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#814

September 18, 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 bifidum* BGN4

Dear Mr. Bonnette,

On behalf of BIFIDO CO., LTD, we are submitting a GRAS notification for *Bifidobacterium bifidum* BGN4 as a food ingredient. The enclosed document provides notice of a claim that the food ingredient, *Bifidobacterium bifidum* BGN4, 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 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)

9/18/2018

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

DETERMINATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF *BIFIDOBACTERIUM BIFIDUM* BGN4

Prepared for BIFIDO CO., LTD.

Prepared by:
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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 *Bifidobacterium bifidum* BGN4 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 bifidum strain BGN4 (*B. bifidum* BGN4)

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

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

B. bifidum BGN4 will be added to non-exempt term infant formula (soy-, milk-, and whey-based) and conventional foods.

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

Non-Exempt Term Infant Formula Applications:

The use level is the same as those described in GRAS notices of other bifidobacteria (GRN 49 for *B. lactis* and GRN 454 for *B. breve*). Powdered non-exempt term infant formulas (milk-, soy-, or whey-based) will contain up to 10^8 colony forming units (cfu) *B. bifidum* BGN4 per g powdered formulas.

Conventional Food Applications:

BIFIDO intends to add *B. bifidum* BGN4 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 and nutritional 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 and nutritional 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. bifidum* BGN4 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)

___ _____ 9/18/2018_____

Name: Susan S. Cho, Ph.D., Date:

Title: President of NutraSource, Inc., Agent for BIFIDO Co., Ltd.

1.I. FSIS/USDA Statement

BIFIDO does not intend to add *B. bifidum* BGN4 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

Bifidobacterium bifidum BGN4

Details of *B. bifidum* BGN4 identification is shown in Appendix A.

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. bifidum* BGN4.

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. bifidum* BGN4 ingredient is illustrated in Figure 1.

The first step involves fermentation of a starter culture of *B. bifidum* BGN4 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. bifidum* BGN4 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 10 ml to 2,000 L 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. bifidum* BGN4 is cooled to 10°C and then centrifuged at 7,500 rpm for 1 h to collect the cells.
6. *B. bifidum* BGN4 is taken to measure the bacterial weight subjected to dilution with a cryoprotective agent (100% maltodextrin), which is 85% (w/w) *B. bifidum* BGN4 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. bifidum* BGN4 ingredients are comprised of 51% *B. bifidum* BGN4 cells, 9% maltodextrin, and 40% corn starch. The number of *B. bifidum* BGN4 cells per one gram of the ingredient is estimated as 1.0×10^{11} cells.

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

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. bifidum* BGN4 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.

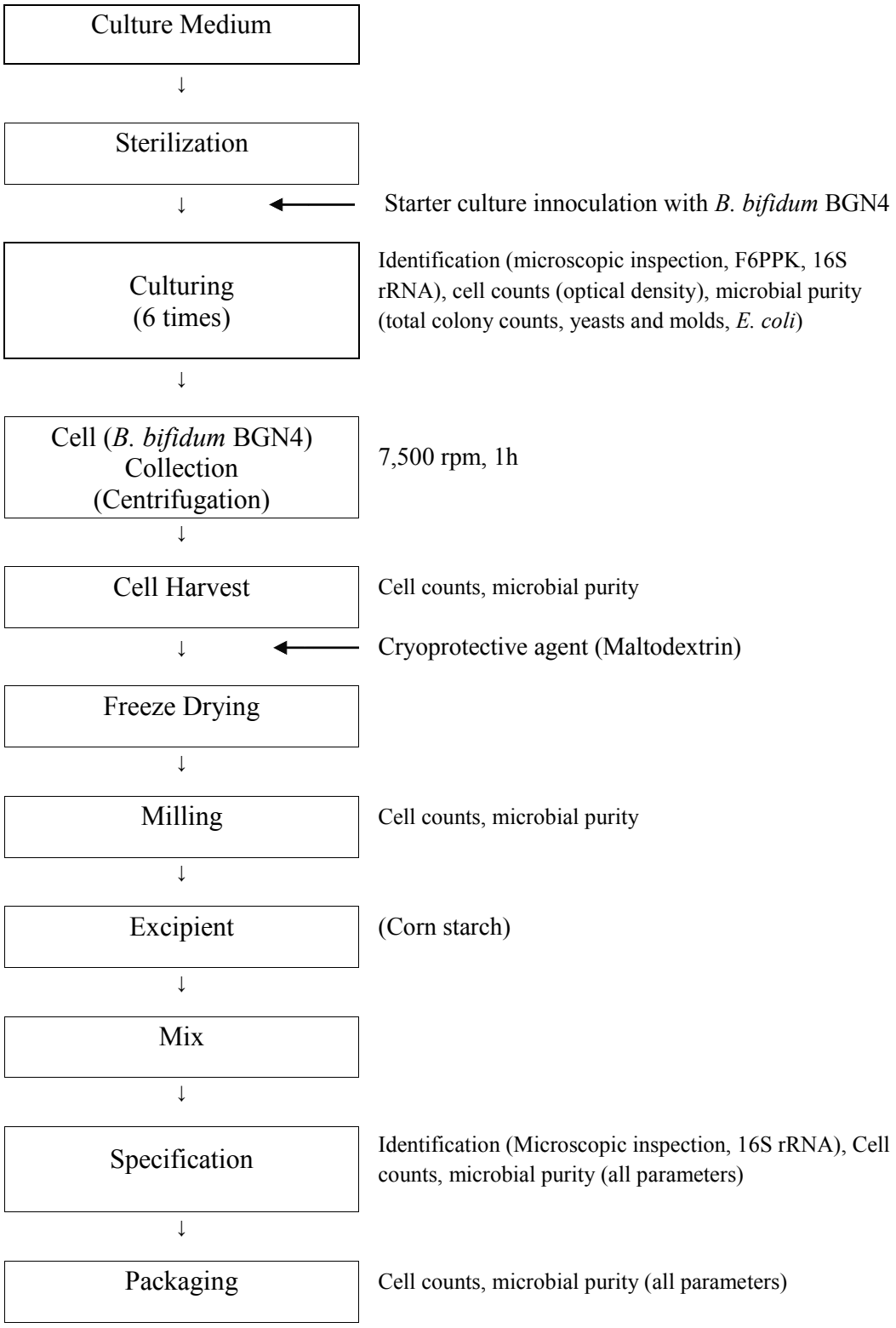

Figure 1. Schematic Overview of Manufacturing Process for *B. bifidum* BGN4

2.C. Specifications and Composition of *B. bifidum* BGN4

Table 3 presents composition and specifications of *B. bifidum* BGN4. Analyses of three non-consecutive lots of the *B. bifidum* BGN4 ingredient confirm that the material produced by the manufacturing process is consistent and complies with the product specifications, meeting appropriate food-grade specifications (Table 3; Appendix B). The analytical data also demonstrate the absence of any chemical impurities or microbiological contamination (Table 4).

Table 3. Composition and Specifications of *B. bifidum* BGN4 Stock Ingredient

Parameter	Specification	Typical composition*	Method of analysis Method number
Appearance	No off-taste and off-flavor	Yellow white powder	Visual
Cell Counts, cfu/g (as *B. bifidum* BGN4)	MT 1.00E+11	1.13E+11	KHFSC 4/3/3-58
Moisture, %	NMT 5.0	3.56	KFSC 7/2/2.1/2.1.1
Heavy metals			
Lead (Pb), ppm	NMT 0.3	0.0149	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	NMT 0.3	0.0227	KFSC 7/9/9.1/9.1.4
Cadmium (Cd), ppm	NMT 0.1	0.0066	KFSC 7/9/9.1/9.1.3
Mercury (Hg), ppm	NMT 0.1	0.0003	KFSC 7/9/9.1/9.1.6
Microbial purity			
Non-Lactic acid bacteria (Total Colony Counts)	NMT 100 cfu/g	Negative	Total Colony Counts 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	1.84	KFSC 7/2/2.1/2.1.2

*Average of 3 analytical values. NA: Not Applicable.
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)

Table 4. Analytical Values of *B. bifidum* BGN4 (3 non-consecutive lots)

Parameter	B4-R-160303	B4-R-161223	B4-R-170210
Appearance	Yellow white powder	Yellow white powder	Yellow white powder
Cell Counts, cfu/g (as *B. bifidum* BGN4),	1.20E+11	1.10E+11	1.10E+11
Moisture, %	3.49	3.52	3.68
Heavy metals			
Lead (Pb), ppm	0.0122	0.0162	0.0162
Arsenic (As), ppm	0.013	0.0159	0.0393
Cadmium (Cd), ppm	0.0058	0.0060	0.0079
Mercury (Hg), ppm	ND	ND	0.001
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, %	-	-	0.42
Protein, %	-	-	12.82
Carbohydrates, %	-	-	81.99
Ash, %	-	-	1.84

KFSC: Korean Food Standards Codex, KHFSC: Korean Health functional Food Standards Codex
ND: Not Detected

2.D. The Stability of the *B. bifidum* BGN4

Bulk ingredient stability data indicate that the number of *B. bifidum* BGN4 cells in the ingredient is stable for up to 2 years at 5 °C and 12 months at 25 ºC when the cells are supplied in excess of 130% of the claim value at the time of shipment. Table 5 presents the stability of *B. bifidum* BGN4 at various temperatures.

Table 5. The Stability of *B. bifidum* BGN4

Temperature /Month	5℃	25℃	35℃
0	1.33E+11	1.33E+11	1.33E+11
2	1.28E+11	1.21E+11	9.80E+10
4	1.32E+11	1.20E+11	8.20E+10
8	1.24E+11	1.03E+11	7.24E+10
10	1.26E+11	1.09E+11	7.00E+10
12	1.18E+11	1.05E+11	6.33E+10
18	1.13E+11	9.00E+10	5.40E+10
24	1.01E+11	8.24E+10	4.40E+10
The viability of *B. bifidum* BGN4 at 24 month compared to the claim value (1.00E+11 cfu/g)	101%	82%	44%

2.E. Intended Technical Effects

The intended effect is to provide *B. bifidum* BGN4 cells to non-exempt term infant formulas 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. bifidum* are known to have several health benefits including improved intestinal health and immune functions (Picard et al., 2005). In particular, *B. bifidum* can also be used as a probiotic supplement.

PART 3. DIETARY EXPOSURE

3.A. Estimated Dietary Intakes (EDIs) of *B. bifidum* BGN4 Under the Intended Use

3.A.1. Non-Exempt Term Infant Formula Applications

The use levels are the same as those described in GRN 454. Since the intended use level in this GRAS determination is the same as GRN 454, these EDI levels are consistent with those reported in GRN 454. Powdered non-exempt term infant formulas (milk-, soy-, or whey-based) will contain up to 10^8 colony forming units (cfu) *B. bifidum* BGN4/g powdered formulas. The intended target intake level will be 10^9 - 10^{10} cfu *B. bifidum* BGN4/day.

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, the caloric requirements of one-month-old and six-month-old infants are 472 kcal/day and 645 kcal/day, respectively (Institute of Medicine (IOM) Panel on Macronutrients and IOM Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). The addition of 10^8 cfu *B. bifidum* BGN4/g infant formula will result in intakes of 9.9×10^9 and 1.35×10^{10} cfu *B. bifidum* BGN4/day, respectively. These formulas will be supplemented appropriately to provide a minimum of 10^9 cfu *B. bifidum* BGN4/day at the end of a 24-month shelf-life at room temperature.

3.A.2. Conventional Food Applications

BIFIDO intends to add *B. bifidum* BGN4 to selected conventional food products for the general population (Table 1). Selected conventional foods will contain up to 1.0×10^9 cfu/serving.

