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#813

September 21, 2018

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

Subject: GRAS Notification – *Bifidobacterium longum* BORI

Dear Mr. Bonnette,

On behalf of BIFIDO CO., LTD, we are submitting a GRAS notification for *Bifidobacterium longum* BORI as a food ingredient. The enclosed document provides notice of a claim that the food ingredient, *Bifidobacterium longum* BORI, described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to non-exempt term infant formulas and other foods. We believe that this determination and notification are in compliance with Pursuant to 21 C.F.R. Part 170, subpart E.

We enclose an original copy of this notification and a virus-free CD Rom for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

9/21/2018

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for BIFIDO CO., LTD

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *BIFIDOBACTERIUM LONGUM* BORI

Prepared for BIFIDO Co., Ltd.

Prepared by: NutraSource, Inc.
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PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 CFR Part 170, subpart E, BIFIDO Co., Ltd. (hereinafter referred to as 'BIFIDO') submits a Generally Recognized as Safe (GRAS) notice and claims that the use of *B. longum* BORI in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.A. Name and Address of the Notifier

Contact: Min-Jung Kim, M.S.
Company: BIFIDO Co., Ltd.
Address: 23-16, Nonggongdanji-gil, Hongcheon-eup, Hongcheon-gun, Gangwon-do, 25117, Republic of Korea

1.B. Common or Trade Name

Bifidobacterium longum BORI (*B. longum* BORI)

1.C. Applicable Conditions of Use of the Notified Substance

1.C.1. Foods in Which the Substance is to be Used

Non-exempt term infant formulas (soy-, milk-, and whey based) and conventional foods.

1.C.2. Levels of Use in Such Foods

Non-exempt Term Infant Formula Applications:

Use level is the same as those described in GRN 454. Powdered non-exempt term infant formulas (soy-, milk-, or whey-based) will contain up to 10^8 colony forming units (cfu) *B. longum* BORI per g powered formulas.

Conventional Food Applications:

BIFIDO intends to add *B. longum* BORI to selected conventional food products (dairy products/dairy-based foods and dairy substitutes, including fermented milk, flavored milk beverages mixes, dried milk powder, imitation milk and yogurt; baby cereals and foods [powder form]; meal replacement powder and nutrition drink mix powder; and sugar substitute [powder form]) for the general population (Table 1). These target foods will contain up to 1 x 10^9 cfu per serving.

Table 1. Proposed Food Categories for Conventional Food Applications

Dairy products/dairy-based foods and dairy substitutes
Fermented milk; includes buttermilk and kefir
Flavored milk beverages mixes, dried milk powder
Imitation milk
Yogurt
Other foods
Baby cereals and foods, powder form
Meal replacement powder and nutrition drink mix powder

Sugar substitute, powder form

1.C.3. Purpose for Which the Substance is Used

The substance will be used as a food ingredient providing *B. longum* BORI to non-exempt term infant formulas and selected conventional foods.

1.C.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of infants and members of general population who consume at least one of the products described above.

1.D. Basis for the GRAS Determination

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

1.E. Availability of Information

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

1.F. Availability of FOIA Exemption

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.G. Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

1.H. Name, Position/Title of Responsible Person Who Signs Dossier, and Signature

(b) (6)

Name: Susan S. Cho, Ph.D. Date: 9/21/2018
Title: President of NutraSource, Inc.
(301-875-6454; susanscho1@yahoo.com)
Agent for BIFIDO Co., Ltd.

1.I. FSIS/USDA Statement

BIFIDO does not intend to add *B. longum* BORI to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

PART 2. IDENTITY, MANUFACTURING, SPECIFICATIONS, AND TECHNICAL EFFECTS

2.A.1. Identity of the Notified Substance

2.A.1.1. Common Name: *B. longum* BORI
Details of *B. longum* BORI identification is presented in Appendix A. *B. longum* BORI and BB536 strains (a reference GRAS strain described in GRN 268, FDA, 2009) have a 99.4% similarity in the 16S rRNA genomic sequence.

2.A.1.2. Chemical Names of Main Component: Not applicable (NA)

2.A.1.3. Chemical Abstract Service (CAS) Registry Number: NA

2.A.1.4. Empirical Formula: NA

2.A.1.5. Structural Formula: NA

2.A.1.6. Molecular Weight: NA

2.A.2. Potential Toxicants in the Source of the Notified Substance
No toxicants are identified from *B. longum* BORI.

2.A.3. Particle Size
NLT 95% pass 20 mesh.

2.B. Method of Manufacture

A schematic diagram of the general manufacturing process used to produce the *B. longum* BORI ingredient is illustrated in Figure 1.

The first step involves fermentation of a starter culture of *B. longum* BORI using a food-grade culture medium, which is composed of sucrose, soy peptone, yeast extract, sodium acetate, sodium phosphate(mono), sodium phosphate(di), L-cysteine HCl, and taurine.

1. The medium is sterilized at 121°C for 30 minutes (min) and cooled to 35°C.
2. The medium is inoculated with *B. longum* BORI and the bacteria are precultured for 10~20 h at 37°C.
3. Additional medium is prepared for the main culture. The pH of the medium is adjusted from 5.8 to 6.0. This culture medium is sterilized at 121°C for 20 min. The medium is cooled to 37°C and then inoculated with the starter culture from Step 2.
4. Culturing consists of six steps (from 10ml to 2,000L maximum), with incubation at 37°C for 10-20 hours until the appropriate concentration is reached at each step.
5. After cultivation, the medium containing *B. longum* BORI is cooled to 10°C and then centrifuged at 7,500 rpm for 1 h to collect the cells.

6. *B. longum* BORI is taken to measure the bacterial weight subjected to dilution with a cryoprotective agent (100% maltodextrin), which is 85% (w/w) *B. longum* BORI and 15% (w/w) maltodextrin. It is then freeze-dried and milled.
7. After milling, the excipient (100% corn starch) is added at a bacteria-to-weight ratio of 2:3, and the ingredient is freed of magnetic contamination prior to packaging.

The final stock of *B. longum* BORI ingredients are comprised of 51% *B. longum* BORI cells, 9% maltodextrin, and 40% corn starch. The number of *B. longum* BORI cells per one gram of the ingredient is estimated as 5.0×10^{10} cells.

Quality Assurance Procedure:

BIFIDO rigorously tests its final production batches to verify adherence to quality control specifications and, thus, are manufactured consistent with current good manufacturing practice (cGMP) for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade. BIFIDO routinely evaluates the quality of the *B. longum* BORI ingredient during the production process to ensure that the genetic identity is consistent with that of the original stock and the finished products are free of contaminants.

The list of raw materials and their regulatory status are summarized in Table 2.

Table 2. The List of Raw Materials and Their Regulatory Status

Raw material	CAS No.	Regulatory status
Fermentation medium		
Sucrose	57-50-1	21CFR 184.1854
Soy peptone	73049-73-7	21 CFR §184.1553
Yeast extract	8013-01-2	21CFR 184.1983
Sodium acetate	127-09-3	21CFR 184.1721
Sodium phosphate (monobasic)	7558-80-7	21 CFR 182.1778
Sodium phosphate (dibasic)	7782-85-6	21 CFR 182.1778
L-cycteine HCl	52-89-1	21 CFR 184.1272
Taurine	107-35-7	GRN 586
Processing aids/Excipients		
Maltodextrin	9590-36-6	21CFR 184.1884
Corn Starch	9005-25-8	21 CFR 184.1854

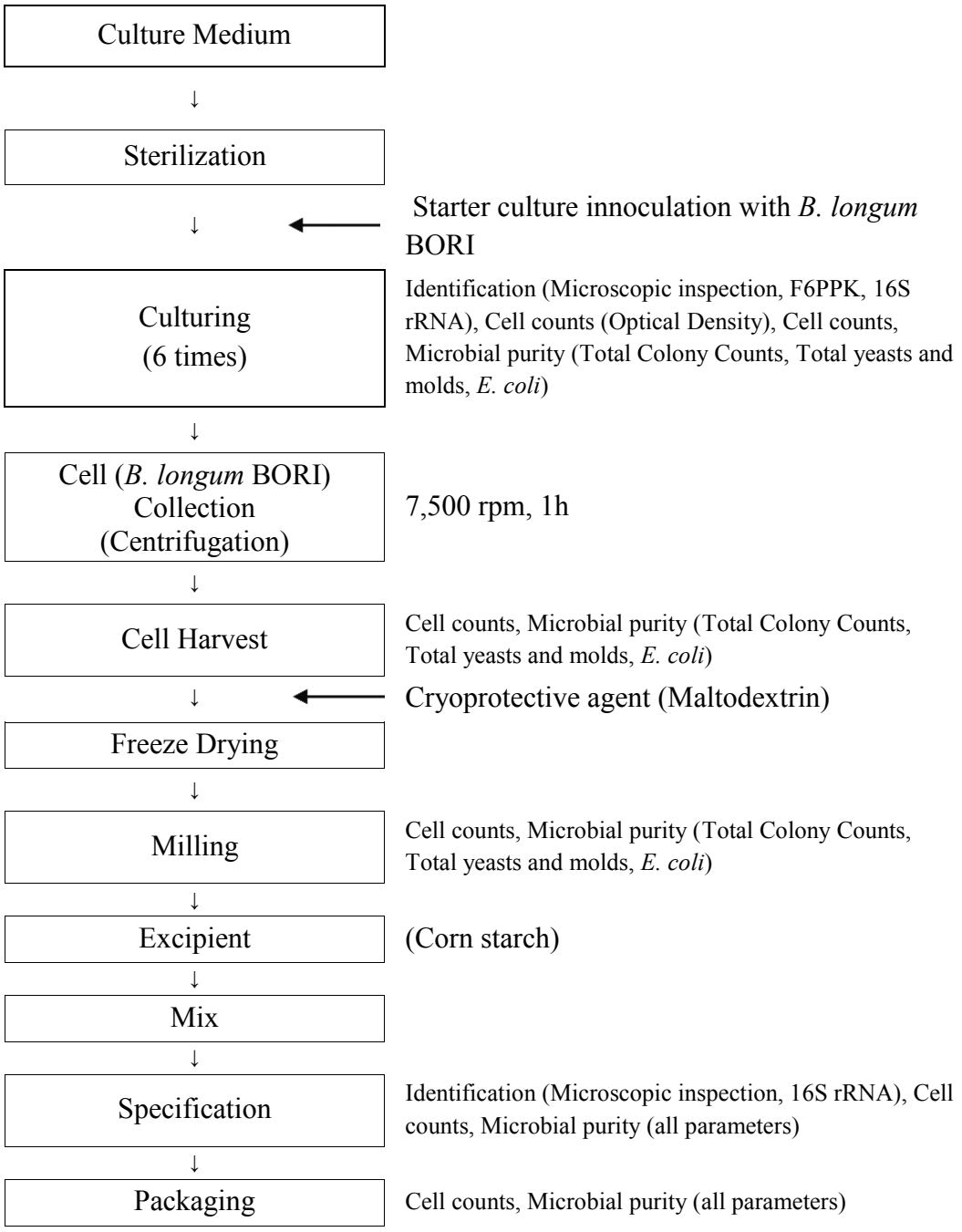

Figure 1. Flow Diagram of *B. longum* BORI Manufacturing Process

2.C. Specifications and Composition of *B. longum* BORI

Tables 3 and 4 present composition and specifications of *B. longum* BORI. Analyses of three non-consecutive lots of the *B. longum* BORI ingredient confirm that the material produced by the manufacturing process is consistent and complies with the product specifications, meeting appropriate food-grade specifications. The analytical data also demonstrate the absence of any

chemical impurities or microbiological contamination. Certificate of analysis (COAs) are shown in Appendix B.

Table 3. Composition and Specifications of *B. longum* BORI Stock Ingredient

Parameter	Specification	Typical composition	Method of analysis Method number
Appearance	No off-taste and off-flavor	Yellow grey powder	Visual
Cell Counts, cfu/g (as *B. longum* BORI),	MT 5.00E+10	7.13E+10	KHFSC 4/3/3-58
Moisture, %	NMT 6.0	5.45	KFSC 7/2/2.1/2.1.1
Heavy metals			
Lead (Pb), ppm	NMT 0.3	0.0136	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	NMT 0.3	0.1090	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	NMT 0.1	0.0077	KFSC 7/9/9.1/9.1.3
Mercury (Hg)	NMT 0.1	0.0003	KFSC 7/9/9.1/9.1.6
Microbial purity			
Non-Lactic acid bacteria (Total Colony Counts)	NMT 1×10^2 cfu/g	Negative	KFSC 7/4/4.5/4.5.1
Total yeasts and molds	NMT 100 cfu/g	Negative	KFSC 7/4/4.10
Escherichia coli	Negative	Negative	KFSC 7/4/4.8
Salmonella	Negative	Negative	KFSC 7/4/4.11
Listeria	Negative	Negative	KFSC 7/4/4.15
Enterobacter sakazakii (*Cronobacter* spp.)	Negative	Negative	KFSC 7/4/4.21
Proximate analysis			
Lipids, %	NA	0.42	KFSC 7/2/2.1/2.1.5/2.1.5.1
Protein, %	NA	12.82	KFSC 7/2/2.1/2.1.3/2.1.3.1
Carbohydrates, %	NA	81.99	KFSC 7/2/2.1/2.1.4/2.1.4.1
Ash, %	NA	1.93	KFSC 7/2/2.1/2.1.2

KFSC: Korean Food Standards Codex, KHFSC: Korean Health functional Food Standards Codex (Available on http://www.foodsafetykorea.go.kr/portal/safefoodlife/food/foodRvlv/foodRvlv.do)

NA: Not Applicable.

Table 4. Analytical Values of 3 Non-consecutive Lots of *B. longum* BORI

Parameter	BI-R-160510	BI-R-161108	BI-R-170910
Appearance	Yellowish gray powder	Yellowish gray powder	Yellowish gray powder
Cell Counts, cfu/g (as *B. longum* BORI)	7.10E+10	7.20E+10	7.10E+10
Moisture, %	5.63	5.32	5.40
Heavy metals			
Lead (Pb), ppm	0.0147	0.0159	0.0102
Arsenic (As), ppm	0.1034	0.0976	0.126
Cadmium (Cd), ppm	0.0078	0.0050	0.0103
Mercury (Hg), ppm	0.001	ND	ND
Microbial purity			
Non-Lactic acid bacteria	Negative	Negative	Negative
Total yeasts and molds	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative
Listeria	Negative	Negative	Negative
Enterobacter sakazakii (*Cronobacter* spp.)	Negative	Negative	Negative
Proximate analysis			
Lipids, %	-	-	0.50
Protein, %	-	-	28.36
Carbohydrates, %	-	-	62.09
Ash, %	-	-	1.93

2.D. The Stability of the *B. longum* BORI

Bulk ingredient stability data indicate that *B. longum* BORI cells in the ingredient are stable for up to 2 years at 5°C and 12 months at 25 ºC, when the cells are supplied in excess of 140% of the claim value at the time of shipment. Table 5 presents the stability of *B. longum* BORI at various temperatures.

