts were placed on identifying conflicts of interest or relevant appearance issues that would potentially bias the outcome of the deliberations of the GRAS Panel; no such conflicts of interest or appearance of conflicts were identified. The GRAS Panel received reasonable honoraria as compensation for its time, and honoraria provided to the GRAS Panel were not contingent upon the outcome of the GRAS Panel's deliberations.

The GRAS Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data pertinent to the safety of *L. rhamnosus* MP108 that had been compiled from the published literature and other sources up to 14 July 2021. This information was summarized by Intertek and presented in a dossier titled, "*Documentation Supporting the GRAS Use of* Lactobacillus rhamnosus *MP108 in Food and Beverage Products*". The information evaluated by the GRAS Panel included information pertaining to the method of manufacture, product specifications and analytical data, the conditions of intended use of *L. rhamnosus* MP108, dietary intake estimates for the intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of *L. rhamnosus* MP108. In addition, the GRAS Panel evaluated other information deemed appropriate or necessary.

Following independent and collaborative critical evaluation of such data and information, the GRAS Panel met *via* teleconference on 27 January 2022. At the conclusion of this meeting, the GRAS Panel unanimously agreed that *L. rhamnosus* MP108, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS for use as an ingredient in conventional food and beverage products under the proposed conditions of use, as described in Table A-1. The GRAS Panel's conclusion on the GRAS status of *L. rhamnosus* MP108 is based on scientific procedures, and a summary of the basis for the GRAS Panel's conclusion is provided below.

CHARACTERIZATION OF *LACTOBACILLUS RHAMNOSUS* MP108

The food ingredient that is the subject of this GRAS evaluation is a lyophilized powder preparation of *L. rhamnosus* MP108. *L. rhamnosus* is a Gram-positive, aerotolerant anaerobe, lactic acid-producing bacteria (LAB) characterized by a rod-shape and facultative heterofermentation activity. The *Lactobacillus* genus has undergone an evolution of classification through advances in molecular techniques and the use of 16S rDNA gene sequencing. *L. rhamnosus* was formerly considered a subspecies of *L. casei*; however, taxonomic characterization by Collins *et al*. (1989) resulted in designation of *L. rhamnosus* as a separate species. The *L. rhamnosus* MP108 strain was initially identified by 16S rRNA and phenylalanyl-tRNA synthetase *alpha* subunit (pheS) gene sequencing. The genome has since been sequenced using *de novo* sequencing and subject to a full functional annotation. The strain was deposited in the Bioresource Collection and Research Center (Taiwan) under BCRC 19616.

More recent polyphasic taxonomic characterization studies by Zheng *et al*. (2020) were followed by reclassification of the genus *Lactobacillus* into 25 genera, and *Lactobacillus rhamnosus* was renamed *Lacticaseibacillus rhamnosus*. Despite this official name change, the nomenclature *Lactobacillus* remains valid, but use of the updated nomenclature is not currently widespread due to the familiarity of the original naming convention. Accordingly, the name *Lactobacillus rhamnosus (L. rhamnosus)* will be used throughout this consensus statement, as it was in the GRAS dossier.

MANUFACTURING AND SPECIFICATIONS

Glac Biotech's *L. rhamnosus* MP108 food ingredient is manufactured using an optimized microbial fermentation process followed by live microbe isolation and freeze drying. Briefly, the culture media is prepared using the components itemized in the "RM for culture medium" list in Figure 2.2-1. Sterilized growth media is prepared for the 200 L seed culture, which is grown *via* shaking at 37°C to a cell density of 1×10^9 colony forming units (CFU)/mL. The seed culture is used to inoculate the 2,500 L production culture, which is grown at 37°C and stirred at 30 RPM for 16 hours. Cells are isolated by centrifugation at 25°C and 16,000 RPM. Isolated cells are then freeze dried and mixed with maltodextrin to a final concentration of $\geq 1 \times 10^{11}$ CFU/g for packaging.

The *L. rhamnosus* MP108 ingredient product specifications are in compliance with best-practice limits for heavy metal and microbial contaminants, and analyses of 3 non-consecutive manufacture lots confirmed that the product is consistent between production lots and the ingredient consistently meets product specifications. Glac Biotech demonstrated product stability and maintenance of viability $\geq 1.0 \times 10^{11}$ CFU/g during storage for 12 weeks at 25°C and for greater than 24 weeks at 4°C.

INTENDED USES AND CONSUMPTION ESTIMATES

L. rhamnosus MP108 is intended for use as an ingredient in conventional foods and beverages, including those intended for infants and children (excluding infant formula), as outlined in Table A-1, at use levels providing up to 1 x 10^9 CFU/serving.

An assessment of the anticipated dietary exposure to *L. rhamnosus* MP108 as an ingredient under the intended conditions of use was conducted using data available in the 2017-2018 cycle of the U.S. National Center for Health Statistics (NCHS)'s National Health and Nutrition Examination Survey (NHANES) (CDC, 2021a,b; USDA, 2021). On a consumer-only basis, the proposed uses of *L. rhamnosus* MP108 in foods and beverages were estimated to result in a mean intake from all proposed food uses of 4 x 10^7 CFU/kg body weight/day (equivalent to 3.2 x 10^9 CFU/day). The heavy consumer (90^{th} percentile) intake from all proposed food uses was estimated to be 9 x 10^7 CFU/day/kg body weight/day (equivalent to 7.2 x 10^9 CFU/day). The largest absolute 90^{th} percentile intake within an individual population group was identified in infants and young children (0 to 2 years of age), who were estimated to consume 1 x 10^9 CFU/kg body weight/day (equivalent to 9.8 x 10^9 CFU/day).

NARRATIVE AND SAFETY INFORMATION

Safety Narrative

In the absence of FDA guidance on the safety evaluation of new food microorganisms, the GRAS evaluation of *L. rhamnosus* MP108 was conducted following consideration of guidance from other regulatory agencies, authoritative scientific bodies, and qualified scientific experts, and therefore included consideration of the European Food Safety Authority (EFSA) Qualified Presumption of Safety guidelines (EFSA, 2007), the guidelines for the Evaluation of Probiotics in Food (FAO/WHO, 2002) issued by the Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food, and the safety decision tree for evaluating microbial cultures intended for human and animal consumption published by Pariza *et al.* (2015). A thorough toxicological investigation of the ingredient in compliance with current best practice was conducted, findings from which corroborate results reported in a product-specific clinical evaluation and in clinical studies of other GRAS strains of *L. rhamnosus*.

History of Safe Use

The MP108 strain does not have a history of use in the food and beverage industry in the U.S.; however, this strain is available for use in infant and children's food in China following an approval by the National Health Commission (NHC) of China (National Health Commission of the PRC, 2021). In the European Union (EU), *L. rhamnosus* has been granted Qualified Presumption of Safety (QPS) status by EFSA which indicates that *"strains should not harbor any acquired antimicrobial resistance genes to clinically relevant antimicrobials"* (EFSA, 2018). As *L. rhamnosus* MP108 is an unmodified commensal bacterium, it is unlikely that the strain would possess traits that meet any of the QPS exclusion criteria cited above.

Metabolic Fate and Colonization

The mucosal lining of a healthy, human gastrointestinal (GI) tract is generally impermeable to translocation by bacteria, either during passage or as resident gut microbes. The translocation of live microorganisms from the lumen of the GI tract to circulation and extraintestinal sites is not common and often associated with increased GI permeability due to compromised integrity of the GI barrier. Several reviews and individual assessments detailing clinical reports of human infections related to oral exposure to *Lactobacillus* species related to *L. rhamnosus* MP108 and other LAB strains were critically evaluated as part of the safety evaluation of the product strain (Gasser, 1994; Borriello *et al.,* 2003; Robin *et al.,* 2010; Gouriet *et al.,* 2012; Vahabnezhad *et al.,* 2013; Goldstein *et al.,* 2015). *L. rhamnosus* species represented the largest species group of reported infections, but this was expected as the species also represents the most commonly used probiotic strains. Reported cases of *L. rhamnosus* strain infections were associated with prior conditions, not limited to but including preexisting infections, compromised immune system, and compromised oral health and dental procedures. These findings corroborate the conclusions drawn by Gasser (1994), Borriello *et al.* (2003), Gouriet *et al.* (2012), and Goldstein *et al.* (2015); these are extremely rare infections, and the safety of consumption of *L. rhamnosus* strains as probiotics is safe and unlikely to result in adverse events. The toxicology assessment published by Zhang *et al.* (2021) concluded that *"[based] on information on Lactobacillus spp., and on L. rhamnosus spp. in particular, there appears to be minimal concern regarding translocation and pathogenicity, at least in healthy populations […]*" when describing the product strain *L. rhamnosus* MP108.