The intended use of 1.0×10^9 cfu *B. bifidum* BGN4/serving in the target food categories would result in the estimated mean and 90th percentile intakes of 1.36 and 3.00 servings of foods per person per day, providing 1.36×10^9 and 3.00×10^9 *B. bifidum* BGN4 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 3.5×10^9 cfu/day. In total population, mean and 90th percentile intakes are estimated to be 0.41 and 1.17 servings per day, providing 0.41×10^9 and 1.17×10^9 cfu/person/day, respectively (Table 6-2).

These estimates are highly amplified since it is not likely that *B. bifidum* BGN4 will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered.

Table 6-1. EDIs of *B. bifidum* BGN4 from Proposed Uses in Conventional Foods in All Users*

	N	% users	Food, serving/d		*B. bifidum* BGN4, cfu/day	
			Mean	90th Pctl	Mean	90th Pctl
Children 1-5	583	39.6	0.72	1.69	0.72×10^9	1.69×10^9
Children, 6-12	486	23.5	0.59	1.11	0.59×10^9	1.11×10^9
Males, 13-18	78	9.6	1.01	2.01	1.01×10^9	2.01×10^9
Females, 13-18	114	15.3	0.60	1.10	0.60×10^9	1.10×10^9
Males, 19-99	1,084	26.0	1.55	3.50	1.55×10^9	3.50×10^9
Females, 19-99	1,626	38.3	1.51	3.00	1.51×10^9	3.00×10^9
Total population	3,971	30.2	1.36	3.00	1.36×10^9	3.00×10^9

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

Table 6-2. EDIs of *B. bifidum* BGN4 from Proposed Uses in Conventional Foods in All Population

	N	% users	Food, serving/d		*B. bifidum* BGN4, cfu/day	
			Mean	90th Pctl	Mean	90th Pctl
Children 1-5	1,587	100	0.29	0.92	0.29×10^9	0.92×10^9
Children, 6-12	2206	100	0.14	0.50	0.14×10^9	0.50×10^9
Males, 13-18	822	100	0.10	NA	0.10×10^9	NA
Females, 13-18	838	100	0.09	0.41	0.09×10^9	0.41×10^9
Males, 19-99	4,294	100	0.40	1.32	0.40×10^9	1.32×10^9
Females, 19-99	4,587	100	0.58	1.67	0.58×10^9	1.67×10^9
Total population	14,334	100	0.41	1.17	0.41×10^9	1.17×10^9

*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.

Summary of Consumption Data
Non-exempt term infant formula applications: The intended target intake level will be a minimum of 10^9 cfu *B. bifidum* BGN4/day since powdered term infant formulas will contain 10^8 cfu *B. bifidum* BGN4/g.

Conventional food applications: The intended use of 1.0×10^9 cfu *B. bifidum* BGN4/serving in the selected food categories would result in the estimated mean and 90th percentile intakes of 1.36×10^9 and 3.00×10^9 cfu/person/day, respectively, in all users. However, these EDIs are overly inflated since it is not expected that all food categories listed under the intended use will contain *B. bifidum* BGN4 and food wastes are also considered.

3.B. Food Sources of *B. bifidum* BGN4

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. bifidum* BGN4 from the diet.

3.C. EDIs of *B. bifidum* BGN4 from Diet

Not applicable.

3.D. Total EDIs of *B. bifidum* BGN4 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 carbohydrates from the diet.

PART 4. SELF LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the *B. bifidum* BGN4 ingredient.

PART 5. HISTORY OF CONSUMPTION

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.

Since 2004, *B. bifidum* BGN4 has been legally marketed in Korea as an ingredient in dietary supplements and as a dietary supplement at the recommended daily dose of up to $1.0x10^{10}$ BGN4 cells per day. The use of *B. bifidum* BGN4 in dietary supplements delivers daily doses up to $1.5x10^{11}$ *B. bifidum* BGN4 cells per day to the Korean population. Over 5,475 kg of the *B. bifidum* BGN4 ingredient (containing $1.0x10^{11}$ *B. bifidum* BGN4 cells per gram) or 688,517 kg of the finished probiotic product (containing 17,115 kg of *B. bifidum* BGN4 cells with 0.10~25%) have been sold in the past 8 years. *B. bifidum* BGN4, as an ingredient for probiotic dietary supplements, is also exported to various countries. No serious side effects of *B. bifidum* BGN4 were reported by consumers in the past 14 years.

PART 6. BASIS FOR GRAS DETERMINATION

6.A. Current Regulatory Status

In the United States, various *Bifidobacterium* species have been determined to be GRAS for use in conventional foods and infant formulas, including:

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).

The FDA did not have questions on the intended uses, use levels, and the summaries of safety of the above listed *Bifidobacterium* species.

According to the 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" in under DSHEA. The bacterial species *B. bifidum* is included in the Old Dietary Ingredient list, i.e., the use of the bacterial species *B. bifidum* is grandfathered under the DSHEA (CRN, 1998).

The European Food Safety Agency (EFSA) considers the bacterial species *B. bifidum* 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. bifidum* 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) has noted that a variety of different *Lactobacillus and Bifidobacterium* species have occasionally been 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 *Lactobacillus and Bifidobacterium* species can be considered nonpathogenic to humans, and therefore, pose no specific safety concerns.

In Korea, *B. bifidum* BGN4 has received the Korean FDA's approval as a functional food ingredient.

6.B. Review of Safety Data

Safety assessment tests required by FAO/WHO were considered when evaluating the safety of *B. bifidum* BGN4. Those tests included assessment of undesirable metabolic activities (e.g., biogenic amine production), determination of antimicrobial resistance factors, mammalian toxin production or hemolytic activity (only if the strain belongs to a species known to be a mammalian toxin producer or to have hemolytic potential), assessment of side effects in human studies, and assessment of postmarket epidemiological surveillance of adverse effects in consumers. The general safety of the *B. bifidum* strains including BGN4 has been confirmed on the basis of sensitivity to a range of antibiotics and the absence of hemolysis, mucolytic activity and biogenic amine production (Kim et al., 2018).

Following summarizes the studies of Kim et al. (2018).

1. The genome of *B. bifidum* BGN4 does not contain regions with significant homology to known antibiotic resistance, pathogenic or toxigenic genes.
2. Functional assays indicate that *B. bifidum* BGN4 exhibits antibiotic susceptibility. The exception was gentamicin resistance for *B. bifidum* BGN4. However, the MIC values of *B. bifidum* BGN4 for gentamicin was higher than that established by EFSA, but equal to the PROSAFE cutoff established for *B. longum* and those of other GRAS strains such as *B. lactis* BB-12 and *B. breve* M-16V.
3. *B. bifidum* BGN4 was not observed to contain plasmids.
4. *B. bifidum* BGN4 was not observed to have hemolytic and mucolytic activity.
5. *B. bifidum* BGN4 was not observed to produce clinically significant levels of biogenic amines and ammonia.
6. *B. bifidum* BGN4 is genetically stable for 25 generations.
7. Human clinical studies found no adverse effects of *B. bifidum* BGN4.
8. No serious adverse effects were reported by consumers in the past 14 years (described in Part 5)

In addition, no animal and human clinical studies reported adverse effects of *B. bifidum* BGN4. This review covers the papers published until June 30, 2018.

6.B.1. Metabolism

Given that *B. bifidum* BGN4 retains its form, it is unlikely that *B. bifidum* BGN4 will enter organs or the systemic circulation from the gastrointestinal tract in normal, healthy individuals. Rather, the fate of *B. bifidum* BGN4 after ingestion is expected to be similar to that seen after consumption of live food-grade bacteria. *B. bifidum* BGN4 is expected to transit through the gastrointestinal tract and be excreted in feces. It has also been shown that live *B. bifidum* BGN4, like other bifidobateria, does not harbor the potential for translocation (AHRQ, 2011; Kim et al., 2018; Picard et al., 2015).

6.B.2. Anti-mutagenicity of *B. bifidum*

Lo et al. (2004) investigated the antimutagenic effects of 6 bifidobacteria (*B. adolescentis, B. bifidum, B. breve, B. infantis, B. lactis,* and *B. longum*) against benzo[α]pyren(B[α]P) by a modified Ames test using *Salmonella typhymurium* TA 100 after acidic and bile treatment mimicking gastrointestinal conditions. When bifidobacteria were treated at pH 2.0 for 3 h or 1% bile for 6 h, their antimutagenic activities against B[α]P were increased as compared to the controls at pH 7.0 for 0 h. *B. bifidum* showed 66.0% of antimutagenic activity at pH 7.0, 62.5% at pH 2.0 and 73.6% % with 1% bile.

6.B.3. Genetic Stability Test

The genetic variation of edible microorganisms possibly results in indel (i.e. gene deletion and insertion) and mutation. A critical consideration of commercializing probiotics is whether it is possible to maintain genetic safety over the long term. Theoretically, an evaluation of genetic stability requires the entire genome sequence of the strain.

The entire genome sequence of *B. bifidum* BGN4 has been published (Yu et al., 2012). It consists of a 2,223,664-bp circular chromosome (62.65% G+C) with no plasmid. The nucleotide sequence identified 1,835 coding sequences (CDSs), 7 pseudogenes, 3 rRNA operons, and 52 tRNAs. This study showed that the similarity in the genomic comparison of the 1st and 25th generations of samples was 99.9996~99.9998% via the Orthologous Average Nucleotide Identity (OrthoANI) analysis (Kim et al., 2018). The difference between 0.0002% and 0.0004% is equivalent to 4.4 bp to 8.8 bp mutations in the entire nucleotide sequence. This difference is assumed to result from sequencing errors or spontaneous evolutionary mutations. These data indicate low 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.4. The Absence of Virulence Genes

The search for virulence factors in *B. bifidum* BGN4 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) (www.genomicepidemiology.org).

The database 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 genomes of *B. bifidum* BGN4. The selected %ID threshold was set at 90% and the

selected minimum length 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 sequence of *B. bifidum* BGN4 was compared with the genome sequences of four well-known pathogens (*E. coli*, *Enterococcus*, *Listeria*, and *Staphylococcus aureus*). The virulence factors included *E. coli* Shiga toxin gene and *S. aureus* exoenzyme genes, host immune alteration or evasion genes, and toxin genes. No virulence factors were found in the genomic sequence of *B. bifidum* BGN4. This result shows that the genomic sequence of *B. bifidum* BGN4 does not include toxic or pathogenic genes related to *E. coli*, *Enterococcus*, *Listeria*, or *S. aureus*.

6.B.5. Susceptibility of *B. bifidum* BGN4 to Antibiotics

To distinguish antibiotic resistance 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. The EFSA based these cut-off values 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. 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.

All *Bifidobacterium* spp. described in the study by Kim et al. (2018) 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). The MIC values of *B. bifidum* BGN4, with the exception of gentamicin, were equal to or lower than the established cut-off values suggested by the EFSA's Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (EFSA, 2012). The MIC values of *B. bifidum* BGN4 for gentamicin was higher than that established by EFSA, but equal to the PROSAFE cutoff established for *B. longum* and those of other GRAS strains such as *B. lactis* BB-12 (GRN 49; FDA, 2002) and *B. breve* M-16V (GRN 453 to 455; FDA, 2013b, 2013c, 2013d).