Table 5. Stability of *B. longum* BORI at Various Temperatures

Temperature /Month	5℃	25℃	35℃
0	7.32E+10	7.32E+10	7.32E+10
2	7.34E+10	6.52E+10	6.00E+10
4	7.22E+10	6.26E+10	5.32E+10
8	7.11E+10	6.01E+10	4.88E+10
10	6.92E+10	5.84E+10	4.32E+10

12	6.76E+10	5.52E+10	4.16E+10
18	5.62E+10	4.84E+10	3.34E+10
24	5.20E+10	4.08E+10	2.00E+10
The viability of *B. longum* BORI at 24 months compared to the claim value (5.00E+10 cfu/g)	104%	82%	40%

The Stability in infant formula and other foods is expected to be comparable to other bifidobacterium, such as *B. longum* BB536. In bifidobacteria-containing milks, milk containing *B. longum* BB536 had 79% of the original concentration measured on the day of manufacture after 14 days of storage (FDA, 2009; GRN 268).

2.E. Intended Technical Effects

The intended effect is to provide a dietary source of *B. longum* BORI to non-exempt infant formula and selected conventional foods.

Bifidobacterium genus is a facultative anaerobic gram-positive bacterium that does not form spores. *Bifidobacteria* comprise up to 25% of the cultivatable fecal bacteria in adults and 80% in infants (Picard et al., 2005). Probiotics including *B. longum* are known to have several health benefits including improved intestinal health and immune functions (Picard et al., 2005). In particular*, B. longum* can also be used as a probiotic supplement. Over the last decade, there has been an increasing number of studies to determine the beneficial effects of various *B. longum* strains in humans (Kim et al., 2018).

PART 3. DIETARY EXPOSURE

3.A. Estimated Dietary Intakes (EDIs) of *B. longum* BORI Under the Intended Use

3.A.1. Non-exempt Term Infant formula applications

The use level for infant formulas is the same as those described in GRN 454. Powdered non-exempt term infant formulas (soy-, milk-, or whey-based) will contain up to 10^8 colony forming units (cfu) *B. longum* BORI /g powered formulas. Intended target intake level will be 10^9 - 10^{10} cfu B. *B. longum* BORI/day since powdered term infant formulas will contain 10^8 cfu *B. longum* BORI/g.

Infant formulas in the US market typically provide 0.67 kcal/ml (20 kcal/fl oz) (Martinez and Ballew, 2011). Assuming that these formulas are the sole source of nutrition, reconstituted at 14.1 g/100 ml with a caloric density of 0.67 kcal/ml, and the caloric requirements of one month-old and a six month-old infant are 472 kcal/day and 645 kcal/day (Institute of Medicine [IOM] Panel on Macronutrients and IOM Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Then the addition of 10^8 cfu *B. longum* BORI /g infant formula will result in intakes of 9.9 x 10^9 and 1.35 x 10^{10} cfu *B. longum* BORI/day. These formulas will be supplemented appropriately to provide a minimum of 10^9 cfu *B. longum* BORI/day at the end of an 18-month shelf-life at room temperature.

Since intended use level in this GRAS determination is the same as GRN 454, these EDI levels are consistent with those reported in other GRAS notification; the addition of 10^8 cfu *B. longum* BORI/g infant formula will result in intakes of 9.9 x 10^9 and 1.35 x 10^{10} cfu *B. longum* BORI/day.

3.A.2. Conventional Food Applications

BIFIDO intends to add *B. longum* BORI to selected conventional food products for the general population (Table 1). Selected conventional foods will contain up to 1.0 x 10^9 cfu/serving.

The intended use of 1.0 x 10^9 cfu *B. longum* BORI/serving in the target food categories would result in the estimated mean and 90th percentile intakes of intakes of 1.36 and 3.00 servings of foods per person per day, providing 1.36 x 10^9 and 3.00 x10^9 *B. longum* BORI cells per person per day, respectively, in all users (Table 6-1). A maximum exposure would occur in males aged 13 to 18 years of age, with a 90th percentile EDI of 3.5 x 10^9 cfu/day. In total population, mean and 90th percentile intakes are estimated to be 0.41 and 1.17 servings per day, providing 0.41 x 10^9 and 1.17 x10^9 cfu/person/day, respectively (Table 6-2). These estimates are highly amplified since it is not likely that *B. longum* BORI will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered.

<u>Summary of Consumption Data</u>

Non-exempt term infant formula applications: Intended target intake level will be 10^9 - 10^{10} cfu *B. longum* BORI/day since powdered term infant formulas will contain 10^8 cfu *B. longum* BORI/g.

Conventional food applications: Intended use of 1.0 x 10^9 cfu *B. longum* BORI/serving in the selected food categories would result in the estimated mean and 90th percentile intakes of 1.4 x 10^9 and 3.0 x10^9 cfu/person/day, respectively, in all users.

Table 6-1. EDIs of *B. longum* BORI from Proposed Uses in Conventional Foods*

	N	% users	Food, serving/d		*B. longum* BORI, cfu/day	
			Mean	90th Pctl	Mean	90th Pctl
Children 1-5	583	39.6	0.72	1.69	0.72 x 10^9	1.69 x 10^9
Children, 6-12	486	23.5	0.59	1.11	0.59 x 10^9	1.11 x 10^9
Males, 13-18	78	9.6	1.01	2.01	1.01 x 10^9	2.01 x 10^9
Females, 13-18	114	15.3	0.60	1.10	0.60 x 10^9	1.10 x 10^9
Males, 19-99	1,084	26.0	1.55	3.50	1.55 x 10^9	3.50 x 10^9
Females, 19-99	1,626	38.3	1.51	3.00	1.51 x 10^9	3.00 x 10^9
Total population	3,971	30.2	1.36	3.00	1.36 x 10^9	3.00 x 10^9

*Based on the 2011-2014 National Health and Nutrition Examination Survey (NHANES)

Table 6-2. EDIs of *B. longum* BORI from Proposed Uses in Conventional Foods in All Population*

	N	% users	Food, serving/d		*B. longum* BORI, cfu/day	
			Mean	90th Pctl	Mean	90th Pctl
Children 1-5	1,587	100	0.29	0.92	0.29 x 10^9	0.92 x 10^9
Children, 6-12	2206	100	0.14	0.50	0.14 x 10^9	0.50 x 10^9
Males, 13-18	822	100	0.10	NA	0.10 x 10^9	NA
Females, 13-18	838	100	0.09	0.41	0.09 x 10^9	0.41 x 10^9
Males, 19-99	4,294	100	0.40	1.32	0.40 x 10^9	1.32 x 10^9
Females, 19-99	4,587	100	0.58	1.67	0.58 x 10^9	1.67 x 10^9
Total population	14,334	100	0.41	1.17	0.41 x 10^9	1.17 x 10^9

*Based on the 2011-2014 National Health and Nutrition Examination Survey (NHANES)
NA-the 90the percentile intake was difficult to calculate due to insufficient number of subjects.

3.B. Food Sources of *B. longum* BORI

Lactic acid bacteria, including bifidobacteria, are commonly consumed in fermented foods throughout the world. However, it is hard to estimate the sources and EDIs of naturally occurring *B. longum* BORI from the diet.

3.C. EDIs of *B. longum* BORI from Diet

Not applicable

3.D. Total EDIs of *B. longum* BORI from Diet and Under the Intended Use

Same as 3.A.

3.E. EDIs of Other Nutrients Under the Intended Use

Corn starch and maltodextrin are subjected to 21 CFR 184.1854 and 21 CFR 184.1884, respectively. Thus, we have not calculated the EDIs of these nutrients from the diet.

PART 4. SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the *B. longum* BORI ingredient.

PART 5. HISTORY OF CONSUMPTION

Since 2004, the *B. longum* BORI has been marketed as a dietary supplement ingredient and as a dietary supplement in Korea. The use of the *B. longum* BORI in dietary supplements delivers daily doses up to $2.0x10^{11}$ *B. longum* BORI cells ($1.0x10^{11}$ *B. longum* BORI cells per serving; two servings a day) for the Korean population. Over 293 kg of the *B. longum* BORI ingredient (containing 3.0×10^{10}~$1.0x10^{11}$ *B. longum* BORI cells per gram) or 651,611 kg of finished probiotics product (containing 13,787 kg of *B. longum* BORI cells with 0.10~5%) have been sold in Korea in the past 8 years. No serious adverse effects were reported by consumers. *B. longum* BORI also has been marketed as a dietary supplement ingredient in Asia (China, Taiwan, Malaysia) and Europe (Germany, Poland). No serious adverse effects were reported by consumers. Additionally, another *B. longum* strain, BB536, has been marketed in Japan since 1977 (FDA, 2009; GRN 268).

However, this GRAS determination is based on the scientific procedure, instead of the history of consumption.

PART 6. BASIS FOR GRAS DETERMINATION

6.A. Current Regulatory Status

USA

Various *Bifidobacterium* species have been determined to be GRAS for use in conventional foods and infant formulas. The FDA did not have questions on the summary of safety of the following *Bifidobacterium* species:

1) *B. animalis* subsp. *lactis* Bf-6 for use in selected foods (GRN 377 [FDA, 2011]; up to 10^{11} cfu/serving of conventional foods),
2) *B. lactis* Bb-12 for use in infant formulas for four months-of-age and older (GRN 49 [FDA, 2002]; 10^{7}-10^{8} cfu/g infant formula),
3) *B. longum* BB536 for use in selected foods and infant formulas (GRN 268 [FDA, 2009]; 10^{10} cfu/serving of conventional foods; 10^{10} cfu/g of milk-based term infant formula for term infants aged 9 months and older),
4) *B. animalis* ssp. *lactis* HNO19, Bi-07, Bi-04, and B420 strains (GRN 445 [FDA, 2013a]; up to 2 x 10^{11} cfu/serving of conventional foods) and
5) *B. breve* M-16V for use in infant formulas and selected conventional foods (GRN 453, [FDA, 2013b]; 5 x 10^{9} cfu/serving of conventional foods.
6) *B. breve* M-16V for use in non-exempt powdered term infant formulas (milk- or soy-based) and exempt powdered term infant formula containing partially-hydrolyzed milk or soy proteins (GRN 454 [FDA, 2013c]; at levels up to 10^{8} colony forming units per gram of infant formula powder).
7) *B. breve* M-16V for use in exempt term powdered amino acid-based infant formulas (GRN 455 [FDA, 2013d]; 10^{8} cfu/g of infant formula powder).

According to Dietary Supplement Health and Education Act (DSHEA), a "new dietary ingredient" means "a dietary ingredient that was not marketed in the United States before October 15, 1994 and does not include any dietary ingredient which was marketed in the United States before October 15, 1994." By this provision, dietary ingredients already in use as of October 15, 1994, were "grandfathered." The bacterial species *B. longum* is included in the Old Dietary Ingredient list, i.e., the use of the bacterial species *B. longum* is grandfathered under the DSHEA (CRN, 1998).

Europe

The European Food Safety Agency (EFSA) considers the bacterial species *B. longum* suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, 2010). The QPS approach is a generic assessment system used within EFSA to harmonize premarket safety assessments of selected groups of microorganisms used in food and food production (EFSA, 2007). The QPS approach establishes the safety of a defined taxon (genus or group of related species) based on four “pillars”: (a) established identity, (b) body of knowledge, (c) possible pathogenicity, and (d) end use. Exclusion or qualification of safety concerns should result in granting QPS status for a given taxonomic group (EFSA, 2007). Those applying for EFSA approval of such “new” strains are required to provide proof of the absence of transferable resistance to therapeutic antibiotics. Other primary criteria for functionality are a strain’s ability to survive passage through the upper gastrointestinal tract and its interaction under typical

conditions in the small intestine. Therefore, *B. longum* strains do not require any specific demonstration of safety other than confirmed absence of any determinants of clinically significant resistance to antibiotics in humans and animals.

The EFSA Scientific Committee (EFSA, 2010) noted that, although a variety of different *Bifidobacterium* species, including *B. longum* have been occasionally isolated from human clinical specimens. However, such occurrences have been rare and were mainly encountered in immune-compromised patients or in those with severe underlying illnesses. The Scientific Committee concluded that most *Bifidobacterium* species can be considered nonpathogenic to humans, and therefore, pose no specific safety concerns.

Korea

In Korea, *B. longum* BORI has received a Korean FDA's approval as a functional food ingredient and as a probiotic dietary supplement.

6.B. Review of Safety Data

The general safety of the *B. longum* BORI has been confirmed on the basis of the following facts: absence of plasmid, susceptibility to a range of antibiotics and the absence of hemolysis, mucin degradation activity and ammonia and biogenic amine production (Kim et al., 2018). In addition, the genome of *B. longum* BORI does not contain regions with significant homology to known antibiotic resistance, pathogenic or toxigenic genes, including those found in other strains of bifidobacteria and lactobacilli.

This review includes the studies of live *B. longum* BORI and BB536 strains but excludes the studies of heat killed or dead *B. longum* strains. Unlike live *B. longum* BORI, dead bacteria are not classified as probiotics. Our review covers the literature published in June 30, 2018.

6.B.1. Metabolism

Given that *B. longum* BORI retains its form, it is unlikely that *B. longum* BORI will enter the organs and the systemic circulation from the gastrointestinal tract in normal healthy individuals. Rather, the fate of *B. longum* after ingestion is expected to be similar to that seen after consumption of live food-grade bacteria (Picard et al., 2005). *B. longum* BORI is expected to transit through the gastrointestinal tract and be excreted in feces. It has also been shown that live *B. longum* BORI from orally-administered preparations does not harbor the potential for translocation (Kim et al., 2018).

6.B.2. *In vitro* Tests

6.B.2.1. Genetic Stability Test (adopted from Kim et al., 2018)

Genetic stability test showed that the similarity in the genomic comparison of the 1st and 25th generations of samples was 99.9996~99.9998% via the 3D coordination of the Orthologous Average Nucleotide Identity (OrthoANI) analysis. The difference between 0.0002 and 0.0004% is equivalent to 4.4 bp to 8.8 bp mutation of the entire nucleotide sequence, which can be assumed to result from sequencing errors or spontaneous evolutionary mutations. These data indicate little genetic mutation, with no change in the genetic information during the process of cultivating 25 generations. Details are described in Kim et al. (2018).

6.B.2.2. Absence of Plasmid

Analysis of whole genomic sequencing (WGS) revealed the absence of a plasmid capable of transferring antibiotic resistance genes (GenBank).