Endpoints for evaluating gut colonization were reported in 4 clinical trials reviewed in the GRAS dossier, "*Documentation Supporting the GRAS use of Lactobacillus rhamnosus MP108 in Food and Beverage Products*", and all led to the same conclusions drawn by the study authors; significantly increased fecal concentrations of administered strains of *L. rhamnosus* were reported during administration phases, ranging from 2 to 28 weeks, ($p < 0.05$) which persisted for a short period following treatment cessation, but had returned to baseline in samples collected weeks after cessation (Firmesse *et al.,* 2008; Dommels *et al.,* 2009; Verdenelli *et al.,* 2009; de Andrade Pires *et al.,* 2020). These data indicate that while *L. rhamnosus* survives passage of the GI tract and persists for up to 2 weeks following treatment, colonization is transient as reported by the return of fecal *L. rhamnosus* concentrations to baseline levels in all cases.

Antibiotic Resistance and Toxigenicity

To establish the safety of *L. rhamnosus* MP108 for use as a food ingredient, the potential for antibiotic resistance transfer, virulence, and pathogenicity were investigated using both empirical and bioinformatics approaches. Data from interrogation of the genome annotation align with the low level of antibiotic resistance observed in the minimum inhibitor concentrations (MIC) analysis, supporting an intrinsic resistance observed for the species. A core-pan gene analysis was conducted using the genomes of 5 closely related strains of *L. rhamnosus* to evaluate the number and identity of genes not common to the species that may confer the phenotypic differences observed between strains. The metabolic fate of *L. rhamnosus* MP108 pertaining to bacterial translocation from the GI tract and the potential for gut colonization has been assessed using relevant data in the public domain, and a conclusion of safety was drawn.

The MICs of a variety of clinically relevant antibiotics against the product strain were determined in compliance with ISO 10932 guidelines for the microdilution method (ISO 10932: 2010 Milk and milk products – Determination of the minimal inhibitory concentration of antibiotics applicable to bifidobacterial and non-enterococcal lactic acid bacteria – ISO, 2010). The MIC values reported were compared to EFSA breakpoint values to determine susceptibility or resistance to the tested antibiotics. *L. rhamnosus* MP108 was sensitive to all antibiotics tested; however, the MIC levels for erythromycin and chloramphenicol were marginally higher than the EFSA breakpoint values for *L. rhamnosus*, indicating low-level resistance to these antibiotics. The GRAS Panel notes that chloramphenicol is no longer widely used in clinical practice in the U.S. due to toxicity concerns related to bone marrow aplasia (Scholar, 2007). The apparent low-level chloramphenicol resistance was attributed to an intrinsic mechanism of resistance not subject to extracellular horizonal gene transfer, and as the clinical significance of chloramphenicol is limited, the resistance is not relevant from a risk-assessment perspective.

The bioinformatic assessment of *L. rhamnosus* MP108 conducted by Glac Biotech corroborates these data, finding no unique genes that confer anti-microbial properties in the product strain. The genome sequence has been solved and functionally annotated. Analysis of the *L. rhamnosus* MP108 genome was conducted to screen for genetic risk factors associated with antimicrobial resistance, virulence factors, and pathogenicity using a variety of homology tools. Of significance to this GRAS notice were the functional annotation results from Basic Local Alignment Search Tool (BLAST) search against the following databases: the virulence factors of pathogenic bacteria (VFDB), the Comprehensive Antibiotic Resistance Database (CARD), pathogen-host interaction database (PHI), and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Interrogation of the genome annotation from comparison to the KEGG database identified 32 genes related to antimicrobial resistance and 11 genes associated with infectious disease. These data align with the low level of antibiotic resistance reported in the MIC analysis, supporting an intrinsic resistance observed for the species. A core-pan gene analysis was conducted using the genomes of 5 closely related strains of *L. rhamnosus* to evaluate the number and identity of genes not common to the species that may confer the phenotypic differences observed between strains. In this analysis, “clustered” genes that are common to all members of the group are distinguished from “unclustered” genes which are unique to each strain. The clustered genes presumably contribute to essential processes required for normal growth and metabolism of the microorganism, whereas the unclustered genes are responsible for the phenotypic differences observed between strains, such as abnormal antimicrobial resistance or alternate metabolic products. The functional annotation of the *L. rhamnosus* MP108 strain genome suggests that antibiotic resistance is intrinsic and demonstrates no concerns of virulence or horizontal antibiotic resistance gene transfer from consumption of the product strain. A study on a strain of *L. rhamnosus* in the human intestinal tract found that a mutation in the 23S rRNA genes that disrupted macrolide activity by decreasing its affinity for ribosomes, effectually conferring resistance in those carrying the mutation, may contribute to the reported resistance, further corroborating this conclusion (Flórez *et al.,* 2007).

Toxicological Studies

A comprehensive toxicological assessment of the product strain, including standard assays for genotoxicity and mutagenicity and acute and 90-day toxicology studies in rats was conducted. The study protocols were consistent with those certified by the Food Safety National Standard (China) which adheres to standards similar to the relevant Organization for Economic Co-operation and Development (OECD) Test Guidelines (TGs), was published by Zhang *et al.* (2021).

L. rhamnosus MP108 was not genotoxic under the conditions tested in a bacterial reverse mutation assay or an *in vivo* mouse spermatocyte chromosome aberration assay and was not mutagenic in an *in vivo* mouse micronucleus assay.

In a 90-day study (Food Safety National Standard [China]; equivalent to OECD TG 408) published by Zhang *et al*. (2021), Sprague Dawley rats (<6 weeks old) were divided into 3 treatment groups (n=10/sex/group) to receive *L. rhamnosus* MP108 at varying doses, and 1 group to serve as a placebo control, for 90 days. The doses for low-, mid-, and high-dose groups were 250, 500, and 1,500 mg/kg body weight/day, respectively, and were administered by gavage; the test item was dissolved in sterile water immediately prior to administration. There were 2 satellite groups (n=5/sex/group) in addition to the 90-day study, in which 1 group was administered a solvent control (reverse osmosis water) and the other group a dose of 1,500 mg/kg body weight/day for 45 days. Clinical observations were made daily, and food consumption and body weights were measured weekly throughout the study. Blood samples for hematology and biochemistry were collected at study initiation and 1 week prior to termination; ophthalmologic observations were made on the same schedule. No treatment-related adverse clinical findings were reported during the study. All test animals exhibited normal activity, growth, and food consumption, as compared to control. There were no significant changes in hematology or blood biochemistry metrics in any treatment group compared to control. At study termination, necropsy of animals in all treatment groups and both 45- and 90-day treated, did not reveal any significant changes in organ weights or other macroscopic observations compared to control. The results of histological examination of high-dose animals did not differ significantly from those of control animals.

The results of the toxicology studies evaluating the safety of *L. rhamnosus* MP108 demonstrate that the administration of up to 1,500 mg/kg body weight/day in rats for 90 days did not cause adverse reactions and the NOAEL is 1,500 mg/kg body weight/day, the highest dose tested.