EFSA cutoffs are not available for the following antibiotics: penicillin, carbenicillin, methicillin, dicloxacillin, kanamycin, neomycin, cephalothin, polymyxin B, metronidazole, rifampicin, phosphomycin, mupirocin, and trimethoprim-sulfamethoxazol. The MICs of penicillin, carbenicillin, methicillin, cephalothin, metronidazole and trimethoprim-sulfamethoxazol for *B. bifidum* BGN4 were 0.063, 0.5, 1, 0.5, 4, 4, and 2, respectively, which were comparable to or lower than the MICs for other GRAS strains (*B. breve* M-16V [FDA, 2013b, 2013c, 2013d; GRNs 453, 454, and 455], *B. lactis* Bb-12 [FDA, 2002; GRN 49], *B. longum* BB536 [FDA, 2009; GRN 268]). The MICs of mupirocin for all strains (*B. bifidum* BGN4, *B. breve* M-16V, *B. lactis* Bb-12, *B. longum* BB536) were >128. The MIC for kanamycin for *B. bifidum* BGN4 was 1024 which was equal to those of another GRAS strains, *B. breve* M-16V *B. lactis* Bb-12, and *B. longum* BB536. The MIC for neomycin for *B. bifidum* BGN4 was 1024 which was equal to that of another GRAS strain *B. breve* M-16V, but higher than that of *B. lactis* Bb-12 and *B. longum* BB536 (both MIC values were 512). The MICs for polymyxin B,

phosphomycin, and rifampicin for *B. bifidum* BGN4 were 512, 128 and 0.5, respectively, and these values were comparable to other GRAS strains which received FDA's no question letters (FDA, 2002, 2009, 2013b, 2013c, 2013d).

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 (Sanders et al., 2010; Zielinska et al., 2018).

Ampicillin, vancomycin, gentamicin, and erythromycin are known as frequently used antibiotics in pediatric patients. For *B. bifidum* BGN4, none of these pediatric antibiotics had MIC values exceeding EFSA breakpoints. The exception was gentamycin. The MIC values of *B. bifidum* BGN4 for gentamycin was higher than that established by EFSA, but equal to the PROSAFE cutoff established for *B. longum* and those of other GRAS strains such as *B. lactis* BB-12 (GRN 49; FDA, 2002) and *B. breve* M-16V (GRN 453 to 455; FDA, 2013b, 2013c, 2013d).

6.B.6. Antibiotic Resistance Transferability Test

Antibiotic resistance transferability studies were conducted to confirm the nature of this resistance. Conjugal transfer of antibiotic resistance was assessed via the methods used in 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. The membrane was placed on the surface of the MRS agar and incubated anaerobically at 37°C for 24 h. 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.

In order to test the transferability of gentamicin resistance of *B. bifidum* BGN4, *L. acidophilus* ATCC 4356 was used as a recipient strain due to its high gentamicin sensitivity. *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. In contrast, *B. bifidum* BGN4 showed resistance to 64 μg/mL gentamicin in this study. Therefore, this proves *B. bifidum* BGN4's resistance to gentamicin was not transferred to the recipient strains.

Table 7. Antimicrobial Susceptibility of *B. bifidum* BGN4 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.7. PCR Assay on Antibiotic Resistance Genes (adopted from Kim et al., 2018)

Even though the whole genome of *B. bifidum* BGN4 has shown that there is no plasmid capable of transferring the antibiotic-resistance gene, PCR analysis was performed on the following antibiotic genes—e.g., 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)).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. No amplicons indicated resistance genes in *B. bifidum* BGN4 or other *Bifidobacterium* spp. (Figure 2).

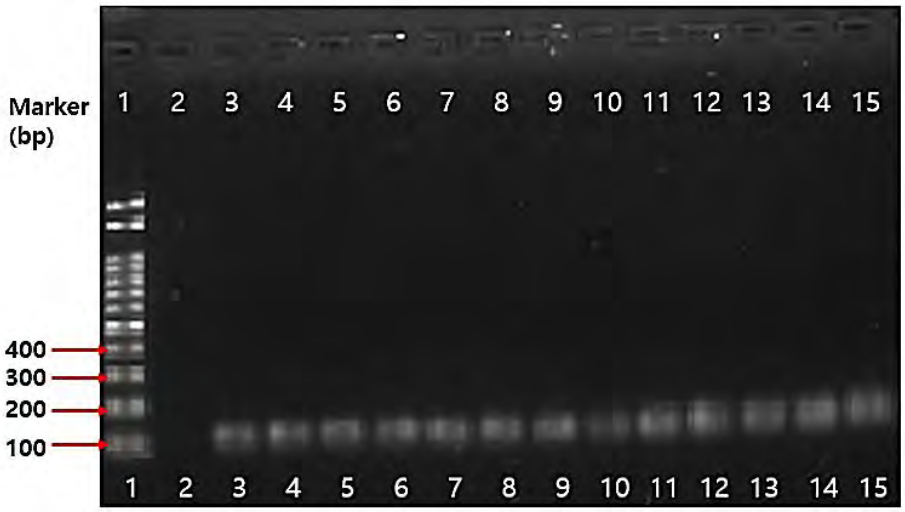

Figure 2. PCR Analysis Results of Various *Bifidobacterium* spp.: Lane 1: marker; Lane 2: without loading; Lane 3: *B. lactis* AS60; Lane 4: *B. bifidum* KCTC 3440; Lane 5: *B. longum* BORI; Lane 6: *B. longum* KCCM 91563; Lane 7: *B. lactis* BB-12; Lane 8: *B. longum* RD47; Lane 9: *B. bifidum* BGN4; 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 pattern of *B. bifidum* BGN4 indicates that overall antibiotic susceptibilities of the strain are similar to patterns of other GRAS strains of bifidobacterial species, and the strain is not likely to have transmissible antibiotic genes. In addition, *B. bifidum* BGN4 does not contain antibiotic resistance genes. These findings indicate that use of *B. bifidum* BGN4 in foods does not present concerns for antibiotic resistance.

6.B.8. 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 (Kim et al., 2018). These naturally-occurring microbiota and artificially-administered flora have a potential to produce various toxic substances during the deamination stage via nitrogen derivatives. Multiple potentially toxic products (i.e., phenol, ammonia, and indole) are possible throughout the proteolytic process, especially in the large intestine. Thus, bacterial ammonia production is highly relevant to human intestinal health and is a necessary component of the safety evaluation of commercial probiotics. In this study, *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 a Brain Heart Infusion (BHI) (BD BBL™, NJ, USA) medium at 37°C for 5 days as described in Kim et al. (2018). The production of ammonia by catalyzed indophenol reaction was determined by the method of Chaney and Marbach as described in Kim et al. (2018).

The ammonia production of *B. bifidum* BGN4 was assessed to verify the safety of these probiotics. In this study, *B. bifidum* BGN4 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. The study found no indication of the production of ammonia by *B. bifidum* BGN4.

6.B.9. 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. bifidum* BGN4 was assessed using the blood agar plating method.

B. bifidum BGN4 was anaerobically cultured in blood agar (BHI broth medium supplemented with 1.5% agar and 5% sheep blood) at 37°C for 2 days as described by Kim et al. (2018). *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 (positive control) showed β-hemolysis colorless zones around the cell colonies, whereas *B. bifidum* BGN4 showed no hemolysis and no change of color in the periphery of the colonies.

6.B.10. Biogenic Amine Production Test

To evaluate if the *B. bifidum* BGN4 would produce bioamines, *B. bifidum* BGN4 was anaerobically cultured 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 15 h. The biogenic amines were extracted and analyzed by HPLC as described by Kim et al. (2018).

The biogenic amine content of the *Bifidobacteria* is shown in Table 8. *B. bifidum* BGN4 and *B. longum* BORI did not produce cadaverine, histamine, or tyramine; however, they produced 24.2 μg/mL and 16.6 μg/mL of putrescine, respectively; the levels were not of concern. Putrescine is a natural substance present in various foods. Small amounts of it are also naturally found in living cells. Putrescine is formed by the decarboxylation of ornithine and arginine.

Putrescine is 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 peas (average 46.3 mg/kg) (Kalač et al., 2002).

Table 8. Biogenic Amine Levels of *B. bifidum* BGN4 and *B. longum* BORI

Strains	Cadaverine (μg/mL)	Histamine (μg/mL)	Putrescine (μg/mL)	Tyramine (μg/mL)
B. bifidum BGN4	N/D[1]	N/D[1]	24.23	N/D[1]
B. longum BORI	N/D[1]	N/D[1]	16.58	N/D[1]

[1]N/D; not detected

6.B.11. 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 capability of *B. bifidum* BGN4 was measured using *in vitro* mucolytic assays (Kim et al., 2018).

B. bifidum BGN4 did not use mucin as a carbon source for their growth. *B. bifidum* BGN4 did not degrade mucin, indicating that the strain is not capable of damaging intestinal surfaces and do not have translocational abilities.

6.B.12. Animal Toxicity Studies

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. bifidum* BGN4 have likely been considered unnecessary and have not been performed.

6.B.13. Animal Efficacy Studies of *B. bifidum* BGN4

Four animal efficacy studies of *B. bifidum* BGN4 were identified from the literature (Table 9). Although these studies were designed to investigate the anti-obesity or anti-allergic effects of *B. bifidum* BGN4, several safety related endpoints were obtained during the experiments; therefore, these studies were reviewed as additional supporting information. This review includes studies of live *B. bifidum* BGN4 strain which have been published by June 30, 2018.

The study by Li et al. (2016) included important safety parameters, such as body weight, 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 probiotics. *B. bifidum* BGN4, *B. longum* BORI, *L. casei* IBS041,

and *L. acidphilus* AD031were individually administered to mice fed a high-fat diet for 8 weeks. No adverse effects of probiotics were reported on measured outcomes.

Kim et al. (2005a) investigated whether orally administered probiotic bacteria (0.2% lyophilized powder of *B. bifidum* BGN4 and *L. casei* in diet, corresponding to 1.8 x 10^7 CFU *B. bifidum*/day or 1.5 x 10^9 cfu/kg bw/day, for 7 weeks) and a gram-negative bacterium (*E. coli*) function as allergic immune modulators to prevent food allergy. C3H/HeJ mice were sensitized with ovalbumin (OVA) and choleratoxin (CT) for 5 weeks. Bacteria-treated mice were fed 0.2% of lyophilized *B. bifidum* BGN4, *L. casei* 911, or *E. coli* MC4100 via a diet pellet. Mice were fed the experimental bacterial powders for 7 weeks, starting 2 weeks before the initial sensitization, until they were finally sacrificed. To determine serum antibody responses, tail vein blood was collected weekly after the initial sensitization. No adverse effects of probiotics were reported on measured outcomes.

In another study, Kim et al. (2005b) investigated the effect of the timing of administration of the probiotic bacteria. C3H/HeJ mice were sensitized with ovalbumin (OVA) and choleratoxin for 5 weeks, and then *B. bifidum* BGN4 (1.8 x 10^7 cfu/day or 1.5 x 10^9 cfu/kg bw/day) was administered continuously for 7 weeks, starting 2 weeks before (pre-treatment group) and 2 weeks after (post-treatment group) the initial sensitization. No adverse effects of *B. bifidum* BGN4 were reported on measured outcomes, such as OVA-specific and total immunoglobins in serum and in fecal samples, IL-6, IL-18 and IFN-γ levels in spleen, and hypersensitivity reactions.

Kim and Ji (2006) investigated the effects of cell viability and integrity of *B. bifidum* BGN4 on suppression of allergy. C3H/HeJ mice were sensitized on weeks 3, 4, 6, and 8 with ovalbumin and choleratoxin to induce an allergic reaction. Mice were fed 0.2% (corresponding to 1.8 x 10^7 cfu/day or 1.5 x 10^9 cfu/kg bw/day) of live, disrupted, or heat-killed *B. bifidum* BGN4 in their diet for 8 weeks starting 2 weeks before initial sensitization. No adverse effects of probiotics were reported on measured outcomes, including OVA-specific IgE, IgG1, IgG2a, and total IgE from sera, OVA-specific and total IgA in fecal samples, hypersensitivity reactions, and body weights.

Overall, daily intake of *B. bifidum* BGN4 at doses up to 1.5 x 10^9 cfu/kg bw/day did not cause any adverse effects in mice.