6.B.2.3. Absence of Virulence Genes (adopted from Kim et al., 2018)

The search for virulence factors in *B. longum* BORI was completed using the VirulenceFinder1.5 Server, which is a component of the publicly available web-based tool for whole-genome sequencing(WGS) analysis hosted by the Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/). The database system detects homologous sequences for the virulence genes related to *E. coli, Enterococcus*, *Listeria*, and *Staphylococcus aureus* in WGS data (Joensen et al., 2014). The output consists of best-matching genes from BLAST analysis of the selected database against the submitted genome of *B. longum* BORI. The selected %ID threshold was set at 90.00 % and the selected minimum length was set at 60 %. In the event of a matching result, the output would show information on the predicted virulence gene, the %ID, the length of query and database gene, the position of the hit in the contig, and the accession number of the hit. The genome sequences of *B. longum* BORI were compared with the genome sequences of four well-known pathogens (*E. coli*, *Enterococcus*, *Listeria*, and *Staphylococcus aureus*).

No virulence factors were found in the genomic sequences of *B. longum* BORI. This result shows that the genomic sequence of *B. longum* BORI does not include toxic or pathogenic genes related to *E. coli*, *Enterococcus*, *Listeria*, and *S. aureus*.

6.B.2.4. Susceptibility of *B. longum* BORI to Antibiotics (adopted from Kim et al., 2018)

In order to distinguish antibiotic-resistant from antibiotic-susceptible microorganisms, the EFSA has established microbiological cut-off values for the antibiotic-resistance of microorganisms used as food and/or feed additives. These microbiological cut-off values were determined based on the distribution of the chosen antimicrobials' minimum inhibitory concentrations (MICs) in cell populations belonging to a single taxonomical unit (EFSA, 2012). The MIC was defined as the lowest concentration of antibiotic giving a complete inhibition of visible growth in comparison to an antibiotic-free control well. The EU-funded research project PROSAFE (Biosafety evaluation of lactic acid bacteria used for human consumption) also defined the criteria, standards, guidelines and regulations ensure safe use of probiotic lactic acid bacteria for humans. Data on antibiotic susceptibility (testing by PROSAFE) established MIC levels for multiple strains of *B. longum* on the basis of MIC distribution for various antibiotics (FDA, 2009).

To compare antibiotic resistance patterns of *B. longum* BORI with those of other bifidobacteria, MIC values for all bacterial isolates were determined by the ISO 10932:2010 broth microdilution procedure as described in Kim et al. (2018). The experiments were replicated three times.

For *Bifidobacterium* species, EFSA has established MIC breakpoints for resistance to ampicillin, gentamicin, streptomycin, tetracycline, erythromycin, vancomycin, chloramphenicol, and clindamycin (EFSA, 2012). For *B. longum*, PROSAFE has also established MIC breakpoints

for gentamicin, streptomycin, erythromycin, vancomycin, and chloramphenicol (GRN 268; FDA, 2009).

All *Bifidobacterium* spp. in this study were susceptible to ampicillin, chloramphenicol, clindamycin, erythromycin, penicillin G, rifampicin, and vancomycin (MIC ranging from 0.01 to 4 μg/ml) and generally resistant to aminoglycoside antibiotics such as gentamicin, kanamycin, neomycin, and streptomycin (MIC > 32 μg/ml, Table 7). For those whose MIC cutoff values are established by EFSA, the MIC values of *B. longum* BORI, with the exception of tetracycline, were equal to or lower than the established EFSA cut-off values suggested by the EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (EFSA, 2012). The susceptibility tendencies of *B. longum* BORI were similar to other Bifidobacterium strains, *B longum* BB536 (GRN 268; FDA, 2009), *B. lactis* Bb-12 (GRN 49; FDA, 2002), and *B. breve* M-16V (GRN 453, 454, 455; FDA, 2013b, 2013c, 2013d) which have received no question letters from FDA.

Tetracycline resistance genes (*tet*) are widely distributed in the *Bifidobacterium* genus; however, they are known as ribosomal protection proteins (Ammor et al., 2007; Gueimonde et al., 2013). The tetracycline resistance gene, *tet (W)*, was found in *B. longum* BORI chromosome DNA. Mättö et al. (2007) reported *Bifidobacterium* strains displayed generally high MICs for streptomycin and gentamicin, and that this outcome suggested intrinsic resistance. In the study of Mättö et al. (2007), human- and probiotic-associated *Bifidobacterium* species (203 strains) showed high MIC values for tetracycline (i.e. ≥16 mg/mL; prevalence of 4-18%). This was attributed to the presence of tetracycline genes (*tet*), where *tet*(W), and *tet*(O) were detected. The *tet*(W), and *tet*(M) were found in 26, and 7%, respectively, of the *Bifidobacterium* isolates. The role of the *tet* (W) gene is presumed to be the translation factor guanosine triphosphatases (GTPase) of the TRAFAC family, which induces a noncovalent modification to the ribosome that destroys the effect of tetracycline inhibiting protein synthesis (UniProt, 2018).

In addition, in GRN 49 for which FDA issued a no question letter (FDA, 2002), Nestle presented data and information to FDA to show that the presence of a tetracycline resistance element in *B. lactis* Bb-12 does not present a safety problem: 1) the *B. lactis tet*(W) gene is unlikely to be transferred to other microorganisms; 2) it was the second most frequently detected tetracycline resistance gene among tetracycline resistant bacteria cultivated from the human oral cavity. In one study, *tet*(W) gene was detected in children who lacked previous exposure to tetracycline. Consequently, the consumption of organisms bearing this gene would not represent new exposure of this gene to humans ingesting food containing *tet*(W)-bearing strains since tetracycline resistance, including that encoded by *tet*(W), is widespread, particularly in farm environments. Nestle also stated that the foods containing *B. lactis* Bb12 had been commonly used in a number of food products in the U.S. and other developed countries for several years. These foods include yogurts and dairy-based drinks, among which yogurt is marketed in the U.S. specifically for infants, and infant formulas containing this ingredient have been consumed for several years overseas. There are no known adverse events attributed to consumption of tetracycline resistant microbes used in food processing. FDA had no questions on the Nestle's conclusion that the *B. lactis* Bb-12 was safe for infant formula applications.

EFSA cutoffs are not available for the following 13 antibiotics: penicillin, carbenicillin, methicillin, dicloxacillin, kanamycin, neomycin, cephalothin, polymyxin B, metronidazole,

rifampicin, phosphomycin, mupirocin, and trimethoprim-sulfamethoxazol. For these antibiotics, the MIC values are comparable among *B. longum* BORI and other GRAS strains, such as *B. breve* M-16V, *B. lactis* Bb-12, and *B. longum* BB536.

Ampicillin, vancomycin, gentamicin, and erythromycin are known as frequently used antibiotics in pediatric patients. For *B. longum* BORI, none of these pediatric antibiotics had MIC values exceeding EFSA breakpoints.

To date, antibiotic resistance has been found in lactic acid bacteria isolated from wine, cheese, milk, and dairy products, such as fermented vegetables, corn oil, and fermented fruit, as well as fermented sausages and other severely aged meat products (Zielinska et al., 2018). It has been suggested that probiotic bacteria used for food and human use should be sensitive to at least two clinically relevant antibiotics (Zielinska et al., 2018).

6.B.2.5. Antibiotic Resistance Transferability Test (adopted from Kim et al., 2018)

Antibiotic resistance transferability studies were conducted to confirm the nature of this resistance. Conjugal transfer of antibiotic resistance was assessed via the methods of Tannock (1987) as described in Kim et al. (2018). Equal bacterial cell volumes (1 ml) of the donor and recipient strains were mixed and centrifuged at 7,000×g for 10 min. After disposing of the supernatant, the bacterial cell pellet was resuspended in the MRS broth medium and cultivated in an anaerobic chamber at 37°C for 12 h. The collected bacterial cells were filtered through a 0.45μm micro-filter membrane and the membrane was placed on the surface of MRS agar and incubated anaerobically at 37°C for 24h. The bacterial cells were washed with 4 ml of 0.9% sterile saline, diluted to 10^{-3}, 10^{-4}, and 10^{-5}, respectively, and then plated on MRS agar containing gentamicin or tetracycline. The plates were incubated aerobically or anaerobically at 37°C for 36 h.

Tetracycline resistance transferability tests were conducted using *L. fermentum* AGBG1, a recipient strain that is highly susceptible to tetracycline. *L. acidophilus* ATCC 4356 has high gentamicin sensitivity and was therefore used as the recipient strain to test the transferability of gentamicin resistance of *B. longum* BORI.

The antimicrobial susceptibility test found that while *B. bifidum* BGN4 was very susceptible to tetracycline (MIC 1.0 μg/mL), *B. longum* BORI was resistant to it (MIC 64 μg/mL) (Table 5). However, the tetracycline resistance of *B. longum* BORI was not transferred to the recipient, *L. fermentum* AGBG1. *L. acidophilus* ATCC 4356, which is highly susceptible to gentamicin, grew well in normal MRS medium; however, *L. acidophilus* ATCC 4356 did not grow in the MRS medium containing gentamicin or the media that was co-cultured with *B. bifidum* BGN4 or *B. longum* BORI. In contrast, *B. longum* BORI showed resistance to 64 μg/mL gentamicin in this study, thereby proving that *B. longum* BORI's resistance to gentamicin and tetracycline were not transferred to the recipient strains.

Table 7. Antimicrobial Susceptibility of *B. longum* BORI and Other *Bifidobacterium* spp. (MIC values)

Data source	EFSA MIC cut off	Kim et al., 2018		GRN 268	Kim et al., 2018			GRN 453, 444, and 455	PROSAFE cutoff (GRN 268)
Antibiotic	*Bifido-bacterium spp.*	*B. longum* BORI	*B. longum* BB536	*B. longum* BB536	*B. bifidum* BGN4	*B. lactis Bb-12*	*B. breve M-16V*	*B. breve M-16V*	*B. longum*
Ampicillin sodium salt	2	0.5	0.25	0.5	0.063	0.125	0.25	0.125-0.25	Inconclusive
Gentamicin sulfate	64	32	64	32	128	128	128	32-128	128
Streptomycin sulfate salt	128	64	128	32	64	128	256	14-128	128
Tetracycline	8	64	1	0.78	1	16	16	0.5-2.0	NA
Erythromycin	1	0.5	0.5	0.032	0.125	0.125	0.125	0.016-0.25	0.25
Vancomycin hydrochloride	2	1	<0.25	0.25	1	0.5	0.5	0.25-0.5	0.5
Chloramphenicol	4	4	2	1	2	2	2	1-2	8
Clindamycin hydrochloride	1	0.125	0.063	0.032	0.063	<0.032	0.063	0.032-0.125	Inconclusive
Penicillin G		1	0.125	0.39	0.063	0.125	0.25	<1.52	Inconclusive
Carbenicllin disodium salt		8	2	3.13	0.5	2	4	NA	NA
Methicillin		16	4	6.25	1	2	8	NA	NA
Dicloxacillin sodium salt hydrate		8	4	6.25	0.5	4	8	NA	NA
Kanamycin sulfate	N/R	512	1024		1024	1024	1024		
Neomycin sulfate		512	512	200	1024	512	1024	>256	NA
Cephalothin sodium salt		32	4	12.5	4	8	16	NA	NA
Polymyxin B sulfate salt		256	32	2000	512	256	1024	15.6-125	NA
Metronidazole		>256	8	400	4	4	8	15.6-31.3	NA
Chloramphenicol	4	4	2		2	2	2		
Rifampicin		0.25	<0.125		0.5	2	1		

Phosphomycin disodium salt		256	256	>800	128	64	32	NA	NA
Mupirocin		>128	>128	>400	>128	>128	>128	NA	NA
Trimethoprim-Sulfamethoxazole		256	256	NA	128	1	2	32-128	Inconclusive

N/R= not required; NA= not applicable.

6.B.2.6. PCR Assay on Antibiotic Resistance Genes (adopted from Kim et al., 2018)

Even though the whole genome shows that *B. longum* BORI does not contain a plasmid capable of transferring the antibiotic-resistance gene, PCR analysis on ten antibiotic genes such as gentamicin (*aaac(6)-aph(2)*), kanamycin (*AphA3, aaaD*), streptomycin (*aadE*), trimethroprim (*dfrA*), and tetracycline (*tet*(K)), *tet*(L), *tet*(M), *tet*(O), and *tet*(S)) were conducted. The experimental conditions are described in Kim et al. (2018).

All the tested *Bifidobacterium* spp. in this study were identified using 16S rRNA *Bifidobacterium* genus specific primers. There were no amplicons that indicate resistance genes in *B. longum* BORI and other *Bifidobacterium* spp. (Figure 2).

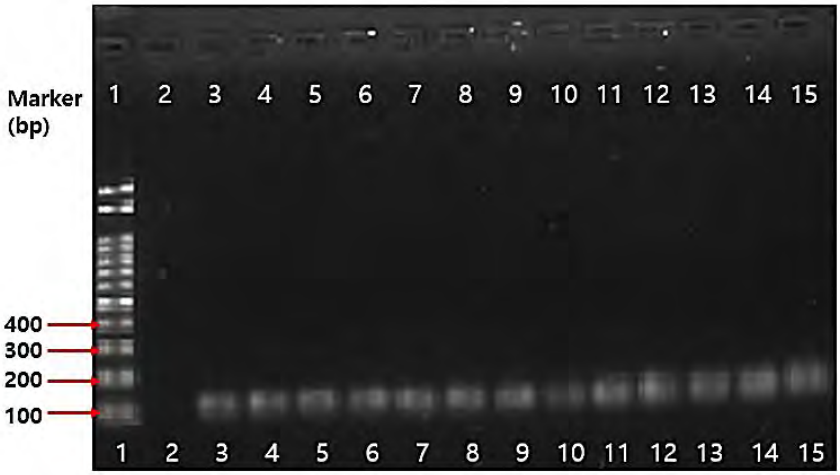

Figure 2. PCR Analysis Results of Various *Bifidobacterium* spp.: Lane 1: marker; Lane 2: without loading; Lane 3: *B. lactis* AS60; Lane 4: *B. bifidum* KCTC 3440; Lane 5: *B. longum* BORI; Lane 6: *B. longum* KCCM 91563; Lane 7: *B. lactis* BB-12; Lane 8: *B. longum* RD47; Lane 9: *B. bifidum* BGN4; Lane 10: *B. thermophilum* KCCM 12097; Lane 11: *B. adolescentis* ATCC 15703; Lane 12: *B. lactis* AD011; Lane 13: *B. infantis* ATCC 15697; Lane 14: *B. breve* M-16V; Lane 15: *B. animalis* ATCC 25527

Summary of Antibiotic Susceptibility

The available information on antibiotic resistance patterns of *B. longum* BORI indicates that the antibiotic susceptibilities of the strain are overall similar to patterns of other bifidobacterial species, and the strain is not likely to have transmissible antibiotic genes. In addition, *B. longum* BORI does not contain antibiotic resistance genes. These findings indicate that use of *B. longum* BORI in foods does not present concerns for antibiotic resistance.