Human Study with *L. rhamnosus* MP108

L. rhamnosus MP108

The safety of *L. rhamnosus* MP108 in humans was evaluated in a double-blind, randomized, placebo-controlled study conducted in children (4 to 48 months) with atopic dermatitis over 8 weeks (Wu *et al.,* 2017). Children in the treatment group (n=30; 80% male subjects, 1.4 ± 1.1 years, 10.5 ± 3.0 kg, 77.1 ± 12.7 cm) received 1 capsule of *L. rhamnosus* MP108 powder [ComProbi – 350 mg *L. rhamnosus* MP108 ($\geq 1.0 \times 10^{11}$ CFU/g) and maltodextrin] once daily; the control group (n=32; 56.3% male subjects, 1.8 ± 1.1 years, 11.6 ± 3.0 kg, 83.0 ± 11.8 cm) received a placebo containing maltodextrin only. The primary efficacy endpoint was a Scoring of Atopic Dermatitis (SCORAD) index using the Hanifin and Rajka criteria (Tada, 2002) at baseline compared to Week 4 and Week 8. A significant difference (decrease) in SCORAD was reported between treated (-23.20 ± 15.24) and control (-12.35 ± 12.82) groups (p=0.002) over 8 weeks of treatment. The safety assessment included the clinical observations of blood pressure, heart and respiratory rate, and ear temperature at 0, 4, and 8 weeks of treatment. No significant changes were reported in any of the safety parameters measured. Adverse events were reported in 42.42% (n=35) of treated subjects and 45.45% (n=37) of control subjects, but "*showed no relation to [the] study products (data not shown)*". The authors concluded that administration of *L. rhamnosus* MP108 is safe for consumption in children ages 4 to 48 months for up to 8 weeks at a dose 175 mg of test article, approximately 3.5×10^{10} CFU/day.

GRAS Strains of *L. rhamnosus*

The strains of *L. rhamnosus* spp. have QPS status in the EU, as designated by EFSA, which is contingent on the absence of transferable antimicrobial elements (EFSA, 2018). The following clinical assessments of *L. rhamnosus* were selected to evaluate similarities between the response to MP108 strain and 2 strains that are GRAS, ***L. rhamnosus* GG** and ***L. rhamnosus* HN001**. The LGG and HN001 strains of *L. rhamnosus* were included in this section because they are GRAS. Representative studies for each strain have been discussed below.

LGG is one of the most extensively studied and well-characterized strains of *L. rhamnosus* and was selected for use as an ingredient in infant formula, as described in GRAS Notice (GRN) 231, for the *"strong safety and scientific profile"* of the strain. LGG has been available in the EU for use in infant formulae (hypoallergenic Stage 1 and Stage 2 formulae) since 2003, and in the U.S. following notification of GRN 231 to the FDA in 2007. While the history of safe use, food categories, and use levels of LGG are consistent with the EFSA QPS status of the species, which is GRAS, relevance of LGG safety data to the safety evaluation of *L. rhamnosus* MP108 has not been established in the literature; however, reported responses to consumption in infants appear to be similar between these strains. A randomized, controlled, blinded clinical trial was conducted to evaluate the effectiveness of LGG as an oral treatment for acute watery diarrhea in children (<2 years of age). No adverse effects related to the test article were reported in treatment group administered up to 2 x 10^{12} CFU/day LGG for 7 days, including the monitoring period following treatment cessation (Basu *et al.,* 2009). A retrospective cohort study that recruited infants for administration of 3.5 x 10^9 CFU/day LGG from 4 days post-natal to 4 to 6 weeks of age reported that treatment was "*microbiologically safe and clinically well tolerated*" with no test article-related adverse events (Manzoni *et al.,* 2011). A 28-week study of children (2 to 6 years of age) administered up to 1.8 x 10^8 CFU/day LGG reported no serious treatment-related adverse events, and the consumption of LGG at this level is not expected to cause adverse reactions (Kumpu *et al.,* 2012). A dose of 1 x 10^{10} CFU/day LGG in infants for 5 days caused no treatment-related incidents for up to 12 months following cessation (Schnadower *et al.,* 2017). These clinical studies evaluating LGG in infants are representative of the 10 infant studies identified in a search of publicly available literature. The duration of treatment and the study initiation points vary among these studies, but all report similar findings of no treatment-related adverse events and a conclusion of safety for use in this demographic at levels as high as 1 x 10^9 CFU/day LGG for at least 4 weeks (Basturk *et al.,* 2020).

The other strain of *L. rhamnosus* with GRAS status is HN001, which was isolated from cheddar cheese and has been maintained in the LAB collection at the Fonterra Research Centre (New Zealand), where it has been used in food products for decades. The tolerability of *L. rhamnosus* HN001 in pregnant women and their neonates from as early as Week 14 of gestation through birth, and in infants post-natal, has been demonstrated in studies evaluating the administration of *L. rhamnosus* HN001 at 6 x 10^9 CFU/day for up to 24 months (Dekker *et al.,* 2009; Barthow *et al.,* 2016; Wickens *et al.,* 2017). These treatments "*had no effect on measures of general growth, health, and tolerance*" and authors concluded that consumption was "*safe and well tolerated*" during treatment and follow-up periods (Dekker *et al.,* 2009; Barthow *et al.,* 2016).

The QPS status of *L. rhamnosus* spp. and the similarity in the reported no adverse effect responses to administration of MP108, LGG, or HN001 in infants corroborate their safety, and it is expected that no adverse effects will occur due to consumption of *L. rhamnosus* MP108 up to 3.5 x 10^{10} CFU/day for 8 weeks, which is greater than the estimated 90th percentile exposure.

Margin of Safety Estimates

A no-observed-adverse-effect level (NOAEL) for *L. rhamnosus* MP108 of 1,500 mg, equivalent to 1.5×10^{11} CFU/kg body weight/day, was determined in the 90-day study in rats by Zhang *et al.* (2021). This NOAEL is 2 orders of magnitude higher than the highest 90th percentile exposure estimates for dietary intakes of *L. rhamnosus* MP108 in infants (1×10^9 CFU/kg body weight/day; <2 years of age).

Application of the Decision Tree Approach (Pariza *et al*., 2015)

The GRAS Panel agreed that available data and information characterizing the identity and hazard of *L. rhamnosus* MP108 were suitable for evaluation of safety using the decision tree approach for microbial cultures intended for human and animal consumption (Pariza *et al.,* 2015). The decision tree is included in Attachment B. Based upon safety considerations evaluated using the Pariza decision tree paradigm, the following were noted:

- The phenotypic and genomic identity of *L. rhamnosus* MP108 is well-characterized, and no phenotypic or genotypic attributes could be identified to suggest that the strain may display pathogenic or toxicogenic potential.
- *L. rhamnosus* MP108 was isolated from human infant feces, and members of this species are present within the gastrointestinal tract of humans from birth through adulthood.
- *L. rhamnosus* MP108 was without evidence of toxicity in a subchronic toxicity evaluation using Sprague-Dawley rats conducted based on the healthy food safety assessment issued by the Chinese Department of Health.
- *L. rhamnosus* MP108 was concluded to be safe based upon findings reported in a product-specific human study, as well as the study of the GRAS strains of *L. rhamnosus,* LGG and HN001. Based on phenotypic and genotypic characterization of *L. rhamnosus* MP108, the GRAS panel concluded that studies conducted using the GRAS *L. rhamnosus* strains were relevant to the safety evaluation of *L. rhamnosus* MP108.

CONCLUSION

We, the undersigned independent qualified members of the GRAS Panel, have individually and collectively, critically evaluated the data and information summarized above, and other data and information that we deemed pertinent to the safety of the proposed use of *Laetobacil/us rhamnosus* MP108 as an ingredient in select food and beverage products, as described in Table A-1, including those intended for infants and young children (excluding infant formula), at a use level of 1.0 x 10^9 CFU/serving. We unanimously conclude that the proposed use of Glac Biotech's *Laetobacil/us rhamnosus* MP108, produced in a manner consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications as presented in the supporting dossier, *"Documentation Supporting the GRAS Use of* Lactobacillus rhamnosus *MP108 in Food and Beverage Products",* is safe.

We further unanimously conclude that the proposed use of Glac Biotech's *Laetobacillus rhamnosus* MP108, produced in a manner that is consistent with cGMP and meeting appropriate food grade specifications as presented in the supporting dossier is Generally Recognized as Safe (GRAS) based on scientific procedures under the conditions of intended use in foods specified herein.