Table 9. Animal studies of *B. bifidum* BGN4

Objective	Animal	Dose	Duration	Measurements	Reference
To investigate the anti-obesity effects of 4 probiotic strains by using a high-fat diet (HFD) mouse model	54 ICR mice (age 7 wk)	3 groups: 1) High fat diet with $5x10^8$ cfu/mL in drinking water; *B. bifidum* BGN4, *B. longum* BORI, *L. casei* IBS041, or *L. acidophilus* AD031; 2) low fat diet; 3) High fat diet	8 wk	Body weight, body weight gain; liver weight; histopathologic evaluation; clinical chemistry (lipid profile, AST, ALT); hepatic lipid analyses	Li et al., 2016
To investigate whether orally administered probiotic bacteria and a gram-negative bacterium function as allergic immune modulators to prevent food allergy	30 C3H/HeJ mice, female, 3 wk old, sensitized with ovalbumin (OVA) and choleratoxin (CT) for 5 wk	0.2% of lyophilized *B. bifidum* BGN4, *L. caesi* 911, or *E. coli* MC 4100 (or 1.8 x 10^7 cfu/d or 1.5 x 10^9 cfu/kg bw/day)	7 wk starting 2 weeks before the initial sensitization	Serum OVA-specific IgE, IgG1, IgG2a, total IgE, IgG1, IgG2a, spleen IL-5, IL-13, IgG1, and IgG2a levels; measurement of OVA-specific and total fecal IgA; histology (Mast cell degranulation during food allergy response); hypersensitivity reactions; allergic symptoms in the tail; body weight gain	Kim et al., 2005a
To investigate the effect of timing of the administration of probiotics	24 C3H/HeJ mice, female, 3 wk old, sensitized with ovalbumin (OVA) and choleratoxin (CT) for 5 wk	0.2% of lyophilized *B. bifidum* BGN4 (or 1.8 x 10^7 cfu/day or 1.5 x 10^9 cfu/kg bw/day)	7 wk starting 2 wk before (pre-treatment group) and 2 wk after (post-treatment group) the initial sensitization.	Ovalbumin (OVA)-specific and total immunoglobins in serum and in fecal samples; IL-6, IL-18 and IFN-γ levels in spleen; hypersensitivity reactions	Kim et al., 2005b

To investigate the effects of the cell viability and integrity of *Bifidobacterium* on suppression of allergy	30 C3H/HeJ female mice, 5 wk old, sensitized with ovalbumin (OVA) and choleratoxin (CT) on weeks 3, 4, 5, and 8	5 groups: 1-3) 0.2% of lyophilized *B. bifidum* BGN4 in diet pellets as live, disrupted or heat-killed cells (or 1.8 x 10^7 cfu/day or 1.5 x 10^9 cfu/kg bw/day); 4) naïve group; 5) sham control	8 wk	Serum ovalbumin (OVA)-specific IgE, IgG1, IgG2a, and total IgE from sera; OVA-specific and total IgA in fecal samples; hypersensitivity reactions; body weights	Kim and Ji, 2006

6.B.14. Human Clinical Studies

Table 10 summarizes human clinical studies of oral administration of *B. bifidum* BGN4 strain in pregnant women with a family history of allergic diseases and their infants at high risk of allergy as well as irritable bowel syndrome (IBS) or ulcerative colitis patients.

In a randomized, double-blind, placebo-controlled trial, Kim et al. (2010) investigated whether supplementation of probiotics prevented the development of eczema in infants at high-risk. Pregnant women with a family history of allergic diseases received a daily supplement of either a mixture of *B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031 (each 1.6 x 10^9 cfu/day) or placebo, starting at 4-8 weeks before delivery to 3 months after delivery. Infants were exclusively breastfed during the first 3 months and were subsequently fed with breastmilk or cow's milk formula from 4 to 6 months of age. Infants were fed the same probiotic powder dissolved in breast milk, infant formula, or sterile water from 4 to 6 months of age. Mothers and infants in the placebo group took maltodextrin and alpha-corn without probiotic bacteria. Clinical symptoms of the infants (including the prevalence of eczema at 1 year) were monitored until 1 year of age, when the total and specific IgE against common food allergens also were measured. Prenatal and postnatal supplementation with a mixture of *B. bifidum* BGN4, *B. lactis* AD011, and L. *acidophilus* AD031 caused no adverse effects on measured outcomes.

Hong et al. (2009) assessed the immunomodulatory effects of probiotics in adults with IBS in a prospective double-blind randomized placebo-controlled clinical study. IBS patients who met Rome III criteria were randomly assigned to receive probiotics with a total of 40 billion lyophilized probiotics daily (*B. bifidum* BGN4, *B. lactis*, *L. acidophilus*, and *L. casei* - each $1x10^{10}$ cfu/day, divided into 2 doses) or placebo for 8 weeks. Measurements included compliance, symptom scores (abdominal pain, flatulence, defecation discomfort, and sum of scores), quality of life, bowel habits, and adverse events. No adverse effects of probiotics were reported.

In a study by Palumbo et al. (2016), 60 patients with moderate-to-severe ulcerative colitis (UC) were enrolled: 30 of them were treated with a single daily oral administration of 1200 mg mesalazine and another 30 patients received a single daily oral administration of mesalazine 1200 mg and a double daily administration of a probiotic blend of *B. bifidum* BGN4 (1.0 x 10^9 cfu/day), *L. salivarius*, and *L. acidophilus*. The treatment was carried out for 2 years and the clinical response evaluated according to the Modified Mayo Disease Activity Index. All patients treated with combination therapy showed better improvement compared to the controls. No adverse effects of therapy were reported.

Overall, daily supplementation of *B. bifidum* BGN4 at doses up to 10 billion cells per day for 8 weeks (Hong et al., 2009) or 1 billion cells per day for 2 years (Palumbo et al., 2016) was not associated with any adverse effects. Additionally, no human clinical studies found adverse effects of any *B. bifidum* strains. Details of studies using other *B. bifidum* strains are presented in Appendix D.

Table 10. Human Clinical Studies of *B. bifidum* BGN4

Objective	Subject	Dose	Duration	Measurements	Reference
To investigate whether supplementation of probiotics prevents the development of eczema in infants at high risk	112 pregnant women with family history of allergic diseases, and 68 infants	2 groups: 1) a mixture of *B. bifidum* BGN4, *B. lactis* AD011, and *L. acidophilus* AD031 (1.6 x 10^9 cfu/d each); 2) placebo	8 wk before expected delivery to 3 mo after delivery	Clinical symptoms (including the prevalence of eczema at 1 year) of the infants; the total and specific IgE against common food allergens	Kim et al., 2010
	68 infants	2 groups: 1) same powder in breast milk, infant formula, or water; 2) placebo	Breast fed for 3 months; intervention from 4 to 6 mo of age, followed up to 1 y of age		
To assess the effects of probiotic strains in Korean adults with irritable bowel syndrome (IBS)	70 patients w/ presence of previous gastrointestinal symptoms suggestive of IBS, age 19-75 y	2 groups: 1) *B. bifidum* BGN4, *B. lactis*, *L. acidophilus*, and *L. casei* (each 1x10^{10} cfu/d or a total of 4x10^{10} cfu/d, divided into 2 doses); 2) placebo	8 wk	Compliance, Symptom scores (abdominal pain, flatulence, defecation discomfort, and sum of scores); quality of life; bowel habits; adverse events	Hong et al., 2009
To evaluate the long-term effects of combination therapy (mesalazine plus probiotic blend) on ulcerative colitis activity	60 patients over 18 y with moderate-to-severe ulcerative colitis (UC)	1200 mg Mesavancol® vs. 1200 mg Mesavancol® + a blend of probiotics (*B. bifidum* BGN4, *L. salivarious,* and *L. acidophilus*; twice daily; *B. bifidum* BGN4 1.0 x 10^9 cfu/day)	2 y	Clinical response measured by modified Mayo Disease Activity Index (MMDAI) for stool frequency; rectal bleeding; mucosal appearance; physician's rating of disease activity	Palumbo et al., 2016

6.C. 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 (Boriello et al., 2003).

6.D. Safety Determination

Studies have demonstrated that intended uses of *B. bifidum* BGN4 is safe based on the following facts:

1. *B. bifidum* BGN4 has a long history of safe consumption in humans. The bacterial species *B. bifidum* is included in the Old Dietary Ingredient list, i.e., the use of *B. bifidum* is grandfathered under the DSHEA.

2. The information/data provided by BIFIDO (specifications, manufacturing process, and intended use) in this report, and supplemented by the publicly available literature/toxicity data on *B. bifidum* BGN4, provide a sufficient basis for an assessment of the safety of *B. bifidum* BGN4 for the proposed use as a food ingredient prepared according to appropriate specifications and used according to cGMP.

   Key findings are summarized as follows:
   1) Animal and human studies showed no adverse effect of *B. bifidum* BGN4.
   2) No *B. bifidum* strains have shown adverse effects in humans and animals.
   3) *In vitro* studies show that antibiotic susceptibility profiles of *B. bifidum* BGN4 are similar to those of the GRAS strains, which have been safely used in the U.S. and Europe for over a decade. *B. bifidum* BGN4 has no hemolytic or mucolytic activities and does not produce biogenic amines.
   4) The genomic sequence of *B. bifidum* BGN4 does not include toxic or pathogenic genes related to *E. coli*, *Enterococcus*, *Listeria*, or *S. aureus.*

3. The *B. bifidum* BGN4 ingredient has been marketed as a dietary supplement ingredient and as a dietary supplement in Korea since May 2004. *B. bifidum* BGN4 at daily doses up to $1.5x10^{11}$ BGN4 cells has not been safely used and no serious adverse events have been reported by consumers.

4. The intended use of *B. bifidum* BGN4 results in levels of exposure significantly below historical human use levels and provides a reasonable certainty of safety.

5. *B. bifidum* BGN4 is well characterized and is free from chemical and other microbial contamination.

It is, therefore, reasonable to conclude that daily intakes of up to 10^8 cfu *B. bifidum* BGN4/g in powdered formulas and $1x10^9$ cfu *B. bifidum* BGN4/serving in other foods are safe.

6.E. Conclusions and General Recognition of the Safety of *B. bifidum* BGN4

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

B. bifidum BGN4 has been safely used as a food ingredient for a decade. As a result, a comprehensive review of the safety of *B. bifidum* BGN4 has been published (Kim et al., 2018).

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

Numerous human and animal studies have reported benefits of *B. bifidum* BGN4 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. There is broad-based and widely disseminated knowledge concerning the safety of *B. bifidum* BGN4 and other bifidobacteria. The literature indicates that *B. bifidum* BGN4 offers consumers benefits without adverse effects. Thus, the intended uses of *B. bifidum* BGN4 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 has concluded that these uses of *B. bifidum* BGN4 are 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. bifidum* BGN4 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. Identification of *B. bifidum* BGN4

Bifidobacterium bifidum BGN4 strain was isolated from healthy infant's feces who is breast feeding. *B. bifidum* BGN4 were cultured anaerobically on modified de Man, Rogosa and Sharpe (MRS) agar (pH 5.0) at 37°C for 48 hours (h). Single colonies were picked from the plates and culture purified on MRS agar. The isolates were gram-stained and characterized by carbohydrate utilization pattern using a Fruncose-6-phosphate phosphoketolase (F6PPK) test [1]. For long-term storage, stock cultures were maintained at −80°C in MRS broth containing 15% glycerol. Later, *B. bifidum* BGN4 was deposited as KCCM 80046 at Korean Culture Center of Microorganisms (http://www.kccm.or.kr) [2]. The whole genome sequence of *B. bifidum* BGN4 was published in GenBank (Accession no.: CP001361.1) in 2012 [3]. The complete sequence of *B. bifidum* BGN4 consists of a 2,223,664-bp circular chromosome (62.65% G+C) with no plasmid.