6.B.2.7. Ammonia Production Test (adopted from Kim et al., 2018)

Intestinal bacteria can degrade various nitrogen sources (e.g., proteins, peptides, and amino acids) present in the feces of the intestinal track. Multiple potentially toxic products (i.e. phenol, ammonia, and indole) are possible products of the proteolytic process, especially in the large intestine. Thus, bacterial ammonia production is highly relevant to human intestinal health and a necessary component of the evaluation to demonstrate the safety of commercial probiotics.

In this study, *B. longum* BORI, *B. bifidum* BGN4, *B. breve* ATCC 15701, *B. bifidum* KFRI 708, *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *C. perfringens* ATCC 13124, *E. cloacae* ATCC 13047, and *E. faecalis* ATCC 19433 were anaerobically cultured in Brain Heart Infusion (BHI) media at 37°C for 5 days as described in Kim et al. (2018). The production of ammonia by catalyzed indophenol reaction was determined according to the method of Chaney and Marbach described in Kim et al. (2018).

B. longum BORI and other probiotic strains did not produce ammonia. In contrast, *Bacteroides* spp., *Clostridium perfringens,* and *Enterobacter* spp., positive controls, produced 12.9 ± 1.3 to 161.0 ± 6.6 μg/mL of ammonia. This study found no indication of the production of ammonia by *B. longum* BORI.

6.B.2.8. Hemolytic Test (adopted from Kim et al., 2018)

Visualizing the physical changes caused by hemolytic activity by culturing the microorganisms on a medium containing animal or human blood is a commonly used tool to evaluate the hemolytic properties of pathogens. In this study, the potential hemolytic activity of *B. longum* BORI and *B. bifidum* BGN4 was assessed using the blood agar plating method as described in Kim et al. (2018). *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 (positive control) showed β-hemolysis colorless zones around the cell colonies, whereas *B. longum* BORI and *B. bifidum* BGN4 showed no hemolysis and no change of color in the periphery of the colonies.

6.B.2.9. Biogenic Amine Production Test

Some edible microorganisms and probiotic strains were reported to produce biogenic amines. Therefore, this study examined the biogenic amine production of *B. longum* BORI by anaerobically culturing the cells in whole milk (Seoul Milk, Korea) or de Man-Rogosa-Sharpe (MRS) broth with supplementation of 0.05% (w/w) L-cysteine-HCl at 37°C for 15h. The biogenic amine analysis extraction procedure was conducted and HPLC analysis of the biogenic amines was performed as described by Kim et al. (2018).

B. longum BORI did not produce cadaverine, histamine, or tyramine; however, they produced 16.6 μg/mL and 24.2 μg/mL of putrescine, respectively. The levels produced were not of concern. Putrescine is a natural substance present in various foods. Putrescine, also naturally found in small amounts in living cells, is formed by the decarboxylation of ornithine and arginine. Putrescine was commonly found in frozen spinach puree (average 12.9 mg/kg), ketchup (average 52.5 mg/kg), concentrated tomato paste (average 25.9 mg/kg), and frozen green pea (average 46.3 mg/kg) (Kim et al., 2018).

6.B.2.10. Mucin Degradation Test

The intestinal mucus gel layer is an important constituent of the intestinal barrier that consists of a glycoprotein family. Bacterial translocation can occur in infants and immunocompromised hosts, even if the intestinal mucus acts as a biological shield from microbes. This bacterial translocation has the potential to cause sepsis and is one of the most serious probiotic safety concerns. In this study, the translocation capabilities of *B. longum* BORI were measured using *in vitro* mucolytic assays as described in Kim et al. (2018).

The growth of both probiotic strains was actively induced when glucose was added as a carbon source. However, when mucin was added instead of glucose, no growth was observed in either strain. These observations clearly indicate that *B. longum* BORI did not use mucin as a carbon source for their growth. *B. longum* BORI did not degrade mucin, indicating that the strains are not capable of damaging intestinal surfaces and do not have translocational abilities.

6.C. Animal Studies of *B. longum*

This review includes the studies of orally administered live *B. longum* BORI and other strains but excludes the studies of heat killed or dead *B. longum* strains and those using other routes of administration. In addition to studies related to the BORI strain, studies of the BB536 strain are included as corroborative data to support the safety of the BORI strain. These two strains have an over 99% homology in genomic sequences of 16S rRNA and antibiotic susceptibility patterns of *B. longum* BORI and BB536 strains are very similar. This review covers the papers published until June 30, 2018.

6.C.1. Animal Toxicity Studies of *B. longum* BORI

Human experience and the available scientific literature concerning the consumption of bifidobacteria by all age groups are remarkably free from any experiences of toxicity. There is no evidence that bifidobacteria produce any toxins or poisonous compounds. Due to the general consensus that bifidobacteria are considered safe for human consumption due to their long history of safe use, traditional safety studies of *B. longum* BORI have likely been considered unnecessary and have not been performed.

However, the study by Li et al. (2016) included important safety parameters, such as body wt, body weight gain, liver weight, histopathologic evaluation, clinical chemistry (lipid profile, aspartate transaminase [AST] and alanine transaminase [ALT]), although this study aimed to evaluate anti-obesity effects of *B. longum* BORI (Table 8). *B. longum* BORI and *B. bifidum* BGN4, *L. casei* IBS041 and *L. acidphilus* AD031 were individually administered to high-fat diet-fed mice for 8 weeks. No adverse effects of probiotics were reported on the measured outcomes including serum activities of AST and ALT and clinical chemistry parameters. Thus, the data from this study support the support the safety of *B. longum* BORI.

Table 8. Animal Toxicity Studies of *B. longum*

Objective	Animal	Dose of *B. longum*	Duration	Measurements or Results	Reference
B. longum BORI					
To investigate the anti-obesity effects of two bifidobacterial and compared them to two lactobacilli by using a high fat diet mouse model	54 ICR mice (age 7 wk)	High fat diet with $5x10^8$ CFU/mL of *B. bifidum* BGN4, *B. longum* BORI, *L. casei*, or *L. acidophilus*	8 wk	Body weight, body weight gain; liver weight; histopathologic evaluation; clinical chemistry (lipid profile, AST, ALT); hepatic lipid analyses	Li et al., 2016
B. longum BB536					
To determine LD_{50} of *B. longum* BB536	Mice	~$5x10^{13}$ CFU/kg bw; details are not available	Single oral dose and 14 d observation	LD_{50} ~$5x10^{13}$ CFU/kg bw	FDA, 2009 (GRN 268)

6.C.2. Animal Efficacy Studies of *B. longum*

An efficacy paper of *B. longum* BORI was identified in the literature (Table 9; Kang et al., 2018). Although this study was designed to investigate the anti-obesity effects of *B. longum* BORI, several safety related endpoints were obtained during the experiments; therefore, this study is reviewed below as additional supporting information.

Kang et al. (2018) evaluated the anti-obesity effect of fermented ginseng and probiotics mixture. The mixture of fermented ginseng and two probiotics, *B. longum* BORI and *L. paracasei* CH88, was administered to high-fat diet fed mice for 9 weeks. No adverse effects of probiotics were reported on the measured outcomes such as food intake, weight gain, morphological analysis of liver and epididymal fat, plasma lipid and glucose profiles.

Table 9. Animal Efficacy Studies of *B. longum*

Objective	Animal	Dose of *B. longum*	Duration	Measurement	Reference
B. longum BORI*					
To evaluate whether fermented ginseng and probiotics mixture had an anti-obesity effect on mice fed a high-fat diet	40 male ICR mice fed low or high fat diet (age 7 wk)	1.25×10^{8}– $5x10^{8}$ CFU/mL *Bifidobacterium longum* BORI and *Lactobacillus paracasei* CH88 (total 0.25-1 $x10^{9}$ CFU/mL+ 0.25 – 1.0% fermented ginseng	9 wk	Food intake, weight gain, morphological analysis of liver and epididymal fat, plasma lipid and glucose profiles	Kang et al., 2018
B. longum BB536*					
To investigate the colon tumor inhibitory activity of *B. longum* BB536	Male F344 rats with azoxymethane (AOM)-induced colon cancer	Doses up to $2x10^{12}$ CFU/kg bw/day	40 wk	body weight, body weight gain, or feed intake	Singh et al., 1997

*Oral administration; CFU=colony forming unit; wk=weeks.

6.C.3. Animal Toxicity Studies of *B. longum* BB536

The LD_{50} of *B. longum* BB536 orally administered to mice was determined to be approximately $5x10^{13}$ CFU/kg bw (FDA, 2009; GRN 268). The LD_{50} of *B. longum* BB536 administered intraperitoneally to mice was determined to be approximately $9x10^{11}$ CFU/kg bw (Table 8; FDA, 2009).

6.C.4. Animal Efficacy Studies of *B. longum* BB536

GRN 268 (FDA, 2009) reported that results from repeated dose studies of *B. longum* BB536 administered to rats showed no treatment effects on body weight, body weight gain, or feed intake at doses up to $2x10^{12}$ CFU/kg bw/day for 40 weeks (Table 9; Singh et al., 1997). This study investigated the colon tumor inhibitory activity of *B. longum* BB536 in male F344 rats with azoxymethane (AOM)-induced colon cancer. Findings for the studies provide support for the safe use of *B. longum* BB536 under the test conditions.

6.D. Human Clinical Studies

6.D.1. Human Clinical Studies of *B. longum* BORI

Table 10 summarizes outcomes from a human clinical study on the oral administration of the *B. longum* BORI strain in infants and/or toddlers with rotavirus-associated symptoms. This review covers the papers published until June 30, 2018.

Park et al. (2017) examined the efficacy of a commercially available probiotic product containing *B. longum* BORI and *L. acidophilus* AD031 in infants and/or toddlers with rotavirus-associated symptoms. The subjects were randomly divided into two treatment groups: a probiotics formula containing both *B. longum* BORI (2×10^{10} CFU/g; twice a day) and *L. acidophilus* AD031 (2×10^{9} CFU/g; twice a day) or a placebo with the standard therapy for diarrhea for 3 days. The duration of the patients' diarrhea was significantly shorter in the probiotics group than the placebo group (5.61 ± 1.23 vs. 4.38 ± 1.29, $P=0.001$). Such symptoms such as duration of fever, frequency of diarrhea, and frequency of vomiting tended to be ameliorated by the probiotic treatment. However, differences between the two groups were not statistically significant. There were no serious adverse events and no differences in the frequency of adverse events in both groups.

6.D.2. Human Clinical Studies of *B. longum* BB536

Since *B. longum* BORI and BB536 strains have a 99.4% similarity in the 16S rRNA genomic sequence, human clinical studies of *B. bifidum* strain BB536 were used to corroborate the safety of *B. longum* BORI. Tables 11-1 and 11-2 summarize human clinical studies of oral administration of *B. longum* BB536 strain in healthy subjects and various patient groups. Table 11-1 shows the studies that employ a single strain of BB536 strain with no other microorganisms. All studies used preparations containing only *B. longum* BB536 with no other microorganisms. Table 11-2 presents the studies that used BB536 strain in combination with other probiotics. This review covers the papers published between January 1, 2009 and June 30, 2018.

The effects of *B. longum* BB536 on influenza (Namba et al., 2010), immune function and intestinal microbiota (Akatsu et al., 2013), and health management (Kondo et al., 2013) were evaluated in elderly subjects. Healthy adults were used to evaluate the effect of *B. longum* BB536 on fecal *B. fragilis* levels (Odamaki et al., 2012). In healthy children, the effects on diarrhea and upper respiratory illnesses were evaluated (Lau et al., 2018). Human studies with a single strain of *B. longum* BB536 tested doses ranging from 4.27×10^{8} to 1×10^{11} CFU/day for durations of 8 weeks to 10 months.

Other human studies evaluated the effects of *B. longum* BB536 combined with another probiotics on digestive tolerance in preterm infants with very low or extremely low birth weights (Rouge et al., 2009), plasma lipids in adult women (Andrade & Borges, 2009), allergic diseases in pregnant Japanese women (Enomoto et al., 2014), diarrhea in pelvic cancer patients (Demers et al., 2014), and fecal and mucosal microbiota and local immune function in patients undergoing elective colorectal resection for cancer (Gianotti et al., 2010). These studies tested doses ranging from 2.7×10^{7} to 1×10^{10} CFU/day for durations of 4 days to 7 months (Table 11-2).