It is our opinion that other qualified experts would concur with these conclusions.

[redacted]

15 February 2022

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[redacted]

11 February 2022

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ATTACHMENT A: INTENDED FOOD USES AND USE LEVELS FOR *LACTOBACILLUS RHAMNOSUS* MP108 IN THE UNITED STATES

Table A-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Food Uses*	Proposed Use Level ($CFUx10^9$/Serving)	RACC[a] (g or mL)	Maximum Intended Use Level ($CFUx10^9$/100 g)
Beverages and Beverage Bases	Energy Drinks	1.0	360	0.28
	Enhanced, Flavored, Carbonated, or Fortified Water Beverages	1.0	360	0.28
	Non-Milk-Based Meal Replacement, Protein, and Nutritional Beverages	1.0	240	0.42
	Sports Drinks	1.0	360	0.28
	Bottled tea	1.0	360	0.28
Breakfast Cereals	Hot Breakfast Cereals (*e.g.*, oatmeal, grits)	1.0	40 to 55	2.50
	Ready-to-Eat Breakfast Cereals			
	Puffed Cereals	1.0	15	6.67
	High-Fiber Cereals	1.0	40	2.50
	Biscuit-Type Cereals	1.0	60	1.67
Cheeses	Cheeses	1.0	30 to 110	3.33
Chewing Gum	Chewing Gum	1.0	3	33.33
Dairy Product Analogs	Non-Dairy Milk (soy-based drinks)	1.0	240	0.42
Gelatins, Puddings, and Fillings	Milk-Based Desserts	1.0	130 to 150	0.77
Grain Products and Pastas	Cereal and Granola Bars	1.0	40	2.50
	Energy Bars, Protein Bars, Meal Replacement Bars and Soy-Based bars	1.0	40	2.50
Hard Candy	Hard Candy	1.0	15	6.67

Table A-1 Summary of the Individual Proposed Food Uses and Use Levels for *L. rhamnosus MP108* in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Food Uses*	Proposed Use Level ($CFUx10^9$/Serving)	RACC[a] (g or mL)	Maximum Intended Use Level ($CFUx10^9$/100 g)
Milk Products	Buttermilk	1.0	240	0.42
	Evaporated, Condensed, and/or Dry Milks	1.0	30	3.33
	Fermented Milks, Plain	1.0	240	0.42
	Flavored Milks, Milk Drinks, and Mixes	1.0	240	0.42
	Milk Shakes	1.0	240	0.42
	Milk-Based Meal Replacement, Nutrition, and Protein Beverages[b]	1.0	240	0.42
	Plain or Flavored Yogurt	1.0	170	0.59
	Yogurt Drinks	1.0	93 to 207[c]	1.08
Plant Protein products	Soy-based Food	1.0	85	1.18
Processed Fruits and Fruit Juices	Fruit Drinks and Ades Including Smoothies	1.0	240	0.42
	Fruit Juices	1.0	240	0.42
	Fruit Nectars	1.0	240	0.42
Soft Candy	Soft Candy, Chocolate, Gummies, Mints, Nougat and Toffees	1.0	30	3.33
Other – Baby Food	Baby food: Cereals			
	Dry Instant	1.0	15	6.67
	Prepared, Ready-to-Serve	1.0	110	0.91
	Baby food: Ready-to-Eat cereals	1.0	100	1.00
	Baby food: Fruits or Vegetables (strained)	1.0	125	0.80
	Baby food: Fruit Juice	1.0	120	0.83

CFR = *Code of Federal Regulations*; CFU = colony forming units; RACC = Reference Amounts Customarily Consumed; U.S. = United States.

* *L. rhamnosus* MP108 is intended for use in unstandardized products and not in foods where standards of identity exist and do not permit its addition.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2021b). When a range of values is reported for a proposed food use, particular foods within that food use may differ with respect to their RACC.

[b] Includes ready-to-drink and powdered forms.

[c] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently on the U.S. market.

ATTACHMENT B: DECISION TREE FOR DETERMINING THE SAFETY OF MICROBIAL CULTURES TO BE CONSUMED BY HUMANS (PARIZA *ET AL.,* 2015)

The decision tree for determining the safety of microbial cultures to be consumed by humans or animals published by Pariza *et al.* (2015) was applied as follows to evaluate the safety of *Lactobacillus rhamnosus* MP108 for human consumption:

1. Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology? (If YES, go to 2. If NO, the strain must be characterized and unambiguously identified before proceeding).

   Answer: Yes

   Taxonomic identity of L. rhamnosus MP108 was confirmed by 16S rRNA and phenylalanyl-tRNA synthetase alpha subunit (pheS) gene sequencing and whole-genome sequencing and annotation, differentiating the strain from other characterized strains of L. rhamnosus.

2. Has the strain genome been sequenced? (If YES, go to 3. If NO, the genome must be sequenced before proceeding to 3.)

   Answer: Yes

3. Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity? (If YES, go to 4. If NO, go to 15.)

   Answer: Yes

   While interrogation of the genome sequence led to the identification of potential genes similar to known virulence factors, they were determined not to be a risk factor due to insufficient sequence identity, or they were demonstrated to represent intrinsic resistance conserved within the species and therefore not likely to represent virulence factors.

4. Is the strain genome free of functional and transferable antibiotic resistance gene DNA? (If YES, go to 5. If NO, go to 15.)

   Answer: Yes

5. Does the strain produce antimicrobial substances? (If NO, go to 6. If YES, go to 15.)

   Answer: No

   The observed resistance to erythromycin and chloramphenicol exhibited by the MP108 strain appears to be intrinsic and not the direct result of production of a known antibiotic resistance compound.

6. Has the strain been genetically modified using rDNA techniques? (If YES, go to 7a or 7b. If NO, go to 8a or 8b.)

   Answer: No

8a. For strains to be used in human food: Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an "incidental isolate")? (If YES, go to 9a. If NO, go to 13a.)

   Answer: No

13a. For strains to be used in human food: Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies? (If YES, go to 15. If NO, go to 14a.)

   Answer: No

   In a 90-day study in rats by Zhang et al. (2021), the NOAEL was determined by the authors to be 1,500 mg/kg body weight/day, equivalent to (>1.5 x 10^{11} CFU/kg body weight/day), the highest dose tested.

14a. The strain is deemed to be safe for use in the manufacture of food, probiotics, and dietary supplements for human consumption.

From: Shawn,Hsia (夏可強)
To: Deng, Kaiping
Cc: Kyle Weston Intertek
Subject: RE: [EXTERNAL] FW: Filing Letter- GRN 001130
Date: Friday, June 9, 2023 1:02:18 PM
Attachments: image002.png
image003.png
China_MP108_Deposition Number_CGMCC 21225.pdf

Dear Dr. Deng,

Thank you for sharing the FDA's views on the use of culture deposit designations vs. trade names as identifiers of microbial ingredients under the agency's voluntary GRAS notification program. The *L. rhamnosus* MP108 is stored in multiple international depositories, including the Taiwanese Bioresource Collection and Resource Center (BCRC), the DSMZ-German Collection of Microorganisms and Cell Cultures, and the China General Microbiological Culture Collection Center. To harmonize the company's global regulatory strategy for the strain, gLac Biotech would like to use the Chinese collection deposit number CGMCC 21225 as the official strain identifier in the GRAS inventory and filing letter. Please find attached the deposit certificate of the MP108 strain at CGMCC in China for your reference. If you need further documentation in this regard please let us know. I am looking forward to receiving your reply.

Best regards

Shawn

From: Deng, Kaiping <Kaiping.Deng@fda.hhs.gov>
Sent: Wednesday, May 24, 2023 4:05 AM
To: Shawn,Hsia (夏可強) <shawn.hsia@glac.com.tw>
Cc: Kyle Weston Intertek <kyle.weston@intertek.com>
Subject: RE: [EXTERNAL] FW: Filing Letter- GRN 001130

Dear Mr. Hsia and Dr. Weston:

Thank you for your emails.