Strain level identification

B. bifidum BGN4 was identified by 16S ribosomal (r) DNA sequence analysis. Chromosomal DNA from *B. bifidum* BGN4 strain was extracted and the 16S rRNA gene was amplified using universal primers. The primers 27F 5' (AGAGTTTGATCMTGGCTCAG) 3' and 1492R 5' (TACGGYTACCTTGTTACGACTT) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 ul reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2minutes, 35 cycles of 95 °C for 1minutes, 55°C, and 72 °C for 1minutes each were performed, finishing with a 10 minute step at 72 °C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Sequence homologies were examined by comparing the obtained sequences with those in the DNA Databases (http://www.ncbi.nlm.nih.gov/BLAST). Strain was identified as *Bifidobacterium bifidum* and was named as *Bifidobacterium bifidum* BGN4.

Primer Information

PCR Primer Name Primer Sequences

27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'

1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Sequencing Primer Name Primer Sequences

785F 5' (GGA TTA GAT ACC CTG GTA) 3'

907R 5' (CCG TCA ATT CMT TTR AGT TT) 3

Standard ID

16S rRNA service report

Order Number : 180119KR-064
Sample name : B_bifidum_BGN4_contig_1

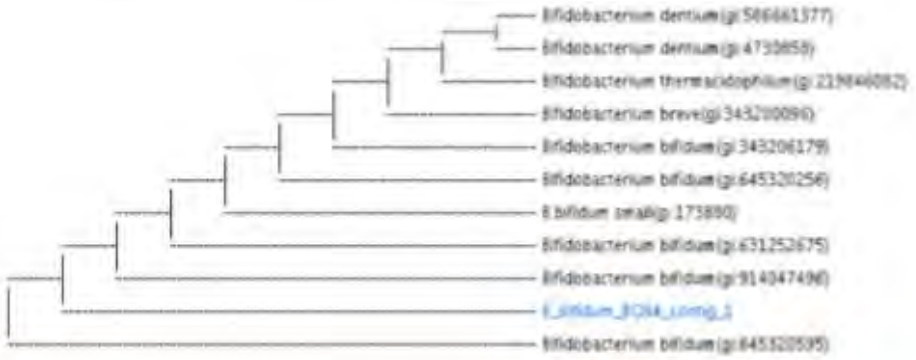
Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
AP012323.1	Bifidobacterium bifidum	2211039	1585030	1583568	0	2588	0.0	1455/1478	98

Kingdom	Family	Genus	Species
Bacteria	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium bifidum



Characterization

Bifidobacterium은 운동성이 없고, 그람 양성이며 종종 가지가 있는 혐기성인 세균이다. 이들은 인간을 포함한 포유동물의 위장관, 질, 구강에서 볼 수 있는 흔한 균이다. Bifidobacteria는 포유류에서 colon flora를 형성하는 주요한 균 중 하나이고, 몇몇 Bifidobacteria는 활생균으로서 사용되곤 한다.

Bifidobacterium bifidum은 Bifidobacterium 속(屬)에 속한 세균이다. B. bifidum은 인간을 포함한 포유 동물의 체내에서 발견 될 수 있는 가장 일반적인 프로 바이오틱 세균 중 하나이다. B. bifidum은 세균총(microflora)의 일부로써 위장관 기능을 돕는다. 이균은 급성 설사 가능성을 감소 시키고, 심지어 대장균 감염에 싸워 도움을 준다.

Characterization-Non-motile, gram-positive, anaerobic bacteria often with branches

3.2.2. Contig Summary

::: BGN4_contig_1 Report :::::::::::::::::::::::::::::::

1. Analysis Report

Name	Read Length (Normal)	Read Length (Q16)	Read Length (Q20)	GC Content
BGN4_contig_1	**1455**	**1373**	**1372**	**59.51890034364261**
BGN4_F	929	927	926	60.60279870828849
BGN4_R	706	688	684	58.78186968838527

2. BlastN Report

- **Query name : BGN4_contig_1**
- **Query length : 1455**

Query		Subject					Score			Identities			
Start	End	Description	AC	Length	Start	End	Bit	Raw	EV	Match	Total	Pct.(%)	Strand
1	1451	Bifidobacterium bifidum BGN4, complete genome	CP001361.1	2223664	1585096	1583646	2675	1448	0.0	1450	1451	99	Plus/Minus
1	1451	Bifidobacterium bifidum S17 strain S17 16S ribosomal RNA, complete sequence	NR_102971.1	1533	32	1482	2669	1445	0.0	1449	1451	99	Plus/Plus
1	1451	Bifidobacterium bifidum S17, complete genome	CP002220.1	2186882	1568403	1566953	2669	1445	0.0	1449	1451	99	Plus/Minus
1	1451	Bifidobacterium bifidum gene for 16S ribosomal RNA, partial sequence, isolate: 4-1-20	AB932541.1	1471	13	1462	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum gene for 16S ribosomal RNA, partial sequence, isolate: 4-1-12	AB932539.1	1474	16	1465	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum gene for 16S ribosomal RNA, partial sequence, isolate: 4-1-11	AB932538.1	1480	21	1470	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum strain NBRC 100015 16S ribosomal RNA gene, partial sequence	NR_113873.1	1453	3	1452	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum strain LCR9 16S ribosomal RNA gene, partial sequence	HQ259744.1	1469	13	1462	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum strain LCR7 16S ribosomal RNA gene, partial sequence	HQ259742.1	1459	3	1452	2651	1435	0.0	1447	1452	99	Plus/Plus
1	1451	Bifidobacterium bifidum strain KCTC 3202 16S ribosomal RNA gene, partial sequence	GU361813.1	1480	3	1452	2651	1435	0.0	1447	1452	99	Plus/Plus

(b) (6)

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Appendix B. Certificate of Analysis for *B. bifidum* BGN4

B I F I D O

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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 bifidum* BGN4	
LOT NO.	B4-R-160303	
PRODUCTION DATE	2016. 03. 03	
CERTIFICATED DATE	2016. 03. 07	
EXPIRATION DATE	2018. 03. 02	
ANALYSIS RESULT		
Parameter	**B4-R-160303**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. bifidum* BGN4), cfu/g	1.20E+11	KHFSC 4/3/3-58
Moisture, %	3.49	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0122	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.013	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0058	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, %	NA	KFSC 7/2/2.1/2.1.5/2.1.5.1
Protein, %	NA	KFSC 7/2/2.1/2.1.3/2.1.3.1
Carbohydrates, %	NA	KFSC 7/2/2.1/2.1.4/2.1.4.1
Ash, %	NA	KFSC 7/2/2.1/2.1.2

QC Manager Ji-Young Shin

(b) (6)

B I F I D O

23-16, Nonggongdanji-gil, Hongcheon-eup, Hongcheon-gun,

Gangwon-do, 25117, Republic of Korea

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CERTIFICATE OF ANALYSIS

NAME OF PRODUCT	*Bifidobacterium bifidum* BGN4
LOT NO.	B4-R-161223
PRODUCTION DATE	2016. 12. 23
CERTIFICATED DATE	2016. 12. 27
EXPIRATION DATE	2018. 12. 22

ANALYSIS RESULT

Parameter	**B4-R-161223**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. bifidum* BGN4), cfu/g	1.10E+11	KHFSC 4/3/3-58
Moisture, %	3.52	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0162	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.0159	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0060	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**

(b) (6)

B I F I D O

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CERTIFICATE OF ANALYSIS

NAME OF PRODUCT	*Bifidobacterium bifidum* BGN4
LOT NO.	B4-R-170210
PRODUCTION DATE	2017. 02. 10
CERTIFICATED DATE	2017. 02. 14
EXPIRATION DATE	2019. 02. 09

ANALYSIS RESULT

Parameter	**B4-R-170210**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. bifidum*	1.10	KHFSC 4/3/3-58
Moisture, %	3.68	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0162	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.0393	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0079	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[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

Analyzed in Korea Health Supplements Institute

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(b) (6)

Appendix C. NHANES Food Codes Used in Exposure Estimates for the General Population Aged 1-99 Years

Foodcode	Description
11115000	Buttermilk, fat free (skim)
11115100	Buttermilk, low fat (1%)
11115200	Buttermilk, reduced fat (2%)
11115300	Buttermilk, whole
11115400	Kefir, NS as to fat content
11120000	Milk, dry, reconstituted, NS as to fat content
11121100	Milk, dry, reconstituted, whole
11121210	Milk, dry, reconstituted, low fat (1%)
11121300	Milk, dry, reconstituted, fat free (skim)
11810000	Milk, dry, not reconstituted, NS as to fat content
11811000	Milk, dry, not reconstituted, whole
11812000	Milk, dry, not reconstituted, low fat (1%)
11813000	Milk, dry, not reconstituted, fat free (skim)
11830100	Hot chocolate / Cocoa, dry mix, not reconstituted
11830110	Cocoa powder with nonfat dry milk and low calorie sweetener, dry mix, not reconstituted
11830115	Hot chocolate / Cocoa, dry mix, no sugar added, not reconstituted
11830120	Cocoa, whey, and low calorie sweetener, fortified, dry mix, not reconstituted
11830140	Chocolate, instant, dry mix, fortified with vitamins and minerals, not reconstituted, Puerto Rican style
11830150	Cocoa powder, not reconstituted (no dry milk)
11830160	Chocolate beverage powder, dry mix, not reconstituted
11830165	Chocolate beverage powder, reduced sugar, dry mix, not reconstituted
11830170	Cocoa (or chocolate) flavored beverage powder with low-calorie sweetener, dry mix, not reconstituted
11830210	Milk, malted, dry mix, fortified, not reconstituted, flavors other than chocolate
11830260	Milk, malted, dry mix, not reconstituted
11830400	Strawberry beverage powder, dry mix, not reconstituted
11830550	Milk beverage, powder, with nonfat dry milk and low calorie sweetener, dry mix, not reconstituted, flavors other than chocolate
11320000	Soy milk
11320100	Soy milk, light
11320200	Soy milk, nonfat
11321000	Soy milk, chocolate
11321100	Soy milk, light, chocolate
11321200	Soy milk, nonfat, chocolate
11340000	Imitation milk, non-soy, sweetened
11350000	Almond milk, sweetened
11350010	Almond milk, sweetened, chocolate
11350020	Almond milk, unsweetened
11350030	Almond milk, unsweetened, chocolate

11360000	Rice milk
11370000	Coconut milk
11512030	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk
11512120	Hot chocolate / Cocoa, ready to drink, made with non-dairy milk and whipped cream
11513310	Chocolate milk, made from dry mix with non-dairy milk
11513750	Chocolate milk, made from syrup with non-dairy milk
11513805	Chocolate milk, made from light syrup with non-dairy milk
11514150	Hot chocolate / Cocoa, made with dry mix and non-dairy milk
11514360	Hot chocolate / Cocoa, made with no sugar added dry mix and non-dairy milk
11519215	Strawberry milk, non-dairy
42401010	Coconut milk, used in cooking (liquid expressed from grated coconut meat, water added)
42402010	Coconut cream (liquid expressed from grated coconut meat), canned, sweetened
11410000	Yogurt, NS as to type of milk or flavor
11411010	Yogurt, plain, NS as to type of milk
11411100	Yogurt, plain, whole milk
11411200	Yogurt, plain, low fat milk
11411300	Yogurt, plain, nonfat milk
11411400	Yogurt, Greek, plain, whole milk
11411410	Yogurt, Greek, plain, low fat
11411420	Yogurt, Greek, plain, nonfat milk
11420000	Yogurt, vanilla, NS as to type of milk
11421000	Yogurt, vanilla, whole milk
11422000	Yogurt, vanilla, low fat milk
11422100	Yogurt, vanilla, low fat milk, light
11423000	Yogurt, vanilla, nonfat milk
11424000	Yogurt, vanilla, nonfat milk, light
11424500	Yogurt, Greek, vanilla, whole milk
11424510	Yogurt, Greek, vanilla, low fat
11424520	Yogurt, Greek, vanilla, nonfat
11426000	Yogurt, chocolate, whole milk
11427000	Yogurt, chocolate, nonfat milk
11428000	Yogurt, Greek, chocolate, nonfat
11430000	Yogurt, fruit, NS as to type of milk
11431000	Yogurt, fruit, whole milk
11432000	Yogurt, fruit, low fat milk
11432500	Yogurt, fruit, low fat milk, light
11433000	Yogurt, fruit, nonfat milk
11433500	Yogurt, fruit, nonfat milk, light
11434000	Yogurt, Greek, fruit, whole milk
11434010	Yogurt, Greek, fruit, low fat
11434020	Yogurt, Greek, fruit, nonfat
11446000	Fruit and low fat yogurt parfait
11480010	Yogurt, whole milk, baby food