Table 10. Human Clinical Studies of *B. longum* BORI

Subject	Dose	Duration	Measurement	Reference
Healthy, full term infants				
57 infants and toddlers infected with rotavirus, 9-16 mo	4 x 10^{10} cfu/day *B. longum* BORI + 4 x 10^{9} cfu/day *L. acidophilus* AD031, divided into 2 doses	3 d	Adverse effects, duration and frequency of diarrhea; frequency of vomiting; duration of fever	Park et al., 2017

Table 11-1. Human Clinical Studies of *B. longum* BB536 (Single Strain Alone)

Objective	Subject	Dose of *B. longum*	Treatment Duration	Measurement	Reference
To evaluate the effects of *B. longum* BB536 administration on influenza infection, influenza vaccine antibody titer, and cell-mediated immunity in the elderly	27 elderly subjects receiving influenza vaccination at wk 3, age 86.7 ± 6.6 yrs	Sachet contains $1x10^{11}$ cfu *B. longum* BB536; once daily	5 wk for control Group; additional 14 wk for test group (test group - total of 19 wk)	Incidence of influenza and fever; Changes in NK cell activity, neutrophil bactericidal activity, and neutrophil phagocytic activity; maintenance of antibody titers to influenza vaccine and cell-mediated immunity	Namba et al., 2010
To examine the effect of yogurt supplemented with a probiotic strain on the cell numbers of fecal enterotoxigenic *Bacteroides fragilis* in a healthy population	32 subjects with enterotoxigenic *Bacteroides fragilis*	160 g of yogurt (~$1.0x10^9$ cfu lactic acid bacteria) supplemented with *B. longum* BB536 (4.27×10^8 cfu after preparation and >$1.12x10^8$ cfu/d at end of consumption period	8 wk intervention, followed by 12 wk post-observation	Fecal sample analysis (changes of enterotoxigenic *Bacteroides fragilis* number and dominant species of *B. fragilis* group)	Odamaki et al., 2012
To evaluate the effects of supplementation with the probiotic *B. longum* BB536 on immune function and intestinal microbiota in the elderly	45 elderly patients, age 81.7 ± 8.7 yrs	5×10^{10} cfu/2 g *B. longum* BB536; twice daily	12 wk intervention, followed by 4 wk post-observation	Clinical observations (body temperature, stool form and consistency using Bristol Stool Form Scale); fecal microbiota; immunological biomarkers in blood-total IgG, IgA, IgM, and IgE conc. in serum NK cell activity; NK cell activity in peripheral blood mononuclear cells (PBMCs)	Akatsu et al., 2013

To investigate if *B. longum* BB536 on modulated the intestinal environment and microbiota in elderly patients receiving enteral feeding	83 patients receiving enteral feeding, 67-101 y	Placebo or 5×10^{10} cfu *B. longum* BB536; once daily	16 wk	Clinical observation (body temperature, occurrence of infection, and frequency of defecation); fecal microbiota; adverse events	Kondo et al., 2013
	123 patients receiving enteral feeding, 65-102 y	Placebo, 5×10^{10} cfu/d *B. longum* BB536 or 1×10^{11} CFU/d *B. longum* B536; divided into two doses			
To investigate the effects of *B. longum* BB536 on intestinal microbiota composition and immune response	264 healthy infants, Days 0 -7 after birth	Formula with 10^7 cfu/g *B. longum* BB536vs. standard formula	From days 0-7 to 6 mo of age, 6 mo post observation	Fecal microbiota; Th2 cytokine secretion; plasma antibody response to vaccines	Wu et al., 2016
To evaluate the effects of *B. longum* BB536 on diarrhea and/or upper respiratory illnesses	520 healthy Malaysian pre-school children, age 2-6 yr	5×10^9 cfu/d *B. longum* BB536; placebo	10 mo	Occurrence of diarrhea and upper respiratory illnesses; gut microbiota;	Lau et al., 2018

Table 11-2. Human Clinical Studies of *B. longum* BB536 in Combination with Another Probiotics

Objective	Subject	Dose	Treatment Duration	Measurement	Reference
To evaluate the effect of the probiotic Bifilact® on moderate and severe treatment-induced diarrhea during pelvic radiation	229 patients with pelvic cancer, mean age 60.6-62.0 y	Bifilact® standard dose – a total of 2.6 billion cfu/d, *B. longum* BB-536 + *L. acidophilus* LAC-361 divided into 2 doses; Bifilact® higher dose – a total of 30 billion cfu/d, *B. longum* BB-536 + *L. acidophilus* LAC-361, divided into 3 doses	45-65 d	Severity of diarrhea; abdominal pain scale; fecal incontinence scale; consistency of stool (Bristol scale)	Demers et al., 2014
To investigate the effects of bifidobacterial supplementation on the risk of developing allergic diseases in the Japanese population	130 pregnant women	Sachets containing $5x10^9$ cfu *B. longum* BB536 [ATCC BAA-999] and *B. breve* M-16V Women: two sachets daily starting 4 wk before delivery Infants: 1 sachet daily	Mothers-7 mo (1 mo prenatally and 6 mo post birth); Infants- from 1 wk to 6 mo of age, 2.5 y post observation	Fecal microbiota from mothers and infants; clinical examination and development of allergic diseases in infants	Enomoto et al., 2014
To evaluate the efficacy of probiotics on the digestive tolerance to enteral feeding in preterm infants born with a very low or extremely low birth weight	94 infants; gestational age <32 wk, a birth weight <1500 g, a postnatal age ≤2 wk	$4x10^8$ cfu/d of *B. longum* BB536 and *L. rhamnosus* GG divided into 4 doses	14 d	Primary endpoint- the percentage of infants receiving 50% of their nutritional needs via enteral feeding on the 14th day of life; others-nosocomial infections, sepsis with positive blood culture, necrotizing enterocolitis, etc.	Rouge et al., 2009
To evaluate the effect of milk	34 women, age 18-65 yr	375 g/d fermented milk containing $2.7x10^7$-$1.0x10^8$ cfu/g *B. longum* BB536 and 1.4-$2.1x10^8$ cfu/g *L.*	4 weeks, with 1-wk washout period;	Blood lipid analysis; health status; body	Andrade and

fermented with *L. acidophilus* 145 and *B. longum* BB536 on plasma lipids in adult women		*acidophilus* 145, divided into 3 doses vs. control-standard yogurt containing *S. thermophilus* and *Lb. bulgaricus*	crossover design	weight, height, and body composition	Borges, 2009
To investigate whether probiotics bacteria, given perioperatively, might reduce concentration of pathogens in stools, and modulate the local immune function	31 subjects undergoing elective colorectal resection for cancer, age 18-80 yrs	Low dose of probiotics: $2x10^7$ cfu/d *B. longum* BB536 and *L. johnsonii* La1 High dose of probiotics: $2x10^9$ cfu/d *B. longum* BB536 and *L. johnsonii* La1	6 d (3 d before operation, post-operatively from day two to day four)	Fecal microbiota; percent of patients with variation of enterococci and enterobacteriaceae; percent of positive dendritic cell subsets	Gianotti et al., 2010

6.E. Potential Infection

Humans are exposed to bifidobacteria by the use of probiotics and eating fermented foods (e.g. yogurt, cheese, fermented vegetables, and olives) as well in the host's own microflora. Even with these sources, bifidobacteria rarely cause infections in humans. This lack of pathogenicity extends to all age groups as well as immunocompromised patients (EFSA, 2010).

6.F. Safety Determination

Studies have demonstrated that a daily dose of *B. longum* BORI at up to 1.0 x 10^{10} cells per day (up to 0.5 x10^{10} per serving; twice a day) is safe based on the following facts:

1. *B. longum* BORI has a long history of safe consumption in humans. The bacterial species *B. longum* is included in the Old Dietary Ingredient list, i.e., the use of *B. longum* is grandfathered under the DSHEA. The *B. longum* BORI ingredient has been marketed as a dietary supplement ingredient and as a dietary supplement in Korea since 2004. *B. longum* BORI at daily doses up to 2 x10^{11} cells has been safely used with no adverse effects. Additionally, other *B. longum* strains which have been successfully marketed in USA, Asia, and Europe with no reported side effects.

2. The information/data provided by BIFIDO (specifications, manufacturing process, identification, and intended use) in this report, and supplemented by the publicly available literature/toxicity data on *B. longum* BORI, provide a sufficient basis for an assessment of the safety of *B. longum* BORI for the proposed use as a dietary supplement ingredient prepared according to appropriate specifications and used according to cGMP. *B. longum* BORI has been tested for parameters outlined in the Food and Agriculture Organization of the United Nations/World Health Organization's (FAO/WHO) guidelines for the evaluation for microbes for probiotic use in foods.

   Key findings are summarized as follows:
   1) Animal and human studies showed no adverse effect from *B. longum* BORI.
   2) No *B. longum* strains have caused adverse effects in humans and animals.
   3) *In vitro* studies show that antibiotic susceptibility profiles of *B. longum* BORI were similar to those of the reference strain BB536, which has been safely used as a probiotic dietary supplement in the U.S. and Europe for over a decade. Available antibiotic susceptibility pattern suggests that *B. longum* BORI does not present concerns for antibiotic resistance in humans.
   4) *B. longum* BORI was not observed to have hemolytic or mucolytic activity.
   5) *B. longum* BORI was not observed to produce clinically significant levels of biogenic amines and ammonia.
   6) Results from comparisons of genomic sequences of known bacterial toxins with sequences of the genomic sequence of other known pathogens with sequences indicate that there is no significant homology.

3. The intended use of *B. longum* BORI results in levels of exposure significantly below historical human use levels and provides a reasonable certainty of safety (Intended target intake level will be 10^9 - 10^{10} cfu *B. longum* BORI/day since powdered term infant formulas will contain 10^8 cfu *B. longum* BORI/g. Intended use of 1.0 x 10^9 cfu *B. longum*

BORI/serving in the selected conventional food categories would result in the estimated mean and 90th percentile intakes of 1.4 x 10^9 and 3.0 x10^9 cfu/person/day, respectively, in all users).

4. *B. longum* BORI is well characterized and is free from chemical and other microbial contamination.

Thus, it is reasonable to conclude that intended use of 10^8 cfu *B. longum* BORI/g powdered term infant formulas and 1.0x10^9 *B. longum* BORI cells per serving of selected foods is safe in infants, children and adults.

6.G. Conclusions and General Recognition of the Safety of *B. longum* BORI

6.G.1. Common Knowledge Element of the GRAS Determination

B. longum BORI has been safely used as a food ingredient around the world for a decade. As a result, a number of comprehensive reviews of the safety of *B. longum* BORI have been published (Kim et al., 2018).

6.G.2. Technical Element of the GRAS Determination (Safety Determination)

Numerous human and animal studies have reported benefits of *B. longum* BORI with no major adverse effects. BIFIDO rigorously tests its final production batches to verify adherence to quality control specifications and, thus, are manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes. There is broad-based and widely disseminated knowledge concerning the safety of *B. longum* BORI and other *B. longum* strains. The literature indicates that *B. longum* BORI and a similar strain offer consumers benefits without adverse effects. Thus, the intended uses of *B. longum* BORI have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the "technical" element of the GRAS determination.

BIFIDO concluded that these uses of *B. longum* BORI is GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions. Therefore, the proposed use is safe within the terms of the Federal Food, Drug, and Cosmetic Act, meeting the standard of reasonable certainty of no harm. It is also Generally Recognized as Safe (GRAS) according to Title 21 Code of Federal Regulations (21 CFR). BIFIDO is not aware of any information that would be inconsistent with a finding that the proposed use of *B. longum* BORI meets appropriate specifications, and its use according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

PART 7. REFERENCES

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7.B. References that are Not Generally Available

Not applicable

Appendix A. Comparison of 16S rRNA Genomic Sequences between *B. longum* BORI and Other *B. longum* strains

Reference strain	Similarity with *B. longum* BORI, %
B. longum BB536	99.40%
B. longum ATCC 15697	99.21%
B. longum ATCC 15707	98.21%
B. longum KCTC 3128	99.59%
B. longum Su 851	99.60%
B. longum ATCC 27533	97.74%

BB536F
Sequence ID: Query_176379Length: 815Number of Matches: 1
Related Information
Range 1: 1 to 815GraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1500 bits(812)	0.0	814/815(99%)	0/815(0%)	Plus/Plus

```
Query  540  AAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCC  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    AAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCC  60

Query  600  GCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGT  659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGT  120

Query  660  GGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTG  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTG  180

Query  720  ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG  779
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG  240

Query  780  TAAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGT  839
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TAAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGT  300
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Query  840   TAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGC  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   TAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGC  360

Query  900   CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGC  959
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361   CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGC  420

Query  960   TTGACATGTTCCCGACGGTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACAGGTGG  1019
             |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  421   TTGACATGTTCCCGACGGTCGTAGAGATACGGCTTCCCTTCGGGGCGGGTTCACAGGTGG  480

Query  1020  TGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA  1079
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481   TGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA  540

Query  1080  CCCTCGCCCCGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAA  1139
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  541   CCCTCGCCCCGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAA  600

Query  1140  CTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGC  1199
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  601   CTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGC  660

Query  1200  ATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAA  1259
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  661   ATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAA  720

Query  1260  CCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAA  1319
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  721   CCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAA  780

Query  1320  TCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCC  1354
             |||||||||||||||||||||||||||||||||||
Sbjct  781   TCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCC  815
```

Query: *B. longum* Bori; subject: *B. longum BB536R*	
Identities	98.92%

BB536_R Sequence

ID: Query_45507Length: 831Number of Matches: 1
Related Information
Range 1: 1 to 831Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1483 bits(803)	0.0	824/833(99%)	5/833(0%)	Plus/Minus

```
Query  10   GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAGG  69
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  831  GGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAAG  772

Query  70   CTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATAC  129
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  771  CTT-GCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATAC  713

Query  130  ACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTTGATCGCATGGTCT  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  712  ACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTTGATCGCATGGTCT  653

Query  190  TCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGTA  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  652  TCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGTA  593

Query  250  ACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGAC  309
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  592  ACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGAC  533

Query  310  TGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA  369
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  532  TGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA  473

Query  370  AGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTAT  429
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            ||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  472  AGCCTGATGCAGCGACACCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTAT  413

Query  430  CGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAGC  489
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  412  CGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAGC  353

Query  490  AGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGT  549
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  352  AGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTAGT  293

Query  550  AGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGTA  609
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  292  AGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGTA  233

Query  610  CGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAA-CGGTGGAATGTGT  668
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  232  CGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAAACGGTGGAATGTGT  173

Query  669  AGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGG  728
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  172  AGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGG  113

Query  729  AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTC-CACGCCGT-AAACGG  786
            |||||||||||||||||||||||||||||||||||||||||||| |||||||| ||||||
Sbjct  112  AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCTCACGCCGTAAAACGG  53

Query  787  TGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGT  839
            |||||||||||||||||||||| |||||||||||| ||||||||||||||||
Sbjct  52   TGGATGCTGGATGTGGGGCCCGTCCCACGGGTTCCG-GTCGGAGCTAACGCGT  1
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Query: *B. longum* Bori; subject: *B. longum* ATCC 15697	
Identities	99.21%

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BORI        TTCGATTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGA 60
15697       TTTGATCATGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGA 60
            ** ***  **************************************************

BORI        TCCATCAGGCTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCT 120
15697       TCCATCGGGCTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCT 120
            ****** **************************************************

BORI        GCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTTGATC 180
15697       GCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGTTCCAGTTGATC 180
            ********************************************** ***********

BORI        GCATGGTCTTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGAC 240
15697       GCATGGTCTTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGAC 240
            ************************************************************

BORI        GGCGGGGTAACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA 300
15697       GGCGGGGTAACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA 300
            ************************************************************

BORI        CATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA 360
15697       CATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA 360
            ************************************************************

BORI        ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAAC 420
15697       ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAAC 420
            ************************************************************

BORI        CTCTTTTATCGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTA 480
15697       CTCTTTTATCGGGGAGCAAGCGTGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTA 480
            ********************** ***********************************

BORI        CGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAA 540
15697       CGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAA 540
            ************************************************************

BORI        AGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCG 600
15697       AGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCG 600
            ************************************************************

BORI        CGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTG 660
15697       CGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTG 660
            ************************************************************

BORI        GAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGA 720
15697       GAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGA 720
            ************************************************************

BORI        CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGT 780
15697       CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGT 780
            ************************************************************

BORI        AAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTT 840
15697       AAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTT 840
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            ************************************************************

BORI        AAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCC 900
15697       AAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCC 900
            ************************************************************

BORI        CGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCT 960
15697       CGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCT 960
            ************************************************************

BORI        TGACATGTTCCCGACGGTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACAGGTGGT 1020
15697       TGACATGTTCCCGACGATCCCAGAGATGGGGTTTCCCTTCGGGGCGGGTTCACAGGTGGT 1020
            ********** ** ******* ** ****************************

BORI        GCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1080
15697       GCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC 1080
            ************************************************************

BORI        CCTCGCCCCGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAAC 1140
15697       CCTCGCCCCGTGTTGCCAGCGGATTGTGCCGGGAACTCACGGGGGACCGCCGGGGTTAAC 1140
            ************************ **********************************

BORI        TCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCA 1200
15697       TCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCA 1200
            ************************************************************

BORI        TGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAAC 1260
15697       TGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAAC 1260
            ************************************************************

BORI        CGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAAT 1320
15697       CGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAAT 1320
            ************************************************************

BORI        CGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAG 1380
15697       CGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAG 1380
            ************************************************************

BORI        TCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCT 1440
15697       TCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCT 1440
            ************************************************************

BORI        AAGGTGAGGCTCGTGATTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGC 1500
15697       AAGGTGAGGCTCGTGATTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGC 1500
            ************************************************************

BORI        TGGATCACCT          1510
15697       TGGATCACCT          1510
            **********
```