While we have referred to bacterial strains in our response letters by their trade name in the past (e.g., *L. rhamnosus* strain "MP108"), we are now referencing strains using deposited designations (e. g., *L. rhamnosus* strain BCRC 19616). It is the FDA standard practice to use the deposit designation in our correspondence rather than a trade name.

We have referred to the bacterial strain in the filing letter of GRN 001130 by the provided depository

name, *L. rhamnosus* strain BCRC 19616. We will reference the strain using the deposited designation in our future correspondence related to this GRAS notice. We do not wish to provide commentary on trade names.

We have addressed the connection of the deposit designation and the trade name through our administrative record as detailed below:

In the January 26, 2023, submission of GRN 001130, trade name of the GRN subject *L. rhamnosus* strain "MP108" is used.
In the GRAS notice (p.8), the notifier states that "The strain was deposited in the Bioresource Collection and Research Center (Taiwan) under BCRC 19616." The provided deposit designation is used for FDA correspondence.

Therefore, we believe the record sufficiently conveys the relationship between *L. rhamnosus* strain "MP108" and strain BCRC 19616.

Should you have any additional questions, please do not hesitate to let me know.

Sincerely,
Kaiping

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18 August 2023

Kaiping Deng, Ph.D.
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Re: GRAS Notice No. GRN 001130

Dear Dr. Deng,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 04 August 2023 pertaining to information provided within Glac Biotech Co., Ltd.'s ("Glac Biotech's") Generally Recognized as Safe (GRAS) Notice for the intended use of *Lactobacillus rhamnosus* (MP108) CGMCC 21225 filed by the Agency under GRAS Notice (GRN) 001130.

FDA.1. *You note that "the genome of the organism was sequenced using bacterial de novo sequencing to generate an assembly map of the genome (Table 2.1.5-1)." (p.8). Please clarify whether its genome data is available in a public domain, e.g., a NCBI accession number.*

The whole genome sequence data has not been uploaded to the NCBI database for public reference; no accession number is currently available.

FDA.2. *You annotate virulence genes in the L. rhamnosus CGMCC 21225 genome with the Virulence Factor Database (VFDB) tool (Table 6.5.2-1, p. 25) and identify 94 genes. Please clearly summarize the putative functions of the 94 annotated genes identified by VFDB and confirm that they are not safety concerns.*

The Virulence Factor Database (VFDB) annotation was generated using the VFDB blast function. The 94 predicted genes and their respective putative functions are presented in Table 1. It should be noted that none of the genes identified shared greater than 80% sequence identity.

Table 1 Virulence Factor Database Annotated Genes – *Lactobacillus rhamnosus* CGMCC 21225

Gene ID	Identity (%)	E-value	Search Type	Putative Function Description
MP108GL001673	77.63	3.00E-95	Predicted	(lisR) two-component response regulator [LisR/LisK (CVF253)] [Listeria monocytogenes EGD-e]
MP108GL001981	77.22	2.00E-164	Predicted	(STER_1222) dTDP-D-glucose 4,6-dehydratase [Capsule (CVF186)] [Streptococcus thermophilus LMD-9]
MP108GL001983	74.39	1.00E-128	Predicted	(rmlA) Glucose-1-phosphate thymidylytransferase, putative [Capsule (CVF186)] [Streptococcus sanguinis SK36]
MP108GL001323	73.4	3.00E-170	Predicted	(tuf) translation elongation factor Tu [EF-Tu (CVF587)] [Mycoplasma mycoides subsp. mycoides SC str. PG1]
MP108GL002189	70.86	0	Predicted	(groEL) chaperonin GroEL [GroEL (CVF403)] [Clostridium thermocellum ATCC 27405]
MP108GL000907	69.74	1.00E-80	Verified	(clpP) ATP-dependent Clp protease proteolytic subunit [ClpP (VF0074)] [Listeria monocytogenes EGD-e]
MP108GL001037	69.57	3.00E-116	Predicted	(hasC) UTP--glucose-1-phosphate uridylyltransferase [Capsule (CVF186)] [Streptococcus pyogenes MGAS10270]
MP108GL000912	67.9	2.00E-173	Predicted	(eno) phosphopyruvate hydratase [Streptococcal enolase (CVF153)] [Streptococcus pneumoniae D39]
MP108GL001571	67.37	6.00E-91	Predicted	(uppS) undecaprenyl diphosphate synthase [Capsule (CVF618)] [Enterococcus faecium Aus0004]
MP108GL001492	63.04	6.00E-108	Predicted	(sigA/rpoV) RNA polymerase sigma factor SigA (Sigma-A) [Sigma A (CVF325)] [Mycobacterium canettii CIPT 140070008]
MP108GL001980	61.87	1.00E-97	Predicted	(STER_1444) dTDP-4-dehydrorhamnose reductase [Capsule (CVF186)] [Streptococcus thermophilus LMD-9]
MP108GL000689	61.58	0	Predicted	(lap) putative alcohol-acetaldehyde dehydrogenase [Listeria adhesion protein (CVF228)] [Listeria ivanovii subsp. ivanovii PAM 55]
MP108GL001765	61.16	0	Verified	(clpE) ATP-dependent protease [ClpE (VF0073)] [Listeria monocytogenes EGD-e]
MP108GL002384	60.99	2.00E-100	Verified	(efaA) endocarditis specific antigen [EfaA (VF0354)] [Enterococcus faecalis V583]
MP108GL002467	60.82	0	Verified	(clpC) endopeptidase Clp ATP-binding chain C [ClpC (VF0072)] [Listeria monocytogenes EGD-e]
MP108GL001552	59.73	0	Predicted	(CT396) molecular chaperone DnaK [MOMP (AI392)] [Chlamydia trachomatis D/UW-3/CX]
MP108GL001330	58.45	8.00E-142	Predicted	(tig/ropA) Trigger factor, putative [Trigger factor (CVF149)] [Streptococcus sanguinis SK36]
MP108GL001627	57.32	3.00E-77	Predicted	(CD1208) putative RNA methyltransferase [Hemolysin (CVF417)] [Clostridium difficile 630]
MP108GL001979	57.29	2.00E-66	Predicted	(epsE) sugar transferase; probable phospho-glucosyltransferase [Polysaccharide capsule (CVF567)] [Bacillus thuringiensis serovar konkukian str. 97-27]
MP108GL001942	57.06	2.00E-121	Verified	(cps4I) UDP-N-acetylglucosamine-2-epimerase [Capsule (VF0144)] [Streptococcus pneumoniae TIGR4]
MP108GL000909	56.6	3.00E-107	Predicted	(plr/gapA) glyceraldehyde-3-phosphate dehydrogenase, type I [Streptococcal plasmin receptor/GAPDH (CVF123)] [Streptococcus pneumoniae D39]
MP108GL000587	54.91	2.00E-105	Predicted	(galE) UDP-glucose 4-epimerase [Polysaccharide capsule (CVF567)] [Bacillus thuringiensis str. Al Hakam]
MP108GL000664	54.74	1.00E-34	Predicted	(ndk) Putative nucleoside diphosphate kinase NdkA (NDK) (NDP kinase) (nucleoside-2-P kinase) [Nucleoside diphosphate kinase (CVF660)] [Mycobacterium canettii CIPT 140070017]
MP108GL001742	54.71	2.00E-68	Predicted	(virR) hypothetical protein [VirR/VirS (CVF252)] [Listeria monocytogenes EGD-e]

Table 1 Virulence Factor Database Annotated Genes – *Lactobacillus rhamnosus* CGMCC 21225