11480020	Yogurt, whole milk, baby food, with fruit and multigrain cereal puree, NFS
67250150	Mixed fruit juice with lowfat yogurt, baby food
67404500	Mixed fruit yogurt dessert, baby food, strained
67408500	Banana yogurt dessert, baby food, strained
67413700	Peach yogurt dessert, baby food, strained
67430500	Yogurt and fruit snack, baby food
57801000	Barley cereal, baby food, dry, instant
57803000	Mixed cereal, baby food, dry, instant
57804000	Oatmeal cereal, baby food, dry, instant
57805000	Rice cereal, baby food, dry, instant
57805090	Rice cereal with mixed fruits, baby food, dry, instant
57805500	Brown rice cereal, baby food, dry, instant
57806000	Mixed cereal with bananas, baby food, dry, instant
57806050	Multigrain, whole grain cereal, baby food, dry, instant
57806200	Oatmeal cereal with fruit, baby food, dry, instant, toddler
57807010	Whole wheat cereal with apples, baby food, dry, instant
95201000	Carnation Instant Breakfast, nutritional drink mix, regular, powder
95201010	Carnation Instant Breakfast, nutritional drink mix, sugar free, powder
95201200	EAS Whey Protein Powder
95201500	Herbalife, nutritional shake mix, high protein, powder
95201600	Isopure protein powder
95201700	Kellogg's Special K20 Protein Water Mix
95202000	Muscle Milk, regular, powder
95202010	Muscle Milk, light, powder
95210000	Slim Fast Shake Mix, powder
95210020	Slim Fast Shake Mix, high protein, powder
95220000	Nutritional drink mix or meal replacement, powder, NFS
95220010	Nutritional drink mix or meal replacement, high protein, powder, NFS
95230000	Protein powder, whey based, NFS
95230010	Protein powder, soy based, NFS
95230020	Protein powder, light, NFS
95230030	Protein powder, NFS
91106000	Sugar substitute, sugar-aspartame blend, dry powder
91107000	Sucralose-based sweetener, sugar substitute
91108000	Sugar substitute, herbal extract sweetener, powder
91200000	Sugar substitute, low-calorie, powdered, NFS
91200020	Sugar substitute, saccharin-based, dry powder
91200040	Sugar substitute, saccharin-based, dry powder and tablets
91201010	Sugar substitute, aspartame-based, dry powder

Appendix D. Human Clinical Studies of Other *B. bifidum* Strains

Summary tables below show that no adverse effects were found in any *B. bifidum* strains in humans, regardless of subjects, daily doses, and the duration of the study (Bartosch et al., 2005; Chitapanarux et al., 2010; Culpepper et al., 2016; Dennis-Wall et al., 2017; Gomi et al., 2015, 2018; Guardamagna et al., 2014; Guglielmetti et al., 2011; Madden et al., 2005; Manzano et al., 2017; Miki et al., 2007; Moroti et al., 2012; Rerksuppaphol and Rerksuppaphol, 2012; Saavedra et al., 1994; Saengtawesin et al., 2014; Schiffrin et al., 1997; Totsu et al., 2014; Wang et al., 2018; Yamasaki et al., 2012). Due to an abundance of the studies reporting no adverse effects of *B. bifidum*, our summary is limited to the studies with the test duration of over 2 weeks with a maximum number of 3 strains including *B. bifidum*. Our literature search covers the papers published until June 30, 2018.

Overall, daily doses up to $2.5x10^9$ cfu/day did not cause any adverse effects in very-low-birthweight (VLBW) infants, term infants and children (Manzano et al., 2017; Rerksuppaphol and Rerksuppaphol, 2012; Saavedra et al., 1994; Saengtawesin et al., 2014; Totsu et al., 2014 Yamasaki et al., 2012. In addition, doses up to $2.5 - 3.5 \times 10^{10}$ cfu/day showed no adverse effects in adults (Bartosch et al., 2005; Madden et al., 2005).

Table AD 1. Human Studies of Other *B. bifidum* – A Single Strain Only

Objective	Treatment Population	Test Substance	Treatment Duration	Measurements	Reference
To evaluate the efficacy and safety of early administration of *B. bifidum* OLB6378 on accelerating enteral feeding and bacterial colonization in very-low-birthweight (VLBW) infants	36 VLBW infants, <1,500 g bw	~$2.5x10^9$ viable cells of *B. bifidum* OLB6378 (divided into 2 doses) within 48 h of birth or at more than 48 h of birth	Until bw reaches 2,000 g	Establishment of enteral feeding (the postnatal day at which the amount of enteral feeding exceeded 100 mL/kg/day); mortality and morbidity; efficacy and safety of *B. bifidum* (episodes of sepsis with positive blood culture, the length of hospital stay, and the level of *B. bifidum* in the fecal samples); gut microbiota	Yamasaki et al., 2012
To evaluate the benefit of *B. bifidum* OLB6378 in VLBW infants for the acceleration of enteral feeding	283 VLBW infants, mean gestational age of 28.5-28.6 wk and 998-1,016 g bw	~$2.5x10^9$ viable cells/d *B. bifidum* OLB6378 within 48 h of birth	Until bw reached 2,000 g	Establishment of enteral feeding; incidence of morbidity and somatic growth before discharge	Totsu et al., 2014
To assess the efficacy of *B. bifidum* MIMBb75 in IBS	122 patients w/mild to moderate IBS, 18-68 y	$1x10^9$ cfu *B. bifidum* MIMBb75	4 wk	IBS symptoms; quality of life; adverse events	Guglielmetti et al., 2011
To evaluate the influence of *B. bifidum* TMC3115 on lipid metabolism in the middle aged and elderly	47 subjects with mild hyperglycemia and dyslipidemia, mean of 64.0 y	$3x10^{10}$ cfu/mL *B. bifidum* TMC3115	3 wk	Serum lipids and glucose; fecal microflora; frequency and wt. of stools	Wang et al., 2018

Table AD2. Studies of Other *B. bifidum* Strains in Pregnant Women, Infants, and Children Using 2 or 3 Probiotic Strains Including *B. bifidum*

Objective	Subject	Dose of *B. bifidum*	Duration	Measurement	Reference
To evaluate the safety and tolerance of 3 probiotic strains (*B. bifidum* R0071, *B. infantis* R0033, and *L. helveticus* R0052)	221 healthy full-tern infants, aged 3-12 mo)	A total of $3x10^9$ cfu/d *B. bifidum* R0071, *B. infantis* R0033, and *L. helveticus* R0052	8 wk	Growth (weight, height and head circumference); adverse events; concentrations of D-lactic acid in urine samples; characteristics of the stools and use of medication	Manzano et al., 2017
To evaluate the efficacy of a formula containing *B. bifidum* and *S. thermophilus* for the prevention of acute diarrhea in infants admitted to hospital	65 infants aged 5-24 mo who were admitted to a chronic medical care hospital	Formula supplemented with $1.9x10^8$ cfu/g *B. bifidum* (or 35.8 cfu/100 kcal) + $0.14x10^8$ cfu/g *S. thermophilus*; strains not specified)	17 mo	Incidence and duration of diarrhea; body wt.; daily energy intake; daily volume of formula intake; number of stools per day; mean daily episodes of regurgitation/vomiting; fecal microflora; rotavirus shedding	Saavedra et al., 1994
To evaluate the efficacy of probiotics supplementation in the prevention of necrotizing enterocolitis (NEC) among VLBW preterm infants	60 preterm infants; gestational age ≤34 wk and birth weight ≤1,500 g	Infloran® (included $2x10^9$ cfu/d *B. bifidum* +$2x10^9$ cfu/d *L. acidophilus,* strains not specified	6 wk	Adverse effects such as sepsis, flatulence or diarrhea; incidence of NEC stage >2	Saengtawesin et al., 2014

To assess the efficacy of a two-strain combination probiotic for prevention of common cold symptoms in healthy schoolchildren	76 healthy children, age 8-13 yrs	Probiotic (included minimum of $2x10^9$ cfu/d *B. bifidum* plus $2x10^9$ cfu/d *L. acidophilus* *(*strains not specified)	3 mo	Occurrence and symptom of cold; school absence; antibiotic use	Rerksuppaphol and Rerksuppaphol, 2012

Table AD 3. Studies of Other *B. bifidum* Strains in Adults Using 2-3 Probiotic Strains Including *B. bifidum*

Objective	Subject	Dose of *B. bifidum*	Duration	Measurement	Reference
To study the effects of ingestion of synbiotic and oligofructose on the composition of intestinal bifidobacteria and *Lactobacillus* population in older people	18 healthy female elderly volunteers; age > 62 y	Synbiotic comprising of ~$3.5x10^{10}$ cfu/d each -*B. bifidum* BB-02 and *B. lactis* BL-01 (Rhodia) with a mixture of inulin and oligofructose, 6 g/d	4 wk intervention, followed by 3 week post observation	Fecal microbiota (counts of total viable anaerobes and bifidobacteria and lactobacilli in feces)	Bartosch et al., 2005
To evaluate the efficacy of probiotics on fecal colonization and other immune modulating factors	28 healthy adults, 23-62 y	Fermented milk providing $1x10^{10}$ cfu *B. bifidum* Bb12 or $7x10^{10}$ cfu *L. acidophilus* La1	3 wk standard milk and 3 wk - fermented milk, followed by 6 wk of standard (no fermented) milk	Fecal colonization (fecal counts of bifidobacterial and lactobacilli); lymphocyte subsets and leukocyte phagocytic activity; bacterial adhesion to enterocytes	Schiffrin et al., 1997
To determine the ability of *L. acidophilus* plus *B. bifidum* to reduce the incidence of radiation-induced diarrhea in locally advanced cervical cancer patients	63 patients undergoing pelvic radiotherapy concurrent with weekly cisplatin, aged 18-65 y	A total of 4×10^9 cfu/d (or 2×10^9 cfu/d each) probiotic including *B. bifidum* and *L. acidophilus*, strains not specified	7 d before radio-therapy and continuing every day during radio-therapy	Severity of diarrhea and radiation-induced diarrhea; stool consistency; white and red blood cells in stool	Chitapanarux et al., 2010