Query: *B. longum* Bori; subject: *B. longum* ATCC 15707

Bifidobacterium longum BORI

Identities	98.21%

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BORI        AGGGTTCGATTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
15707       AGGGTTCGATTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
            ************************************************************

BORI        GGGATCCATCAGGCTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCG 120
15707       GGGATCCATCAAGCT-TGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCG 119
            *********** *** ******************************************

BORI        ACCTGCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTT 180
15707       ACCTGCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGTTCCAGTT 179
            ************************************************* *******

BORI        GATCGCATGGTCTTCTGGGAA--AGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAG 238
15707       GATCGCATGGTCTTCTGGNGAAAGCNTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAG 239
            ******************  *     *********************************

BORI        CTTGACGGCGGGGTAACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGAC 298
15707       CTTGACGGNGGGGTAACGGCNNACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGAC 299
            ******** ***********  ************************************

BORI        CGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT 358
15707       CGGCCACATTGGGACTGAGATACGGCCCNGACTCCTACGGGAGGCAGCAGTGGGGAATAT 359
            ************************** ******************************

BORI        TGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTT 418
15707       TGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTT 419
            ************************************************************

BORI        GTAAACCTCTTTTATCGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGC 478
15707       GTAAACCTCTTTTATCGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGC 479
            ************************************************************

BORI        TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGG 538
15707       TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCNAGCGTTATCCGGAATTATTGG 539
            ************************************ ********************

BORI        GCGTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTG 598
15707       GCGTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTG 599
            ************************************************************

BORI        GATCCGCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTA 658
15707       GATCCGCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTA 659
            ************************************************************

BORI        ACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGT 718
15707       ACGGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGT 719
            ************************************************************

BORI        TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCA 778
15707       TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCA 779
            ************************************************************
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BORI    CGCCGTAAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAA 838
15707   CGCCGTAAACGGTGGATGCTGGATGTGGGGCCNGTTCCACGGGTTCCGTGTCGGAGCTAA 839
        ********************************* **************************

BORI    CGCGTTAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACG 898
15707   CGCGTTAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACG 899
        ************************************************************

BORI    GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC 958
15707   GGGGCCNGCACAAGCGGCGGAGCATGCGGATTAATTCGATGNAACGCGAAGAACCTTACC 959
        ****** ********************************** ******************

BORI    TGGGCTTGACATGTTCCCGACGGTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACA 1018
15707   TGGGCTTGACATGTTCCCGACGGTCGTAGAGATACGGCNTCCCTTCGGGGCGGGTTCACA 1019
        ********************************* **** ********************

BORI    GGTGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG 1078
15707   GGTGGNGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG 1079
        ***** ****************************************************

BORI    CGCAACCCTCGCCCCGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGG 1138
15707   CGCAACCCTCGCCCCGTGTTGCCAGCGGATTATGCCGGNAACTCACGGGNNACCGCCGGG 1139
        ************************************ **********  *********

BORI    GTTAACTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTT 1198
15707   GTTAACTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTT 1199
        ************************************************************

BORI    CACGCATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCT 1258
15707   CACGCATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCT 1259
        ************************************************************

BORI    GAAAACCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCT 1318
15707   GAAAACCNGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCT 1319
        ******* **************************************************

BORI    AGTAATCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCC 1378
15707   AGTAATCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCNGGCCTTGTACACACCGCCC 1379
        ************************************** ******************

BORI    GTCAAGTCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAG 1438
15707   GTCAAGNCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGANGGAG 1439
        ****** ********************************************** ****

BORI    CCGTCTAAGGTGAGGCTCGTGATTGGGAC 1467
15707   CCGTCTAAGGTGAGGCTCGTGATTGGGAC 1468
        ****************************
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Query: *B. longum* Bori; subject: *B. longum* KCTC 3128

Bifidobacterium longum BORI

Identities	99.59%

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BORI    GATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAGGCTTTGCT 60
3128    GATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAAGC-TTGCT 59
        ************************************************** ** *****

BORI    TGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATACACCGGAA 120
3128    TGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATACACCGGAA 119
        ************************************************************

BORI    TAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTTGATCGCATGGTCTTCTGGGA 180
3128    TAGCTCCTGGAAACGGGTGGTAATGCCGGATGTTCCAGTTGATCGCATGGTCTTCTGGGA 179
        ******************************** ***************************

BORI    AAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGTAACGGCCC 240
3128    AAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGTAACGGCCC 239
        ************************************************************

BORI    ACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGAGATA 300
3128    ACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGACTGAGATA 299
        ************************************************************

BORI    CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA 360
3128    CGGCCCATACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA 359
        ******* ************************************************

BORI    TGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTATCGGGGAG 420
3128    TGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTATCGGGGAG 419
        ************************************************************

BORI    CAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCG 480
3128    CAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCG 479
        ************************************************************

BORI    GTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGT 540
3128    GTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCGTAGGCGGT 539
        ************************************************************

BORI    TCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGTACGGGCGG 600
3128    TCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGTACGGGCGG 599
        ************************************************************

BORI    GCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCG 660
3128    GCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTGGAATGTGTAGATATCG 659
        ************************************************************

BORI    GGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAG 720
3128    GGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGGAGCGAAAG 719
        ************************************************************

BORI    CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGATGCTGG 780
3128    CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGATGCAGG 779
        ********************************************************** **
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BORI    ATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCTGGG 840
3128    ATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCCCGCCTGGG 839
        ************************************************************

BORI    GAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCGGAG 900
3128    GAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAGCGGCGGAG 899
        ************************************************************

BORI    CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGTTCCCGACG 960
3128    CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGTTCCCGACG 959
        ************************************************************

BORI    GTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACAGGTGGTGCATGGTCGTCGTCAG 1020
3128    GTCGTAGAGATACGGCTTCCCTTCGGGGCGGGTTCACAGGTGGTGCATGGTCGTCGTCAG 1019
        *********** **********************************************

BORI    CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCCGTGTTGC 1080
3128    CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCCGTGTTGC 1079
        ************************************************************

BORI    CAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAACTCGGAGGAAGGTGGGG 1140
3128    CAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAACTCGGAGGAAGGTGGGG 1139
        ************************************************************

BORI    ATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCATGCTACAATGGCCGGT 1200
3128    ATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCATGCTACAATGGCCGGT 1199
        ************************************************************

BORI    ACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAACCGGTCTCAGTTCGGAT 1260
3128    ACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAACCGGTCTCAGTTCGGAT 1259
        ************************************************************

BORI    CGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAACGT 1320
3128    CGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGAATCAGCAACGT 1319
        ************************************************************

BORI    CGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAAGTGGGCAG 1380
3128    CGCGGTGAATGCGTTCCCGGGCCTTGTACACACTGCCCGTCAAGTCATGAAAGTGGGCAG 1379
        ********************************** *************************

BORI    CACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCTAAGGTGAGGCTCGTGA 1440
3128    CACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCTAAGGTGAGGCTCGTGA 1439
        ************************************************************

BORI    TTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGG   1478
3128    TTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGG   1477
        ************************************
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Query: *B. longum* Bori; subject: *B. longum* Su 851	
Identities	99.60%

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BORI    TGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCAG 60
851     TGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGGATCCATCGA 60
        ************************************************************

BORI    GCTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATA 120
851     GC-TTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCGACCTGCCCCATA 119
        ** ************************************************************

BORI    CACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTTGATCGCATGGTC 180
851     CACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGTTCCAGTTGATCGCATGGTC 179
        ************************************** ********************

BORI    TTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGT 240
851     TTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCTTGACGGCGGGGT 239
        ************************************************************

BORI    AACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGA 300
851     AACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACATTGGGA 299
        ************************************************************

BORI    CTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGC 360
851     CTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGC 359
        ************************************************************

BORI    AAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTA 420
851     AAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGTAAACCTCTTTTA 419
        ************************************************************

BORI    TCGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAG 480
851     TCGGGGAGCAAGCGTGAGTGAGTTTACCCGTTGAATAAGCACCGGCTAACTACGTGCCAG 479
        ************** *********************************************

BORI    CAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCG 540
851     CAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGCGTAAAGGGCTCG 539
        ************************************************************

BORI    TAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGT 600
851     TAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGATCCGCGCCGGGT 599
        ************************************************************

BORI    ACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTGGAATGTGT 660
851     ACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAACGGTGGAATGTGT 659
        ************************************************************

BORI    AGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGG 720
851     AGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTACTGACGCTGAGG 719
        ************************************************************
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BORI    AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTG  780
851     AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTG  779
        ************************************************************

BORI    GATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCC  840
851     GATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACGCGTTAAGCATCC  839
        ************************************************************

BORI    CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAG  900
851     CGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGGGGCCCGCACAAG  899
        ************************************************************

BORI    CGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGT  960
851     CGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGCTTGACATGT  959
        ************************************************************

BORI    TCCCGACGGTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACAGGTGGTGCATGGTC 1020
851     TCCCGACGGTCGTAGAGATACGGCTTCCCTTCGGGGCGGGTTCACAGGTGGTGCATGGTC 1019
        ******************* ****************************************

BORI    GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCC 1080
851     GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCC 1079
        ************************************************************

BORI    CGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGTTAACTCGGAGGA 1140
851     CGTGTTGCCAGCGGATTGTGCCGGGAACTCACGGGGGACCGCCGGGGTTAACTCGGAGGA 1139
        ***************** ****************************************

BORI    AGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCATGCTACAA 1200
851     AGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCACGCATGCTACAA 1199
        ************************************************************

BORI    TGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAACCGGTCTCA 1260
851     TGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGAAAACCGGTCTCA 1259
        ************************************************************

BORI    GTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGAATC 1320
851     GTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAGTAATCGCGAATC 1319
        ************************************************************

BORI    AGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAA 1380
851     AGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCATGAAA 1379
        ************************************************************

BORI    GTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCTAAGGTGAG 1440
851     GTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCCGTCTAAGGTGAG 1439
        ************************************************************

BORI    GCTCGTGATTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGG       1495
851     GCTCGTGATTGGGACTAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGG       1494
        ********************************************************
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Query: *B. longum* Bori; subject: *B. longum* ATCC 27533	
Identities	97.74%

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BORI        AGGGTTCGATTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
27533       AGGGTTCGATTCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
            ************************************************************

BORI        GGGATCCATCAGGCTTTGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCG 120
27533       GGGATCCATCAAGCT-TGCTTGGTGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCG 119
            *********** *** ****************************************

BORI        ACCTGCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGCTCCAGTT 180
27533       ACCTGCCCCATACACCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGGATGTTCCAGTT 179
            ************************************************* *******

BORI        GATCGCATGGTCTTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGCGTCCTATCAGCT 240
27533       GATCGCATGGTCTTCTGGGAAAGCTTTCGCGGTATGGGATGGGGTCGNGTCCTATCAGCT 239
            ************************************************ ************

BORI        TGACGGCGGGGTAACGGCCCACCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCG 300
27533       TGACGGNGGGGTAACGGCNNNCCGTGGCTTCGACGGGTAGCCGGCCTGAGAGGGCGACCG 299
            ****** ***********   ***************************************

BORI        GCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG 360
27533       GCCACATTGGGACTGAGATACGGCCNNGACTCCTACGGGAGGCAGCAGTGGGGNATATTG 359
            ************************  ************************* ******

BORI        CACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGT 420
27533       CACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGGAGGCCTTCGGGTTGT 419
            ************************************************************

BORI        AAACCTCTTTTATCGGGGAGCAAGCGAGAGTGAGTTTACCCGTTGAATAAGCACCGGCTA 480
27533       AAACCTCTTTTATCGGGGAGCAAGCGTGAGTGAGTTTACCCGTTGAATAAGCACCGGCTA 479
            ************************* ********************************

BORI        ACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTATCCGGAATTATTGGGC 540
27533       ACTACGTGCCAGCAGCCNCGGTAATACGTAGGGTGCNAGCGTTATCCGGAATTATTGGGC 539
            ***************** ****************** ***********************

BORI        GTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGA 600
27533       GTAAAGGGCTCGTAGGCGGTTCGTCGCGTCCGGTGTGAAAGTCCATCGCTTAACGGTGGA 599
            ************************************************************

BORI        TCCGCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAAC 660
27533       TCCGCGCCGGGTACGGGCGGGCTTGAGTGCGGTAGGGGAGACTGGAATTCCCGGTGTAAC 659
            ************************************************************

BORI        GGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCCGTTA 720
27533       GGTGGAATGTGTAGATATCGGGAAGAACACCAATGGCGAAGGCAGGTCTCTGGGCNGNNA 719
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BORI        CTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACG 780
27533       CTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACG 779
            ************************************************************

BORI        CCGTAAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACG 840
27533       CCGTAAACGGTGGATGCTGGATGTGGGGCCCGTTCCACGGGTTCCGTGTCGGAGCTAACG 839
            ************************************************************

BORI        CGTTAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGAAATTGACGGG 900
27533       CGTTAAGCATCCCGCCTGGGGAGTACGGCCGCAAGGCTNAAACTCAAAGAAATTGACGGG 899
            ************************************ ********************

BORI        GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTG 960
27533       GGCCNGCACAAGCGGCGGAGCATGCGGATTAATTCGATGNAACGCGAAGAACCTTACCTG 959
            **** ********************************** ********************

BORI        GGCTTGACATGTTCCCGACGGTCGTAGAGATGCGGCTTCCCTTCGGGGCGGGTTCACAGG 1020
27533       GGCTTGACATGTTCCCGACGGCCGTAGAGATACGGCTTCCCTTCGGGGCGGGTTCACAGG 1019
            ********************* ********* ****************************

BORI        TGGTGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG 1080
27533       TGGNGCATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG 1079
            *** ********************************************************