Gene ID	Identity (%)	E-value	Search Type	Putative Function Description
MP108GL000889	53.96	6.00E-83	Predicted	(lgt) prolipoprotein diacylglyceryl transferase [Lipoprotein diacylglyceryl transferase (CVF248)] [Listeria monocytogenes SLCC2376]
MP108GL000931	51.52	9.00E-68	Predicted	(sugC) ABC transporter ATP-binding protein [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium smegmatis str. MC2 155]
MP108GL001976	49.92	4.00E-170	Verified	(clpE) ATP-dependent protease [ClpE (VF0073)] [Listeria monocytogenes EGD-e]
MP108GL002668	49.6	0	Verified	(EF3023) polysaccharide lyase, family 8 [Hyaluronidase (VF0359)] [Enterococcus faecalis V583]
MP108GL002778	49.56	4.00E-59	Predicted	(regX3) two component transcriptional regulator [RegX3 (CVF667)] [Mycobacterium vanbaalenii PYR-1]
MP108GL000945	49.36	3.00E-63	Predicted	(sugC) maltodextrin import ATP-binding protein MsmX [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium abscessus subsp. bolletii str. GO 06]
MP108GL000711	49.32	2.00E-141	Predicted	(dltA) D-alanine-activating enzyme [D-alanine-polyphosphoribitol ligase (CVF676)] [Listeria seeligeri serovar 1/2b str. SLCC3954]
MP108GL001982	49.18	5.00E-44	Predicted	(rmlC) dTDP-6-deoxy-D-xylo-4-hexulose-3,5-epimerase [Capsular polysaccharide (CVF282)] [Vibrio vulnificus YJ016]
MP108GL001896	49.15	1.00E-09	Verified	(cylR2) cytolysin regulator R2 [Cytolysin (VF0356)] [Enterococcus faecalis str. MMH594]
MP108GL002325	49.06	6.00E-68	Predicted	(srt2) Srt2 [Bee (biofilm enhancer in enterococci) (AI134)] [Enterococcus faecalis str. E99]
MP108GL000744	48.28	4.00E-47	Predicted	(slrA) peptidyl-prolyl cis-trans isomerase, cyclophilin-type [Streptococcal lipoprotein rotamase A (CVF129)] [Streptococcus pneumoniae TIGR4]
MP108GL001460	48.21	5.00E-88	Predicted	(lplA1) putative lipoate protein ligase A [Lipoate protein ligase A1 (CVF238)] [Listeria ivanovii subsp. ivanovii PAM 55]
MP108GL001372	47.73	5.00E-13	Predicted	(ML1683) histone-like protein [histone-like protein (Hlp)/laminin-binding protein (LBP) (AI354)] [Mycobacterium leprae TN]
MP108GL002054	47.66	2.00E-24	Predicted	(fabZ) beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ [LPS (CVF383)] [Brucella melitensis ATCC 23457]
MP108GL002770	47.61	1.00E-89	Predicted	(htrA/degP) serine peptidase HtrA [Serine protease (CVF148)] [Streptococcus agalactiae A909]
MP108GL001570	47.57	1.00E-63	Predicted	(EFAU085_01747) phosphatidate cytidylyltransferase [Capsule (CVF618)] [Enterococcus faecium Aus0085]
MP108GL002001	47.54	4.00E-42	Predicted	(oppF) oligopeptide ABC transporter, permease component [Capsule (CVF591)] [Mycoplasma mycoides subsp. mycoides SC str. PG1]
MP108GL001431	46.83	1.00E-137	Predicted	(fbp54) fibronectin/fibrinogen binding protein [Fibronectin-binding proteins (CVF113)] [Streptococcus suis 05ZYH33]
MP108GL001361	46.39	5.00E-42	Predicted	(scpB) segregation and condensation protein B [Fibronectin-binding protein (AI186)] [Streptococcus agalactiae 2603V/R]
MP108GL001347	46.11	0	Verified	(clpC) endopeptidase Clp ATP-binding chain C [ClpC (VF0072)] [Listeria monocytogenes EGD-e]

Table 1 Virulence Factor Database Annotated Genes – *Lactobacillus rhamnosus* CGMCC 21225

Gene ID	Identity (%)	E-value	Search Type	Putative Function Description
MP108GL001616	45.93	1.00E-57	Predicted	(stp) serine/threonine protein phosphatase family protein [Serine-threonine phosphatase (CVF245)] [Listeria welshimeri serovar 6b str. SLCC5334]
MP108GL002323	45.56	3.00E-34	Predicted	(aatC) ABC transporter ATP-binding protein AatC [ABC transporter for dispersin (CVF737)] [Escherichia coli O78:H11:K80 str. H10407]
MP108GL002057	45.49	9.00E-53	Predicted	(flmH) 3-oxoacyl-ACP reductase [Polar flagella (VF0473)] [Aeromonas hydrophila ML09-119]
MP108GL000572	45.45	1.00E-23	Predicted	(mgtC) MgtC/SapB transporter [Magnesium transport (CVF313)] [Mycobacterium sp. KMS]
MP108GL000311	45.03	4.00E-83	Predicted	(manA) mannose-6-phosphate isomerase [Polysaccharide capsule (CVF567)] [Bacillus thuringiensis str. Al Hakam]
MP108GL000096	45.01	1.00E-98	Predicted	(lysA) diaminopimelate decarboxylase [Lysine synthesis (CVF310)] [Mycobacterium abscessus subsp. bolletii str. GO 06]
MP108GL000961	44.9	3.00E-56	Predicted	(CbuG_0446) hypothetical protein [T4SS effectors (CVF803)] [Coxiella burnetii CbuG_Q212]
MP108GL000957	44.2	6.00E-90	Predicted	(mrsA/glmM) predicted phosphomannomutase [Exopolysaccharide (CVF495)] [Haemophilus influenzae PittEE]
MP108GL000983	44.2	4.00E-33	Predicted	(regX3) two component transcriptional regulator [RegX3 (CVF667)] [Mycobacterium vanbaalenii PYR-1]
MP108GL001606	44.12	2.00E-11	Verified	(acpXL) acyl carrier protein [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]
MP108GL000926	44.07	3.00E-10	Verified	(cylR2) cytolysin regulator R2 [Cytolysin (VF0356)] [Enterococcus faecalis str. MMH594]
MP108GL002239	43.71	0	Predicted	(mgtB) hypothetical protein [Mg2+ transport (CVF005)] [Salmonella enterica subsp. arizonae serovar 62:z4,z23:-- str. RSK2980]
MP108GL001993	43.58	2.00E-46	Predicted	(BCE_5398) capsular exopolysaccharide family protein [Polysaccharide capsule (CVF567)] [Bacillus cereus ATCC 10987]
MP108GL001299	43.49	1.00E-77	Predicted	(pdhB) pyruvate dehydrogenase E1 component beta subunit [PDH-B (CVF588)] [Mycoplasma mobile 163K]
MP108GL000564	43.48	5.00E-41	Verified	(cylA) ABC (ATP-binding cassette) transporter CylA [Beta-hemolysin/cytolysin (CVF171)] [Streptococcus agalactiae 2603V/R]
MP108GL002550	43.1	5.00E-10	Verified	(cylR2) cytolysin regulator R2 [Cytolysin (VF0356)] [Enterococcus faecalis str. MMH594]
MP108GL001944	43.01	6.00E-36	Predicted	(BCE_5393) UDP-galactose phosphate transferase [Polysaccharide capsule (CVF567)] [Bacillus cereus ATCC 10987]
MP108GL000439	42.86	7.00E-44	Predicted	(kpsF) arabinose-5-phosphate isomerase [Capsule biosynthesis and transport (CVF393)] [Campylobacter jejuni subsp. doylei 269.97]
MP108GL000540	42.86	1.00E-52	Predicted	(pchH) ABC transporter, ATP-binding/permease protein [Pyochelin (CVF553)] [Pseudomonas fluorescens Pf-5]
MP108GL001420	42.74	2.00E-55	Predicted	(hisF) imidazole glycerol phosphate synthase subunit HisF [LPS (VF0171)] [Legionella pneumophila subsp. pneumophila str. Philadelphia 1]

Table 1 Virulence Factor Database Annotated Genes – *Lactobacillus rhamnosus* CGMCC 21225