To examine whether three different probiotics could normalize self-reported stress-associated GI discomfort and reduce overall self-reported stress	581 undergraduate students, age 19.9 ± 0.1 yrs	$3x10^9$ cfu/d of *B. bifidum* R0071, *Lactobacillus helveticus* R0052, and *B. longum* ssp. *infantis* R0033	6 wk	Daily stress level (salivary cortisol analysis); stress related gastrointestinal discomfort; three diarrhea-related symptoms using gastrointestinal Symptom Rating Scale	Culpepper et al., 2016
To determine whether consuming a probiotic would improve quality of life during allergy season	173 healthy volunteers who self-identified as having seasonal allergies	A total of $3x10^9$ cfu/d of *B. bifidum* G9-1 ($0.3x10^9$ cfu/d), *Lactobacillus gasseri* KS-13 ($2.4x10^9$ cfu/d), and *B. longum* MM- ($0.3x10^9$ cfu/d); divided into 2 doses	8 wk	Quality of life as measured by Mini Rhinoconjunctivitis Quality of Life Questionnaire (MRQLQ); fasting serum conc. of total IgE, IL-10, and regulatory T cells; Gastrointestinal Symptom Rating Scale; bacterial DNA in stools	Dennis-Wall et al., 2017
To examine how fermented milk containing the probiotic affects gastric and lower abdominal symptoms in adults taking no medication	Trial 1- 305 adults, 50.6 ± 7.4 y Trial 2 – 27 adults, 35.3 ± 11.3 y	100 mL/d fermented milk containing $1x10^7$ cfu/mL *B. bifidum* YIT10347 + 1×10^7 cfu/mL of *S. thermophilus* YIT 2021 vs. placebo milk containing 1×10^7 cfu/mL of *S. thermophilus* YIT 2021 only	2 wk	Frequency and severity of gastric and lower abdominal symptoms	Gomi et al., 2015

To investigate the effects of *B. bifidum* YIT 10347 on gastrointestinal symptoms	100 healthy Japanese adults, 41.1-41.6 y	100 mL/d fermented milk containing >3 × 10^7 cfu/mL of YIT10347 plus >1×10^7 cfu/mL of *S. thermophilus* YIT 2021 vs. placebo milk containing >1×10^7 cfu/mL of *S. thermophilus* YIT 2021 only.	4 wk	Gastric symptoms (using Japanese version of the Gastrointestinal Symptom Rating Scale [GSRS] questionnaire); changes in the scores of psychological symptoms and quality of life; salivary stress markers (cortisol); gastric emptying; adverse events	Gomi et al., 2018
To study the effects of probiotic supplement on the intestinal microflora in response to antibiotic therapy	22 patients with *H. pylori* infection	triple-therapy with placebo or probiotics -2.5x10^{10} cfu/d *B. bifidum* (CUL17 and Rhodia -2 strains) plus *L. acidophilus* (CLT60 and CUL21-2 strains)	Probiotics-7 or 15 d	Fecal microbiota	Madden et al., 2005
To evaluate the efficacy of fermented milk containing *B. bifidum* on *H. pylori* and gastric health	79 healthy Japanese adults	100 mL fermented milk containing 1-5x10^8 cfu/mL *B. bifidum* YIT 4007 plus 1x10^7 cfu/mL *S. thermophilus* YIT 2021	12 wk	*H. pylori* urease activity and gastric situation using a urea breath test (UBT); the difference of the UBT value from the baseline value; upper gastrointestinal symptoms; serum pepsinogen levels as biomarkers for inflammation or atrophy	Miki et al., 2007
To evaluate the effect of the consumption of a synbiotic shake on glycemia and	20 mildly hyper-cholesterol-emic and hyper-	Synbiotic shake containing 2x10^{10} cfu/d each of *B. bifidum* (strain not	30 d	Fasting serum lipid profile; fasting glycemia	Moroti et al., 2012

cholesterol levels in elderly people	glycemic adults, age 50-60 y	specified) and *L. acidophilus* + 2 g oligofructose			
To evaluate the effects of three *Bifidobacterium* strains on lipid profiles in children affected by primary dyslipidemia	38 children with dyslipidemia, mean age 10.8 y	$1x10^9$ cfu/d each – *B. bifidum* MB 109B, *B. animalis* subsp. *lactis* MB 2409, and *B. longum* subsp. *longum* BL04	12 wk; crossover	Blood lipid profile	Guardama-gna et al., 2014

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March 16, 2019

Dr. Denis Wafula
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

Dear Dr. Wafula,

In response to FDA Comments and Questions for GRN 814, we have prepared our answers as follows.

FDA's Comments and Questions for GRN 814

1. The Picard et al., 2005 reference is missing from the bibliography although it is cited several times in the notice. Please provide the reference.
   Response:
   We apologize for the missing reference. The reference is listed below:
   Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. Aliment Pharmacol Ther. 2005;22(6):495-512.

2. The last paragraph on page 14 reads; "These estimates are highly amplified since it is not likely that *B. bifidum* BGN4 will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered." The last sentence does not make sense in terms of the intended uses in food, as we do not typically consume food wastes. It also isn't clear what "highly amplified" means in this context, though we presume that means that they are very conservative. The paragraph seems incomplete please revise it for clarity.
   Response:

a) About 'Also, food wastes should be considered." - We agree with your viewpoint. Since NHANES is based on dietary survey, a food waste is not an issue. We will amend the notice by deleting this sentence.

b) About 'highly amplified' - We have amended the statement as follows (bold font indicates the changes): '**It is possible that these estimates are overestimated** since it is not likely that *B. bifidum* BGN4 will be used at maximum levels for all food categories under the intended uses.'

We believe that current EDI calculation method leads to overestimation. The current exposure estimates are based on the assumption that all foods in a specified food category will have the maximum intended use levels. We believe it is far from the realistic situations. For example, the intended use includes term infant formulas and conventional food products (dairy products/dairy-based foods and dairy substitutes, including fermented milk, flavored milk beverages and mixes, dried milk powder, imitation milk and yogurt; baby cereals and foods (powder form); meal replacement and nutritional drink mix powder; and sugar substitute. We are not aware that all foods belonging to the food categories listed above contain *B. bifidum,* bifidobacteria, or probiotics in general.

3. In section 6A (page 19), when discussing the basis for GRAS determination for your ingredient you state that; "According to the 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" in under DSHEA. The bacterial species *B. bifidum* is included in the Old Dietary Ingredient list, i.e., the use of the bacterial species *B. bifidum* is grandfathered under the DSHEA (CRN, 1998)." Please note that the safety standard for dietary ingredients differs from that for food ingredients, so we did not evaluate this statement because it relates to dietary supplements and not food ingredients.
   Response:
   Thank you for pointing it out. We will not include such statements for future submission.

4. In section 6.B.1 on page 21, you state that because *B. bifidum* BGN4 retains its 'form' it is unlikely for it to enter organs or systemic circulation from the intestinal tract of normal, healthy individuals. Please provide a reference that supports this assertion.
   Response:
   We have translated the Probiotic concept which was summarized in Picard et al. (2005) as follows: "Probiotics are defined as '**live micro-organisms** which confer a health benefit on the host when administered in adequate amounts'. --- As already mentioned, prebiotics are defined as **a non-digestible food ingredient** that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon."

If probiotics would enter organs and systemic circulations from the intestinal tract of normal, healthy individuals, they may cause opportunistic infection. But there is no evidence that probiotics is a significant factor for opportunistic pathogenicity in health individuals. For instance, cases of infection due to lactobacilli and bifidobacteria are extremely rare and are estimated to represent 0.05%–0.4% of cases of infective endocarditis and bacteremia
Increasing consumption of probiotic lactobacilli and bifidobacteria has not led to an increase in such opportunistic infections in consumers (Borriello et al., 2003).

Thus, we would like to amend the statement as follows: '**It is unlikely** that *B. bifidum* BGN4 will enter organs or the systemic circulation from the gastrointestinal tract in normal, healthy individuals (**Borriello et al., 2003; Picard et al., 2015**).'

References:

Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, et al. Safety of probiotics that contain lactobacilli or bifidobacteria. Clin Infect Dis 2003; 36:775-80.

Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. Aliment Pharmacol Ther. 2005 Sep 15;22(6):495-512.

5. You have presented the wrong figure for the PCR Assay for antibiotic resistance genes (page 26). You state that PCR analysis was performed to test the presence of 10 genes encoding for antibiotic resistance in *B. bifidum* BGN4 and other *Bifidobacterium* spp. and the PCR results indicated the absence of antibiotic resistance genes in the bacteria. You further state that the results are presented in Figure 2. However, the legend in Figure 2 does not indicate which genes were tested and the presence of amplicons in the image contradicts the finding that the tested antibiotic genes were not present. However, our review of the Kim et al., 2018 publication finds that the image shown in Figure 2 is for 16S rRNA PCR analysis and not for antibiotic resistance genes analysis. Please provide the correct gel image, which can be found in the publication.

   Response:

   We agree that Figure 2 is not a correct figure. The correct figure for antibiotic resistance genes in *B. bifidum* BGN4 is Figure 3 A of the Kim et al. (2018). Thus, we want to amend the notice by deleting Figure 2 and correcting a statement as follows (bold font indicates changes):

   'Even though the whole genome of *B. bifidum* BGN4 ---and tetracycline (*tet*(K)), *tet*(L), *tet*(M), *tet*(O), and *tet*(S)). The experimental conditions are described in Kim et al. (2018) **and the result for *B. bifidum* BGN4 is presented in the Figure 3 (a) of the cited**

paper. No amplicons indicated resistance genes in *B. bifidum* BGN4 or other *Bifidobacterium* spp**.'**

6. We do not entirely concur with your conclusions regarding the results of the Antibiotic Resistance Transferability Test. The publication that you cite (Kim et al., 2018) provides significant data on antibiotic resistance. However, the conclusion on page 23 ('Therefore this proves that *B. bifidum* BNF4's resistance to gentamicin was not transferred to the recipient strains') is overstated, because there was no positive control to show that DNA had been transferred. Please revise the conclusion to include this observation so as to accurately describe the results.
   Response:
   We would like to amend the statement (the last sentence of page 23) as follows: 'It appears that *B. bifidum* BGN4's resistance to gentamicin was not transferred to the recipient strain under the test conditions.

7. On page 26 (Summary of Antibiotic Susceptibility), you state that; "…GRAS strains of bifidobacterial species, and the strain is not likely to have transmissible antibiotic genes…" We presume by 'transmissible antibiotic genes' you mean transmissible antibiotic **resistance** genes.
   Response:
   Thank you for catching an error. We would like to amend it as follows: '…GRAS strains of bifidobacterial species, and the strain is not likely to have transmissible antibiotic **resistance** genes…'

We hope we have properly answered your questions and comments. In addition, we are sending you a pdf files of amended pages (blue font indicates changes). We would appreciate your kind attention to this matter. Please let me know if you have further questions.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
NutraSource
An agent for Bifido
Susanscho1@yahoo.com
(301) 87506454

PART 3. DIETARY EXPOSURE

3.A. Estimated Dietary Intakes (EDIs) of *B. bifidum* BGN4 Under the Intended Use

3.A.1. Non-Exempt Term Infant Formula Applications

The use levels are the same as those described in GRN 454. Since the intended use level in this GRAS determination is the same as GRN 454, these EDI levels are consistent with those reported in GRN 454. Powdered non-exempt term infant formulas (milk-, soy-, or whey-based) will contain up to 10^8 colony forming units (cfu) *B. bifidum* BGN4/g powdered formulas. The intended target intake level will be 10^9 - 10^{10} cfu *B. bifidum* BGN4/day.

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, the caloric requirements of one-month-old and six-month-old infants are 472 kcal/day and 645 kcal/day, respectively (Institute of Medicine (IOM) Panel on Macronutrients and IOM Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). The addition of 10^8 cfu *B. bifidum* BGN4/g infant formula will result in intakes of 9.9×10^9 and 1.35×10^{10} cfu *B. bifidum* BGN4/day, respectively. These formulas will be supplemented appropriately to provide a minimum of 10^9 cfu *B. bifidum* BGN4/day at the end of a 24-month shelf-life at room temperature.

3.A.2. Conventional Food Applications

BIFIDO intends to add *B. bifidum* BGN4 to selected conventional food products for the general population (Table 1). Selected conventional foods will contain up to 1.0×10^9 cfu/serving.