BORI        CAACCCTCGCCCCGTGTTGCCAGCGGATTATGCCGGGAACTCACGGGGGACCGCCGGGGT 1140
27533       CAACCCTCGCCCNGTGTTGCCAGCGGATTGTGCCGGNNACTCACGGGNGACCGCCGGGGT 1139
            ************ ***************** ******  ********* ************

BORI        TAACTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCA 1200
27533       TAACTCGGAGGAAGGTGGGGATGACGTCAGATCATCATGCCCCTTACGTCCAGGGCTTCA 1199
            ************************************************************

BORI        CGCATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGA 1260
27533       CGCATGCTACAATGGCCGGTACAACGGGATGCGACGCGGCGACGCGGAGCGGATCCCTGA 1259
            ************************************************************

BORI        AAACCGGTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAG 1320
27533       AAACCNNNCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGGCGGAGTCGCTAG 1319
            *****   **************************************************

BORI        TAATCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGT 1380
27533       TAATCGCGAATCAGCAACGTCGCGGTGAATGCGTTCCCGGGCCTTGTACACACCGCCCGT 1379
            ************************************************************

BORI        CAAGTCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCCTTGTGGGATGGAGCC 1440
27533       CAAGTCATGAAAGTGGGCAGCACCCGAAGCCGGTGGCCTAACCCTT-GCGGGAGGGAGCC 1438
            ******************************************* * * **** ******