Gene ID	Identity (%)	E-value	Search Type	Putative Function Description
MP108GL002582	42.69	6.00E-47	Predicted	(sugC) sugar ABC transporter [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium sp. JDM601]
MP108GL001644	42.53	1.00E-96	Predicted	(glnA1) glutamine synthetase [Glutamine synthesis (CVF311)] [Mycobacterium tuberculosis RGTB327]
MP108GL002060	42.47	2.00E-06	Verified	(acpXL) acyl carrier protein [LPS (CVF383)] [Brucella melitensis bv. 1 str. 16M]
MP108GL002005	42.11	3.00E-113	Predicted	(oppA) oligopeptide ABC transporter substrate-binding protein [Oligopeptide-binding protein (CVF240)] [Listeria seeligeri serovar 1/2b str. SLCC3954]
MP108GL002823	42.04	1.00E-41	Predicted	(sugC) ABC transporter, ATP-binding protein SugC [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium intracellulare str. MOTT36Y]
MP108GL001518	41.94	2.00E-174	Predicted	(relA) GTP pyrophosphokinase [(p)ppGpp synthesis and hydrolysis (CVF335)] [Mycobacterium smegmatis str. MC2 155]
MP108GL000633	41.71	2.00E-38	Predicted	(phoP) DNA-binding response regulator [PhoP/R (CVF331)] [Mycobacterium tuberculosis CCDC5180]
MP108GL000877	41.56	4.00E-50	Predicted	(regX3) response regulator with CheY-like receiver domain and winged-helix DNA-binding domain [RegX3 (CVF667)] [Mycobacterium smegmatis JS623]
MP108GL001422	41.4	1.00E-41	Predicted	(hisH) imidazole glycerol phosphate synthase subunit HisH [LPS (VF0171)] [Legionella pneumophila subsp. pneumophila str. Philadelphia 1]
MP108GL001411	41.36	3.00E-49	Predicted	(virR) DNA-binding response regulator [VirR/VirS (CVF252)] [Listeria welshimeri serovar 6b str. SLCC5334]
MP108GL000273	41.07	2.00E-24	Verified	(kfiC) lipopolysaccharide biosynthesis protein [LOS (CVF494)] [Haemophilus influenzae Rd KW20]
MP108GL002100	40.95	1.00E-38	Predicted	(fbpC) iron(III) ABC transporter ATP-binding protein [ABC transporter (CVF197)] [Neisseria meningitidis M01-240149]
MP108GL000084	40.92	3.00E-65	Predicted	(trpD) anthranilate phosphoribosyltransferase [Tryptophan synthesis (CVF308)] [Mycobacterium abscessus subsp. bolletii 50594]
MP108GL002386	40.82	3.00E-53	Predicted	(sitB) SitB protein [Iron/managanease transport (CVF459)] [Escherichia coli UTI89]
MP108GL000682	40.79	5.00E-29	Predicted	(luxS) S-ribosylhomocysteinase [Autoinducer-2 (CVF628)] [Vibrio fischeri ES114]
MP108GL001133	40.79	1.00E-49	Predicted	(pchH) ABC transporter, ATP-binding/permease protein [Pyochelin (CVF553)] [Pseudomonas fluorescens Pf-5]
MP108GL001380	40.69	3.00E-35	Predicted	(hlyIII) hypothetical protein [Hemolysin III (CVF560)] [Bacillus subtilis subsp. subtilis str. 168]
MP108GL000070	40.68	1.00E-43	Predicted	(sugC) ABC transporter, ATP-binding protein SugC [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium intracellulare MOTT-02]
MP108GL001112	40.58	2.00E-19	Predicted	(mf3) MF3 [Mitogenic factor 3 (CVF139)] [Streptococcus pyogenes MGAS8232]
MP108GL000267	40.51	3.00E-59	Predicted	(lytR) membrane-bound transcriptional regulator LytR [Polysaccharide capsule (CVF567)] [Bacillus cereus ATCC 10987]
MP108GL000895	40.49	7.00E-28	Predicted	(fbpC) ABC transporter, ATP-binding protein, iron related [ABC transporter (CVF197)] [Neisseria gonorrhoeae FA 1090]

Table 1 Virulence Factor Database Annotated Genes – *Lactobacillus rhamnosus* CGMCC 21225

Gene ID	Identity (%)	E-value	Search Type	Putative Function Description
MP108GL002735	40.46	9.00E-72	Predicted	(sugC) ABC transporter--like protein [Trehalose-recycling ABC transporter (CVF651)] [Mycobacterium vanbaalenii PYR-1]
MP108GL001672	40.41	2.00E-87	Predicted	(lisK) putative two-component sensor histidine kinase [LisR/LisK (CVF253)] [Listeria ivanovii subsp. ivanovii PAM 55]
MP108GL002095	40.38	3.00E-13	Predicted	(srtA) sortase, truncated [Sortase A (CVF130)] [Streptococcus thermophilus LMG 18311]
MP108GL002403	40.38	8.00E-34	Predicted	(hitC) iron(III) ABC transporter, ATP-binding protein [Haemophilus iron transport locus (CVF501)] [Haemophilus somnus 129PT]
MP108GL002096	40.34	1.00E-15	Verified	(gtcA) wall teichoic acid glycosylation protein GtcA [GtcA (VF0448)] [Listeria monocytogenes EGD-e]
MP108GL001657	40.28	8.00E-37	Predicted	(devR/dosR) two-component system response regulator [DevR/S (CVF334)] [Mycobacterium indicus pranii MTCC 9506]
MP108GL000871	40.26	4.00E-119	Predicted	(secA2) preprotein translocase ATPase secA2 [Accessory secretion factor (CVF299)] [Mycobacterium avium subsp. paratuberculosis MAP4]
MP108GL001977	40.16	1.00E-55	Predicted	(BCE_5397) capsular polysaccharide biosynthesis protein [Polysaccharide capsule (CVF567)] [Bacillus cereus ATCC 10987]
MP108GL002457	40.1	4.00E-33	Predicted	(ahpC) putative alkylhydroperoxidase C [AhpC (CVF322)] [Mycobacterium abscessus ATCC 19977]

FDA.3. *In Part 8 of Appendix B "Comparative Genomics" (p. 20 of Appendix B), you state that "Compare sequencing strain with reference strains by using their genome sequence and gene sequence. The result shows some information of the structural differences, mutation and evolution relationship between them". Please clarify the following:*

(a) What were the reference strain(s)?

(b) What were the genome sequence homology between L. rhamnosus CGMCC 21225 and the reference strain(s)?

(a) The reference strains for the comparative genomics assessment of identity for *L. rhamnosus* CGMCC 21225 were *L. rhamnosus* 4B15, *L. rhamnosus* ATCC8530, *L. rhamnosus* GG, *L. rhamnosus* LOCK900, and *L. rhamnosus* NCTC13710 (ATCC 7469). These strains are also probiotic strains intended for human consumption, some of which were isolated from human sources; of special note is *L. rhamnosus* GG, which is GRAS.

(b) Sequence homology results are provided in Table 2 below.

Table 2 Genome Sequence Comparison with Reference Strains of *Lactobacillus rhamnosus*

Comparator Strain	Homology with *L. rhamnosus* CGMCC21225 [MP108] (%)	GC (%)
L. rhamnosus 4B15	95.6	47.5
L. rhamnosus ATCC8530	95.8	47.2
L. rhamnosus GG	95.9	47.4
L. rhamnosus LOCK900	95.6	47.0
L. rhamnosus NCTC13710 (ATCC 7469)	99.3	47.1

GC = guanine-cytoside content.

FDA.4. *Does L. rhamnosus CGMCC 21225 produce any undesirable or toxic secondary metabolites? If so, please identify them and discuss if they present as a safety concern from the intended uses.*

In the review of existing scientific literature described in the response to Question 12, there are no data to suggest that *L. rhamnosus* produces any toxic secondary metabolites. Furthermore, *L. rhamnosus* strains have been safely used in food products for many years and are also listed on the European Union's Qualified Presumption of Safety (QPS) positive list, underscoring the recognized safety profile of the species for use in food.