BORI        GTCTAAGGTGAGGCTCGTGATTGG       1464
27533       GTCTAAGGTGAGGCTCGTGATTGG       1462
            ************************
```

Appendix B. Certificate of Analysis for *B. longum* BORI

B I F I D O
23-16, Nonggongdanji-gil, Hongcheon-eup, Hongcheon-gun,
Gangwon-do, 25117, Republic of Korea
TEL +82-33-435-4962 FAX +82-33-435-4963

CERTIFICATE OF ANALYSIS

NAME OF PRODUCT		*Bifidobacterium longum* BORI
LOT NO.		BI-R-160510
PRODUCTION DATE		2016. 05. 10
CERTIFICATED DATE		2016. 05. 14
EXPIRATION DATE		2018. 05. 09
ANALYSIS RESULT		
Parameter	**BI-R-160510**	**Method of analysis/Method**
Appearance	Yellowish gray powder	Visual
Cell Counts (as *B. longum* BORI), cfu/g	7.10	KHFSC 4/3/3-58
Moisture, %	5.63	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0147	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.1034	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0078	KFSC 7/9/9.1/9.1.3
Mercury (Hg)	0.001	KFSC 7/9/9.1/9.1.6
Microbial purity		
Non-Lactic acid bacteria	Negative	KFSC 7/4/4.5/4.5.1
Total yeasts and molds	Negative	KFSC 7/4/4.10
Escherichia coli	Negative	KFSC 7/4/4.8
Salmonella	Negative	KFSC 7/4/4.11
Listeria	Negative	KFSC 7/4/4.15
Enterobacter sakazakii	Negative	KFSC 7/4/4.21
Proximate analysis		
Lipids, %	-	KFSC 7/2/2.1/2.1.5/2.1.5.1
Protein, %	-	KFSC 7/2/2.1/2.1.3/2.1.3.1
Carbohydrates, %	-	KFSC 7/2/2.1/2.1.4/2.1.4.1
Ash, %	-	KFSC 7/2/2.1/2.1.2

QC Manager **Ji-Young Shin** (b) (6)

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Gangwon-do, 25117, Republic of Korea
TEL +82-33-435-4962 FAX +82-33-435-4963

CERTIFICATE OF ANALYSIS

NAME OF PRODUCT	*Bifidobacterium longum* BORI
LOT NO.	BI-R-161108
PRODUCTION DATE	2016. 11. 08
CERTIFICATED DATE	2016. 11. 12
EXPIRATION DATE	2018. 11. 07

ANALYSIS RESULT

Parameter	**BI-R-161223**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. longum* BORI), cfu/g	7.20E+10	KHFSC 4/3/3-58
Moisture, %	5.32	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0159	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.0976	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0050	KFSC 7/9/9.1/9.1.3
Mercury (Hg)	ND	KFSC 7/9/9.1/9.1.6
Microbial purity		
Non-Lactic acid bacteria	Negative	KFSC 7/4/4.5/4.5.1
Total yeasts and molds	Negative	KFSC 7/4/4.10
Escherichia coli	Negative	KFSC 7/4/4.8
Salmonella	Negative	KFSC 7/4/4.11
Listeria	Negative	KFSC 7/4/4.15
Enterobacter sakazakii	Negative	KFSC 7/4/4.21
Proximate analysis		
Lipids, %	-	KFSC 7/2/2.1/2.1.5/2.1.5.1
Protein, %	-	KFSC 7/2/2.1/2.1.3/2.1.3.1
Carbohydrates, %	-	KFSC 7/2/2.1/2.1.4/2.1.4.1
Ash, %	-	KFSC 7/2/2.1/2.1.2

QC Manager **Ji-Young Shin**

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CERTIFICATE OF ANALYSIS

NAME OF PRODUCT		*Bifidobacterium longum* BORI
LOT NO.		BI-R-170910
PRODUCTION DATE		2017. 07. 10
CERTIFICATED DATE		2017. 02. 14
EXPIRATION DATE		2019. 02. 09
ANALYSIS RESULT		
Parameter	**BI-R-170210**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. longum* BORI), cfu/g	7.10E+10	KHFSC 4/3/3-58
Moisture, %	5.40	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0102	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.126	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0103	KFSC 7/9/9.1/9.1.3
Mercury (Hg)	ND	KFSC 7/9/9.1/9.1.6
Microbial purity		
Non-Lactic acid bacteria	Negative	KFSC 7/4/4.5/4.5.1
Total yeasts and molds	Negative	KFSC 7/4/4.10
Escherichia coli	Negative	KFSC 7/4/4.8
Salmonella	Negative	KFSC 7/4/4.11
Listeria	Negative	KFSC 7/4/4.15
Enterobacter sakazakii	Negative	KFSC 7/4/4.21
Proximate analysis[1]		
Lipids, %	0.42	KFSC 7/2/2.1/2.1.5/2.1.5.1
Protein, %	12.82	KFSC 7/2/2.1/2.1.3/2.1.3.1
Carbohydrates, %	81.99	KFSC 7/2/2.1/2.1.4/2.1.4.1
Ash, %	1.84	KFSC 7/2/2.1/2.1.2

[1]Analyzed in Korea Health Supplements Institute

QC Manager **Ji-Young Shin**

(b) (6)

Appendix C. **Human Studies of Other *B. longum* Strains**

The purpose of the summary tables below is to present that none of *B. longum* strains were associated with any adverse effects. Daily doses up to $4x10^{11}$ CFU/day did not result in any adverse effects (Steed et al., 2010). Due to abundance of the literature, our summary is limited to the studies using a single strain probiotic. Our summary covers the literature published until June 30, 2018.

Human Studies of Other *B. longum* Strains Reporting No Adverse Events Due to Treatment

Objective	Subject	Dose of Test Group	Duration	Measurement	Reference
To investigate the effects of synbiotic consumption on the colonic microbiota, immune function and health status in older people	43 older volunteers, aged 65-90 y	$4x10^{11}$ CFU/d *B. longum* (strain not specified) + 12 g a mixture of inulin and oligofructose, divided into 2 doses	4 wk	Fecal microbiota; clinical measurements (fasting blood conc of CRP, lipids, glucose, insulin and immunoglobulins and full blood counts); immune parameters (IL-1b, IL-6, TNF-a, IL-10, IFN-c, IL-4, IL-8 and MCP-1 cytokine and chemokine); bacterial metabolites in fecal samples	Macfarlane et al., 2013
To evaluate the potential effects of *B. longum* CECT 7347 in children with newly diagnosed coeliac disease	33 children age 2-17 y with newly diagnosed coeliac disease	10^9 CFU/capsule/d *B. longum* CECT 7347	3 mo	Clinical assessments; lymphocyte phenotyping; serum cytokine and Ig quantification; fecal secretory IgA quantification; microbiota analysis	Olivares et al., 2014
To investigate the effects of synbiotic consumption on disease processes in patients with Crohn's disease	35 patients with active Crohn's disease, age 18-79 y	$4x10^{11}$ CFU/d freeze-dried viable *B. longum* (strain not specified)	6 mo	Transcription levels of pro-inflammatory cytokines in mucosal tissue; microbiological analysis of tissue biopsies	Steed et al., 2010
To evaluate the effects of a 4-wk treatment regimen with probiotics, prebiotics, or synbiotics in patients with ulcerative colitis in remission	94 patients with ulcerative colitis	$2x10^9$ CFU/d *B. longum* (strain not specified)	4 wk	Inflammatory Bowel Disease Questionnaire scores; blood analysis	Fujimori et al., 2009

To investigate the possible effect of anxiolytic-like activity of probiotic formulation (PF) on anxiety, depression, stress and coping strategies in healthy human volunteers	55 subjects, mean age 42.4 y	Probio'Stick® containing $3x10^9$ CFU/stick probiotic (including *B. longum* R0175)	30 d	Hopkins Symptom Checklist-90 questionnaire; Hospital Anxiety and Depression Scale; Perceived Stress Scale; Coping Checklist questionnaire; urinary free cortisol analysis	Messaoudi et al., 2011
To assess the effect of probiotic supplementation in the first 6 months of life on eczema and allergic sensitization at 1 year of age in Asian infants at risk of allergic disease	253 infants with a family history of allergic disease	$9.26x10^7$ CFU/d *B. longum* BL999	6 mo	Clinical assessment (incidence of eczema and allergen sensitization); serum total immunoglobin E and skin prick tests	Soh et al., 2009
To determine the impact of 2 probiotic bifidobacteria on the fecal microbiota of premature infants fed either human milk or formula	12 premature infants on formula feeding (birth weight <1500 g, gestational age <33 wk)	Increasing dose *B. lactis* (strain not specified) starting from $1x10^8$ to $8.4x10^9$ CFU/d	5 wk	Fecal microbiota analysis	Underwood et al., 2013
	9 premature infants on mother's milk (birth weight <1500 g, gestational age <33 wk)	$8x10^9$ organisms/d *B. infantis* or *B. lactis* (strains not specified)	2 wk for each treatment		
To test whether probiotic-supplemented feeding to extremely low-birth-weight infants will improve growth	101 premature infants, birth weight 501-1000 g, ≤14 d of age	Probiotic (including 500 million CFU/d *B. infantis*, strain not specified)	until discharge or 34 wk of age	Clinical data; adverse event	Al-Hosni et al., 2012

To examine whether oral administration of prophylactic *L. acidophilus* and *B. infantis* to neonates would decrease the incidence of necrotizing enterocolitis	1237 newborns	Infloran Berna7 (including 250 million live each of *B. infantis*, strain not specified)	until discharge	Stool color and consistency; incidence of necrotizing enterocolitis	Hoyos, 1999
To evaluate the efficacy of probiotics in reducing the incidence and severity of necrotizing enterocolitis in very low birth weight infants	417 VLBW infants, <1500 g	Infloran (including minimum of 1,015,697 of *B. infantis*, units and strain not specified), 125 mg/kg, twice daily	until discharge	Incidence of necrotizing enterocolitis	Lin et al., 2005
To assess the effect of probiotics on the incidence of necrotizing enterocolitis (NEC) in premature infants born to HIV-positive and HIV-negative women	74 HIV-exposed and 110 HIV-unexposed premature and VLBW infants; <34 weeks' gestation, <1250 g	$0.35x10^9$ CFU each of *B. infantis* (strain not specified) and *L. rhamnosus* GG; control; 5 drops daily in breast milk	4 wk	Clinical data; Apgar scores; development necrotizing enterocolitis	Van Niekerk et al., 2015
To compare the effect of administration of probiotics on feeding tolerance and growth outcomes of HIV-exposed (but uninfected) vs HIV non-exposed preterm infants	74 HIV-exposed and 110 non-exposed premature infants; <34 wk gestation, between 500 and 1250 g	Probiotics including $0.35x10^9$ CFU *B. infantis* (strain not specified), 5 drops daily in breast milk	28 d	Clinical data; Apgar score; development of necrotizing enterocolitis	Van Niekerk et al., 2014

To study the effect on the gut flora of feeding two different commercially-available preparations of lactic acid bacteria and bifidobacteria to normal healthy volunteers	24 healthy volunteers, age 19-59 y	Idoform capsule (including $3.2x10^8$ CFU/d *B. longum*, strain not specified)	7 d	Bacteriological examinations	Nielsen et al., 1994
To evaluate the treatment of pediatric functional chronic intestinal constipation with a probiotic goat yogurt	59 students with functional chronic intestinal constipation, age 5-15 y	10^9 CFU/d *B. longum* (strain not specified)	5 wks for each treatment	Defecation frequency; stool consistency (Bristol Stool Scale); abdominal or defecation pain assessment	Guerra et al., 2011
To evaluate the short-term efficacy and safety of probiotics as an aid in the treatment of *Candida*-associated stomatitis	65 patients with *Candida*-associated stomatitis, age 18-75 y	$6x10^7$ CFU/d *B. longum*, strain not specified	4 wk	Clinical assessment of pain level (visual analogue scale) and hyperemia; resting saliva analysis; lingual dorum swab analysis	Li et al., 2014
To investigate whether maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of developing eczema in high-risk infants	205 mother-infant pairs; pregnant women with atopic sensitization and history or active allergic disease	$1x10^9$ CFU/d probiotic (including *B. longum*, strain not specified)	4 mo	Clinical examination of infants; cumulative incidence of eczema in infants; skin prick tests in infants	Rautava et al., 2012
To examine whether three different probiotics could normalize self-reported stress-associated gastrointestinal discomfort and reduce overall self-reported stress	581 undergraduate students, age 19.9 ± 0.1 y	$3x10^9$ CFU *B. infantis* R0033	6 wk	Number and consistency of stools (Bristol Stool Form Scale); stress level; Gastrointestinal Symptom Rating Scale (GSRS); salivary cortisol analysis	Culpepper et al., 2016

To determine whether consuming *L. gasseri* KS-13, *B. bifidum* G9-1, and *B. longum* MM-2 would result in beneficial effects on Mini Rhinoconjunctivitis Quality of Life Questionnaire (MRQLQ) scores throughout allergy season in individuals who typically experience seasonal allergies	173 healthy volunteers who self-identified as having seasonal allergies	Probiotic capsule including 0.30 billion CFU/d *B. longum* MM-2	8 wk	MRQLQ scores; Gastrointestinal Symptom Rating Scale; fecal bacterial DNA; regulatory T-cells analysis in fasting whole blood samples; serum total IgE and IL-10 concentrations in fasting whole blood samples	Dennis-Wall et al., 2017
To determine the effect of administering a specific combination of probiotics to very preterm infants on culture-proven late-onset sepsis	1099 infants <32 wk gestation, <1500 g	$350x10^6$ organism of *B. lactis* BB-12; 2 mL in 3 mL breast milk or formula	until discharge or term corrected age	Incidence of sepsis; incidence of necrotizing enterocolitis; mortality	Jacobs et al., 2013
To determine the efficacy of a probiotic combination for the prevention of antibiotic-associated diarrhea in children	78 children, age 5 mo to 16 y with acute otitis media, and/or respiratory tract infection, and/or urinary tract infection	$2x10^8$ CFU/d *B. longum* PL03	duration of antibiotic treatment	Frequency of diarrhea; discontinuation of antibiotic treatment; need for hospitalization; intravenous rehydration; adverse events	Szymanski et al., 2008
To determine whether Ecologic®Relief is effective on constipation during pregnancy	20 women, 12-34 weeks into pregnancy, with functional constipation, aged ≥ 18 y	Ecologic®Relief (total $4x10^9$ CFU, including *B. longum* W108 and *B. lactis* W52)	4 wk	Change in defecation frequency; baseline characteristics; adverse effects; bisacodyl use	de Milliano et al., 2012

To investigate the efficacy of probiotic therapy in alleviating small intestinal bacterial overgrowth and permeability in chronic liver disease	50 patients with chronic liver disease, age 18-65 y	Duolac Gold probiotic ($1x10^{10}$ viable cells/d, including *B. longum* KCTC 12200BP and *B. lactis* KCTC 11904BP)	4 wk	Intestinal permeability assessment; small intestinal bacterial overgrowth assessment; clinical symptoms assessment; fecal microbiota analysis	Kwak et al., 2014
To investigate the effects of synbiotic consumption on the colonic microbiota, immune function and health status in older people	43 older volunteers, age 65-90 y	$4x10^{11}$ CFU/d freeze-dried viable *B. longum* (strain not specified)	4 wk for each treatment	Microbiological analysis; clinical measurements; immune parameters analysis; bacterial metabolites analysis in fecal samples	Macfarlane et al., 2013
To determine efficacy of synbiotic in reducing average infant crying time	50 breastfed infants aged 15-150 days with infantile colic	Synbiotic (1 billion CFU, including *B. infantis*, strains not specified)	30 d	Daily cry time; stool consistency and frequency; side effects	Kianifar et al., 2014
To assess the safety and tolerability of an experimental formula	97 healthy, full-term infants, <14 d old, 2500-4500 g	$2x10^{7}$ CFU *B. longum* BL999	until 112 d old	Weight gain; tolerability in terms of gastrointestinal symptoms	Puccio et al., 2007
To compare growth and development of toddlers fed milk containing synbiotics and long-chain polyunsaturated fatty acids (LCPUFA) or a control milk	393 healthy 12-mo toddlers	Synbiotics milk (including 10^{7} CFU/g *B. longum* BL999); 400 mL/d	12 mo	Weight gain; stool characteristics; microbiota composition; hematology assessment; motor, cognitive, and behavioral development; immune response	Firmansyah et al., 2011
To test the efficacy of the synbiotic food supplement Probiotical in children with acute diarrhea	111 children with acute diarrhea (age between 3 and 186 mo)	Probiotic capsule including $6.5x10^{9}$ *B. infantis* (unit and strain not specified)	7 d	Degree of dehydration; duration of diarrhea; Bristol stool score	Vandenplas et al., 2011

To assess the effect of a synbiotic mixture on the duration of diarrhea and the length of hospital stay in children with acute watery diarrhea	209 hospitalized children with acute watery diarrhea, age 3 to 120 mo	NBL Probiotic Gold® (including $2.5x10^9$ CFU of live bacteria of *B. longum*, strain not specified)	5 d	Frequency and consistency of stools; length of hospitalization; duration of diarrhea	Dinleyici et al., 2013
To determine optimal time efficiency of a synbiotic in controlling respiratory infections and wheezing disease	78 children, age <5 y	$5x10^9$ CFU synbiotic (including *B. infantis* Rosell-33)	-	Assessment of immune status; skin prick	Stojkovic et al., 2016
To compare the effect of two probiotic products in the treatment of diarrhea in children less than 2 y of age	64 children with acute diarrhea, age 1-23 mo	$1.75x10^7$ lyophilized cells/d *B. longum*	5 d	Length of diarrhea; presence of stools and their consistency	Grandy et al., 2010
To evaluate the efficacy of probiotic strains isolated from Koreans for the treatment of viral gastroenteritis in young children and against rotavirus *in vitro*	29 pediatric patients with symptoms of viral gastroenteritis, age 3 mo to 7 y	Sachet including 10^8 CFU *B. longum* and *B. lactis* (strains not specified)	1 wk	Symptoms assessments; fecal rotavirus detection; physical and hematological examinations	Lee et al., 2015
To measure the efficacy of a probiotic formulation on time to reach full enteral feeds in very low birth weight (VLBW) newborns	104 VLBW newborns, 750-1499 g, on enteral feeds	Probiotic (total $1.25x10^9$ CFU/d including *B. longum*, strain not specified)	until discharge	Time to reach full enteral feed; feed intolerance; incidence of necrotizing enterocolitis; weight gain; mortality during hospital stay	Shashidhar et al., 2017
To investigate the effects of intake of yogurt containing *B. longum* BB536-y and fructo-oligosaccharides (FOS)	27 healthy persons	Yogurt containing BB536; yogurt containing BB536 and FOS	5 wk	Microbial community, short chain fatty acids, and pH analyses	Ohara and Suzutani, 2018

Human Studies of Other *B. longum* Strains Reporting Side or Adverse Effects

Objective	Subject	Dose of *B. longum*	Duration	Measurement	Adverse Events	Reference
To evaluate the effects of *B. longum* with fructo-oligosaccharides in the treatment of nonalcoholic steatohepatitis	66 patients, age 30-65 y	2.5 g *B. longum* (strain not specified)	24 wk	Biochemical analysis; body mass index; serum endotoxin levels; blood analysis	1 nausea, 1 moderate headache, and 1 abdominal pain; No clinically relevant changes in hematology, clinical chemistry, and renal function	Malaguarnera et al., 2012
To evaluate the bifidogenic effect of a mainly whey protein study formula low in phosphate and protein, allowing a composition closer to that of human milk	190 healthy full-term infants	$2x10^7$ CFU/g *B. longum* BL999	4 mo	Fecal microbiota analysis; stool IgA levels	13 gastrointestinal events, 4 upper respiratory events, 2 lower respiratory events, 1 other adverse events; No differences in occurrence of adverse events between different groups	Hascoet et al., 2011
To assess the safety and acceptability of three different regimens of *L. reuteri* DSM 17938 and *B. longum* subspecies *infantis* 35624 given over one month to very	113 healthy infants, age 4-12 wks	Probiotic including 10^9 CFU *B. longum* subspecies *infantis* 35624 daily, weekly, or biweekly dose	29 d	Occurrence of gastrointestinal and respiratory symptoms	Cough and congestion are the most common reported symptoms	Hoy-Schulz et al., 2016

young health infants						
To evaluate the effects of a probiotic formulation containing three *Bifidobacterium* strains on lipid profiles in children affected by primary dyslipidemia	38 children with dyslipidemia, age 10.8 ± 2.1 y	Probiotic capsule including $1x10^9$ CFU/d each of *B. lactis MB* 2409 and *B. longum* BL04	12 wk for each treatment	Biochemical analysis	No serious adverse events were detected; 3 intestinal symptoms (2 in probiotic and 1 in placebo group): occurred at start of treatment, plausible to exclude cause and effect relationship	Guardamagna et al., 2014
To evaluate infant formulas containing probiotics and synbiotics for safety and tolerance	227 healthy full-term infants; ≤14 d old, 2500-4500 g	$1.29x10^6$ or $2.58x10^6$ CFU/mL *B. longum* BL999 (BL999)	from 1-14 d to 112 d of age	Weight gain; symptoms of digestive tolerance; frequency of adverse events	2 in BL999 + *L. rhamnosum* LPR had milk allergy that could possibly be related to treatment	Chouraqui et al., 2008

References for Appendix C

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Analytical Report

Sample Code	**502-2018-00025081**	**Report date**	**30-May-2018**
Certificate No.	**AR-18-SU-025011-02**		

***This analytical report replaces the previous issued analytical report no.: AR-18-SU-025011-01**

BIFIDO.CO.,LTD.

Our reference:	502-2018-00025081/ AR-18-SU-025011-02
Client Sample Code:	BI-R-171004
Sample described as:	Bifidobacterium longum BORI
Sample Packaging:	Sealed plastic bag
Sample reception date:	15-May-2018
Analysis starting date:	15-May-2018
Analysis ending date:	22-May-2018

Arrival Temperature (°C)	22.8	**Sample Weight**	250g
Sample Type	Powder		

		Results	Unit	LOQ	LOD
☆ **VV053**	Allergen – Soya (ELISA) Method: Neogen Test-Combination 8410				
Soya protein		**<2.5**	mg/kg	2.5	

SIGNATURE

(b) (6)

Shine Xie
Food Chemistry Manager

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
☆ means the test is subcontracted within Eurofins group
◎ means the test is subcontracted outside Eurofins group
Sum compounds results are calculated from the results of each quantified compound as set by regulation
The result(s) relate(s) only to the item (s) tested.
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END OF REPORT

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Analytical Report

Sample Code	**502-2018-00025080**	**Report date**	**30-May-2018**
Certificate No.	**AR-18-SU-025010-02**		

***This analytical report replaces the previous issued analytical report no.: AR-18-SU-025010-01**

BIFIDO.CO.,LTD.

Our reference:	502-2018-00025080/ AR-18-SU-025010-02
Client Sample Code:	BI-R-170416
Sample described as:	Bifidobacterium longum BORI
Sample Packaging:	Sealed plastic bag
Sample reception date:	15-May-2018
Analysis starting date:	15-May-2018
Analysis ending date:	22-May-2018

Arrival Temperature (°C)	22.8	**Sample Weight**	220g
Sample Type	Powder		

		Results	Unit	LOQ	LOD
☆ **VV053**	Allergen – Soya (ELISA) Method: Neogen Test-Combination 8410				
Soya protein		**<2.5**	mg/kg	2.5	

SIGNATURE

(b) (6)

Shine Xie
Food Chemistry Manager

EXPLANATORY NOTE

LOQ: Limit of Quantification
< LOQ: Below Limit of Quantification
N/A means Not applicable
☆ means the test is subcontracted within Eurofins group
◎ means the test is subcontracted outside Eurofins group
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END OF REPORT

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From:	susanscho1@yahoo.com
To:	Zhu, Jianmei
Subject:	Re: Questions for GRN813
Date:	Thursday, February 21, 2019 12:49:06 PM
Attachments:	Eurofin BORI soy allergen 170416.pdf Eurofin BORI soy allergen 180106.pdf Eurofin BORI soy allergen171004.pdf

---

Dear Dr. Zhu,

In response your questions of Feb 20, 2019, we have prepared our answers as follows:

Answer to Question 1.

Please see the attached three COAs from Eurofin which show negative results, i.e., the concentrations of soya allergen were below detection limits (<2.5 ppm) in all 3 non-consecutive lots.

Answer to Question 2.

Those thresholds (%ID of 90% and minimum length 60%) were default settings. When you check the link below, you will find those threshold settings. https://cge.cbs.dtu.dk/services/VirulenceFinder/

Thank you very much for your kind attention to these matters. Have a nice day!

Sincerely,

Susan

Susan Cho, Ph.D., NutraSource

On Wednesday, February 20, 2019, 03:24:26 PM EST, Zhu, Jianmei <Jianmei.Zhu@fda.hhs.gov> wrote:

Dear Susan,

Our reviewers have convened and discussed about GRN813 on *B. longum* BORI for its use in conventional foods and non-exempt term infant formula. We have the following two questions that need to be addressed:

1. On Page 7, in the section of Method of Manufacture, it states that soy peptone is used during fermentation. Soy is one of the eight major allergens in the US. Please clarify whether soy peptone is removed during processing and whether any allergen is present in the final product. If not, please provide a statement to confirm that.

2. On Page 19, in the section of 6.B.2.3. Absence of Virulence Genes, it states "The output consists of best-matching genes from BLAST analysis of the selected database against the submitted genome of *B. longum* BORI. The selected %ID threshold was set at 90.00 % and the

selected minimum length was set at 60 %.”

Are these thresholds (%ID of 90% and minimum length 60%) default settings or have they been set by the notifier? If they have been specifically set by the notifier, please explain the rationale for choosing these thresholds. If not, please provide a citation for the use of these thresholds.

We respectfully request a response within 10 business days. If you need extra time, please contact me as soon as possible. Thank you.

Best regards,

Jianmei(Jamie) Zhu, Ph.D.
Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1953
Jianmei.Zhu@fda.hhs.gov

From: Susan S Cho
To: Zhu, Jianmei
Subject: Re: Additional Question for GRN813
Date: Monday, May 20, 2019 2:33:26 PM

Magnetic contamination means any metal contamination. They wanted to remove any metals. There is not reason why metals are present during the manufacturing process, since they do not use any metal sources (Fe, Mn, etc). Yet it is an almost standard process in Korea before the final packaging in case any foreign metals are introduced by any reasons. Hope it naswers your questions. Thank you

Sincerely,
Susan
Susan Cho, Nutrasource--301-875-6454

On Monday, May 20, 2019, 02:11:50 PM EDT, Zhu, Jianmei <Jianmei.Zhu@fda.hhs.gov> wrote:

Dear Dr. Cho,

Your response did not quite address the question. Please explain what “magnetic contamination ” is present and/or **why** this step is needed.

Thank you,

Jamie

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Tuesday, May 14, 2019 6:23 PM
To: Zhu, Jianmei <Jianmei.Zhu@fda.hhs.gov>
Subject: Re: Additional Question for GRN813

Dear Dr Zhu,

It meant that it went through magnetic separator and is free of metals. Hope it answers your question. Thank you

Sincerely,

Susan

Susan Cho, Ph.D. NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

On Tuesday, May 14, 2019, 03:54:22 PM EDT, Zhu, Jianmei <Jianmei.Zhu@fda.hhs.gov> wrote:

Dear Dr. Cho,

Our technical reviewers identified another question for you to address about GRN813:

On Page 8, in step 7 of the section of Method of Manufacture, you state “…the ingredient is freed of magnetic contamination prior to packaging.” Please explain what “magnetic contamination ” is present and/or why this step is needed.

Please address this question at your earliest convenience and let me know if you need any clarification.

Regards,

Jamie