In relation to CGMCC 21225 specifically, Glac Biotech has conducted a detailed toxicological evaluation as documented in the study "*Lactobacillus rhamnosus* MP108: Toxicological evaluation" [J Food Sci, 2021. 86(1): p. 228-241] by Zhang *et al.* (2021). This study centered around a lyophilized powder of *L. rhamnosus* CGMCC 21225, a strain derived from infant feces, and aimed to comprehensively evaluate its safety. A battery of toxicity tests, including a bacterial reverse mutation assay, *in vivo* mouse micronucleus assay, and *in vivo* mouse spermatocyte chromosome aberration assays, were conducted on the *L. rhamnosus* CGMCC 21225 ingredient. The findings confirmed that *L. rhamnosus* CGMCC 21225 was not genotoxic or mutagenic under the conditions of the assays conducted at the highest doses tested, as described in the GRAS notice. Furthermore, oral toxicity of *L. rhamnosus* CGMCC 21225 in Sprague-Dawley rats at up to 1,500 mg/kg body weight/day for 90 days was tested and no adverse events were reported. These study outcomes and the considerable history of safe use of *L. rhamnosus* in food support the GRAS conclusion for *L. rhamnosus* CGMCC 21225 under the intended conditions of use.

The bioinformatics analysis of *L. rhamnosus* CGMCC 21225 and functional annotations using the VFDB, Comprehensive Antibiotic Resistance Database (CARD), and Kyoto Encyclopaedia of Genes and Genomes (KEGG) did not reveal any known toxic metabolites produced by the CGMCC 21225 strain.

FDA.5. *For the administrative record, please briefly specify how the purity of the L. rhamnosus CGMCC 21225 inoculum for the manufacturing process is ensured.*

During each critical stage of the manufacturing process, a small sample is taken for quality control testing. Quality control test items include visual inspection under fluorescence microscopy, universal polymerase chain reaction (PCR), and species-specific PCR; morphological assessments of the culture using microscopy were conducted at critical points of the manufacturing process. The universal PCR detects the presence of any potential contaminating strains, while the species-specific PCR confirms the correct production strain.

FDA.6. *Please clarify whether the medium components added during the fermentation process are present in the final product or if they are removed.*

Bacterial cells are isolated from the culture media by centrifugation and washed in buffer prior to drying; the resulting mass of the bacterial powder after separation (45 kg) is approximately 2.69% of the weight before separation (1,670 kg). Trace levels of media components (*e.g.,* milk protein) may remain in the finished raw material; however, the methods for isolation of cells from media for the *L. rhamnosus* CGMCC 21225 ingredient are commonly used in the food and biotechnology industry for microbial ingredients and are GRAS. Labeling requirements for trace amounts of media ingredients that are allergens will be met.

FDA.7. *Please confirm that the fermentation process is continuously monitored for contaminants.*

Yes, Glac Biotech confirm that the fermentation process is continuously monitored for contaminants. As illustrated in the production flowchart (Figure 2.2.2-1), from the "Raw Materials Combined" to "Centrifugation," there are 4 processes where quality control steps (plate culturing and microscopic observation) are carried out. Once the raw material production is complete, microbial testing will be conducted batch-by-batch following the Taiwan Food and Drug Administration (TFDA) inspection method. The factory's production process follows the food safety management systems Food Safety System Certification (FSSC) 22000, International Organization for Standardization (ISO) 9001, and Hazard Analysis and Critical Control Points (HACCP).

FDA.8. *For the product specifications and batch analysis listed in Tables 2.3.1-1 and 2.3.2-1 (pp. 10-11), please address the following questions:*

(a) Please confirm that all analytical methods in Table 2.3.1-1 (p. 10) used for testing the batches of L. rhamnosus CGMCC 21225 are validated for their intended use.

(b) We note that the sampling size for MOHWM0025.01 method of detecting Salmonella in food is 25 g. Please clarify whether the limit of detection for MOHWM0025.01 method is 1 CFU/g or 1 CFU /25 g (Table 2.3.1-1, p. 10).

(a) Glac Biotech confirms that all analytical methods referenced in Table 2.3.1-1 for testing the batches of *L. rhamnosus* CGMCC 21225 have been validated and are in line with the methods officially announced by the TFDA. For microbial testing, the methods primarily reference the U.S. FDA's *Bacteriological Analytical Manual* (BAM).

(b) To clarify, based on the methods provided by the TFDA, the detection limit for *Salmonella* testing should be represented as colony-forming units (CFU)/25 g, and for *Staphylococcus aureus* should be represented as CFU/50 g. This oversight has been brought to the attention of our quality assurance team for correction.

FDA.9. In Part 1.3 (p. 5), you state that the intended use of *L. rhamnosus* CGMCC 21225 excludes the use in infant formula. However, in Part 6.7 (p. 27) you state that "Glac Biotech has concluded that *L. rhamnosus* MP108 is GRAS for use in non-exempt term infant formula and specified conventional food products, as described in Section 1.3, on the basis of scientific procedures." Please confirm that *L. rhamnosus* CGMCC 21225 is not intended for use in infant formula.

Glac Biotech confirms that *L. rhamnosus* CGMCC21225 is not intended for use in infant formula.

FDA.10. *Please confirm that the uses of L. rhamnosus CGMCC 21225 are substitutional for current uses of L. rhamnosus. If not, please provide a cumulative dietary exposure based on current uses of L. rhamnosus and the intended use of your ingredient.*

Glac Biotech confirms that the uses of *L. rhamnosus* CGMCC21225 are substitutional for current uses of *L. rhamnosus*.

FDA.11. *You intend to use L. rhamnosus CGMCC 21225 in beverage products. Please confirm that the beverage products do not include alcoholic beverages.*

Glac Biotech confirms that the intended use of *L. rhamnosus* CGMCC 21225 in beverage products does not include alcoholic beverages.

FDA.12. *Please provide information on the literature search(es) performed to prepare the notice. This includes the date(s) (e.g., month and year) of the search(es), the resource database(s) used (e.g., PubMed), the principal search terms used, and the time period that the search spanned (e.g., 1/2000 to 7/2023). If needed, please also perform an updated literature search from the last date noted to present and discuss if any new data or information were found that would contradict the current GRAS conclusion.*

A comprehensive literature search was conducted using the electronic search tool ProQuest Dialog™ to identify literature related to the safety of *L. rhamnosus* CGMCC 21225. A search was conducted to identify literature related to the safety of *L. rhamnosus* in humans published since 2008 as well as the safety of *L. rhamnosus* CGMCC 21225 published in the literature without data restrictions. The principal search terms used to identify pertinent literature were "*Lactobacillus rhamnosus*" and similar terms appearing in the title of the literature. Additional search terms used to identify endpoints relevant to the safety of *L. rhamnosus* were "toxicity, " "genotoxicity," "mutagenicity," "teratogenicity," "carcinogenicity," "allergenicity," "*in vitro*," "*in vivo*," "preclinical," "clinical," "oral," and "ADME." A separate search was conducted to identify articles pertaining to the *L. rhamnosus* strain MP108 (CGMCC 21225) by using the principal search term "*Lactobacillus rhamnosus* MP108." The searches were conducted using the following databases: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, Toxicology Abstracts, and ToxFile®. The searches were originally conducted in

July 2021 and repeated to capture additional studies published through August 2023. No new data were identified in the updated literature search that would contradict the current GRAS conclusion.

FDA.13. *On p. 33, you provide a "Table of CFR Sections Referenced" including a list of regulations, GRAS substances, and food additives. We note that the majority of the regulations listed in the table are not referenced in GRN 001130. Please clarify whether the GRAS substances and food additives listed in the table are used as components of the fermentation medium or for another purpose in the manufacture of L. rhamnosus CGMCC 21225. Please also clarify the purpose of citing 21 CFR 169.181 for vanilla-vanillin flavoring in the table.*

The GRAS substances and food additives listed in the table are used as components of the culture medium or as processing aids.

The reference to 21 CFR 169.181 for vanilla-vanillin flavoring in the table was made in error.

FDA.14. *In Table of CFR Sections Referenced on p. 33, we note that the "Maltoxetrin" is a typo. The 21 CFR184.1444 is for Maltodextrin. This is for the administrative record and no correction is needed.*

Confirmed.

We hope this information adequately addresses the Agency's questions regarding GRN 001130. If there is any additional information or clarification required, Glac Biotech will be happy to provide such information or clarification upon request.

Sincerely,

[redacted]

Sheng-Hung Huang, ate
General Manager,
Glac Biotech Co., Ltd.