The intended use of 1.0×10^9 cfu *B. bifidum* BGN4/serving in the target food categories would result in the estimated mean and 90th percentile intakes of 1.36 and 3.00 servings of foods per person per day, providing 1.36×10^9 and 3.00×10^9 *B. bifidum* BGN4 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 3.5×10^9 cfu/day. In total population, mean and 90th percentile intakes are estimated to be 0.41 and 1.17 servings per day, providing 0.41×10^9 and 1.17×10^9 cfu/person/day, respectively (Table 6-2).

It is possible that these estimates are overestimated since it is not likely that *B. bifidum* BGN4 will be used at maximum levels for all food categories under the intended uses.

6.B.1. Metabolism

It is unlikely that *B. bifidum* BGN4 will enter organs or the systemic circulation from the gastrointestinal tract in normal, healthy individuals (Borriello et al., 2003; Picard et al., 2015). Rather, the fate of *B. bifidum* BGN4 after ingestion is expected to be similar to that seen after consumption of live food-grade bacteria. *B. bifidum* BGN4 is expected to transit through the gastrointestinal tract and be excreted in feces. It has also been shown that live *B. bifidum* BGN4, like other bifidobateria, does not harbor the potential for translocation (AHRQ, 2011; Kim et al., 2018; Picard et al., 2015).

6.B.2. Anti-mutagenicity of *B. bifidum*

Lo et al. (2004) investigated the antimutagenic effects of 6 bifidobacteria (*B. adolescentis, B. bifidum, B. breve, B. infantis, B. lactis,* and *B. longum*) against benzo[α]pyren(B[α]P) by a modified Ames test using *Salmonella typhymurium* TA 100 after acidic and bile treatment mimicking gastrointestinal conditions. When bifidobacteria were treated at pH 2.0 for 3 h or 1% bile for 6 h, their antimutagenic activities against B[α]P were increased as compared to the controls at pH 7.0 for 0 h. *B. bifidum* showed 66.0% of antimutagenic activity at pH 7.0, 62.5% at pH 2.0 and 73.6% % with 1% bile.

6.B.3. Genetic Stability Test

The genetic variation of edible microorganisms possibly results in indel (i.e. gene deletion and insertion) and mutation. A critical consideration of commercializing probiotics is whether it is possible to maintain genetic safety over the long term. Theoretically, an evaluation of genetic stability requires the entire genome sequence of the strain.

The entire genome sequence of *B. bifidum* BGN4 has been published (Yu et al., 2012). It consists of a 2,223,664-bp circular chromosome (62.65% G+C) with no plasmid. The nucleotide sequence identified 1,835 coding sequences (CDSs), 7 pseudogenes, 3 rRNA operons, and 52 tRNAs. This study showed that the similarity in the genomic comparison of the 1st and 25th generations of samples was 99.9996~99.9998% via the Orthologous Average Nucleotide Identity (OrthoANI) analysis (Kim et al., 2018). The difference between 0.0002% and 0.0004% is equivalent to 4.4 bp to 8.8 bp mutations in the entire nucleotide sequence. This difference is assumed to result from sequencing errors or spontaneous evolutionary mutations. These data indicate low 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.4. The Absence of Virulence Genes

The search for virulence factors in *B. bifidum* BGN4 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) (www.genomicepidemiology.org).

The database 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 genomes of *B. bifidum* BGN4. The selected %ID threshold was set at 90% and the

phosphomycin, and rifampicin for *B. bifidum* BGN4 were 512, 128 and 0.5, respectively, and these values were comparable to other GRAS strains which received FDA's no question letters (FDA, 2002, 2009, 2013b, 2013c, 2013d).

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 (Sanders et al., 2010; Zielinska et al., 2018).

Ampicillin, vancomycin, gentamicin, and erythromycin are known as frequently used antibiotics in pediatric patients. For *B. bifidum* BGN4, none of these pediatric antibiotics had MIC values exceeding EFSA breakpoints. The exception was gentamycin. The MIC values of *B. bifidum* BGN4 for gentamycin was higher than that established by EFSA, but equal to the PROSAFE cutoff established for *B. longum* and those of other GRAS strains such as *B. lactis* BB-12 (GRN 49; FDA, 2002) and *B. breve* M-16V (GRN 453 to 455; FDA, 2013b, 2013c, 2013d).

6.B.6. Antibiotic Resistance Transferability Test

Antibiotic resistance transferability studies were conducted to confirm the nature of this resistance. Conjugal transfer of antibiotic resistance was assessed via the methods used in 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. The membrane was placed on the surface of the MRS agar and incubated anaerobically at 37°C for 24 h. 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.

In order to test the transferability of gentamicin resistance of *B. bifidum* BGN4, *L. acidophilus* ATCC 4356 was used as a recipient strain due to its high gentamicin sensitivity. *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. In contrast, *B. bifidum* BGN4 showed resistance to 64 μg/mL gentamicin in this study. It appears that *B. bifidum* BGN4's resistance to gentamicin was not transferred to the recipient strain under the test conditions.

6.B.7. PCR Assay on Antibiotic Resistance Genes (adopted from Kim et al., 2018)

Even though the whole genome of *B. bifidum* BGN4 has shown that there is no plasmid capable of transferring the antibiotic-resistance gene, PCR analysis was performed on the following antibiotic genes—e.g., 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)).The experimental conditions are described in Kim et al. (2018) and the results for *B. bifidum* BGN4 are presented in the Figure 3 (a) of the cited paper. No amplicons indicated resistance genes in *B. bifidum* BGN4 or other *Bifidobacterium* spp.

Summary of Antibiotic Susceptibility

The available information on antibiotic resistance pattern of *B. bifidum* BGN4 indicates that overall antibiotic susceptibilities of the strain are similar to patterns of other GRAS strains of bifidobacterial species, and the strain is not likely to have transmissible antibiotic resistance genes. In addition, *B. bifidum* BGN4 does not contain antibiotic resistance genes. These findings indicate that use of *B. bifidum* BGN4 in foods does not present concerns for antibiotic resistance.

6.B.8. 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 (Kim et al., 2018). These naturally-occurring microbiota and artificially-administered flora have a potential to produce various toxic substances during the deamination stage via nitrogen derivatives. Multiple potentially toxic products (i.e., phenol, ammonia, and indole) are possible throughout the proteolytic process, especially in the large intestine. Thus, bacterial ammonia production is highly relevant to human intestinal health and is a necessary component of the safety evaluation of commercial probiotics. In this study, *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 a Brain Heart Infusion (BHI) (BD BBL™, NJ, USA) medium at 37°C for 5 days as described in Kim et al. (2018). The production of ammonia by catalyzed indophenol reaction was determined by the method of Chaney and Marbach as described in Kim et al. (2018).

The ammonia production of *B. bifidum* BGN4 was assessed to verify the safety of these probiotics. In this study, *B. bifidum* BGN4 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. The study found no indication of the production of ammonia by *B. bifidum* BGN4.

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7.B. References That Are Not Generally Available

Not applicable

Tables

Figures

Wafula, Denis

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Thursday, April 04, 2019 10:29 AM
To: Wafula, Denis
Subject: Re: GRN 814 Questions and Comments for Notifier
Attachments: B. bifidum BGN4 COA-revised 4-4-2019.docx

Dear Dr. Wafula,

In response to your additional questions, we have prepared our responses as follows:
1. Please see a revised COA in the attached.
2. What it meant was that the ingredient is free of metal contamination since metals are separated using magnetic separators prior to packaging.
We would like to re-phrase it as 'the ingredient is subjected to magnetic separators prior to packaging.'

I hope we have properly answered your questions and comments. Thank you very much. Have a nice day!

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 Wednesday, April 3, 2019, 01:04:01 PM EDT, Wafula, Denis <Denis.Wafula@fda.hhs.gov> wrote:

Dear Dr. Cho,

I sent a comment earlier; and missed sending one more question. Therefore, the questions should read as a written below.

1. On page 45 (in Appendix B), we suspect that the cell count was supposed to be 1.10E+10 and not 1.10. Additionally, provide the units for the cell counts under the ‘parameters’ as you have done for the other certificates in the appendix where you have provided the counts as cfu/g. Please confirm the cell numbers and provide a new certificate if possible.
2. On page 9, under item 7 in the section labelled ‘Method of Manufacture’ you state that; “…the ingredient is freed of magnetic contamination prior to packing.” Please provide a brief explanation of what you mean by ‘magnetic contamination’.

Best Regards,

Denis

From: Wafula, Denis
Sent: Wednesday, April 03, 2019 9:57 AM
To: 'Susan S Cho' <susanscho1@yahoo.com>
Subject: RE: GRN 814 Questions and Comments for Notifier

Hello Dr. Cho,

On page 45 (in Appendix B), we suspect that the cell count was supposed to be 1.10E+10 and not 1.10. Also provide the units for the cell counts under the parameters as you have done for the other certificates in the appendix where you have provided the counts as cfu/g. Please confirm the cell numbers and provide a new certificate if possible.

Best Regards,

Denis

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Saturday, March 16, 2019 8:24 PM
To: Wafula, Denis <Denis.Wafula@fda.hhs.gov>
Subject: Re: GRN 814 Questions and Comments for Notifier

Dear Dr. Wafula,

Thank you very much for your kind, thorough review. In response to your comments and questions, we have prepared our answers in the attached documents. The first document has our answers and responses. The second document is a pdf file of amended pages. We hope we have properly answered your questions. We would appreciate our kind attention to this matter. If you have any further questions, please let me know. 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 Friday, March 15, 2019, 04:08:31 PM EDT, Wafula, Denis <Denis.Wafula@fda.hhs.gov> wrote:

Dear Dr. Cho,

We have completed the preliminary review of GRN 814. Find attached the questions and comments from the reviewers.

The questions and comments deal with issues we think that you can resolve in 10 working days (i.e. COB March 29, 2019). Please provide your response by then to enable us to complete the evaluation of your notice.

When answering the questions, please provide the answers after the questions and **do not submit** a new notice as a way of answering the questions.

Let me know if you have any questions or need any further assistance with the questions/comments.

Best Regards,

Denis

From: Wafula, Denis
Sent: Tuesday, November 27, 2018 3:49 PM
To: 'susanscho1@yahoo.com' <susanscho1@yahoo.com>
Subject: GRN 000814 Filing Letter

Dear Dr. Cho,

Find attached the filing letter for GRAS Notice GRN 000814 that you submitted to FDA. If you have any questions, do not hesitate to contact us.

Best Regards,

Denis

Denis Wafula, Ph.D.
Staff Fellow

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
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CERTIFICATE OF ANALYSIS

NAME OF PRODUCT	*Bifidobacterium bifidum* BGN4
LOT NO.	B4-R-170210
PRODUCTION DATE	2017. 02. 10
CERTIFICATED DATE	2017. 02. 14
EXPIRATION DATE	2019. 02. 09

ANALYSIS RESULT

Parameter	**B4-R-170210**	**Method of analysis/Method**
Appearance	Yellow white powder	Visual
Cell Counts (as *B. bifidum* BGN4), cfu/g	1. 10E+11	KHFSC 4/3/3-58
Moisture, %	3.68	KFSC 7/2/2.1/2.1.1
Heavy metals		
Lead (Pb), ppm	0.0162	KFSC 7/9/9.1/9.1.2
Arsenic (As), ppm	0.0393	KFSC 7/9/9.1/9.1.4
Cadmium (Cd)	0.0079	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[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

Analyzed in Korea Health Supplements Institute

QC Manager **Ji-Young Shin**

(b) (6)

Wafula, Denis

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Thursday, April 25, 2019 12:57 AM
To: Wafula, Denis
Subject: GRN 814-Soy allregen ttest results for B. bifidum BGN4
Attachments: Eurofin BGN4 soy allergen180328.pdf; Eurofin BGN4 soy allergen 170623.pdf; Eurofin BGN4 soy allergen 171122.pdf

Dear Dr. Wafusa,

Please see the COAs issued by Eurofin. Thank you very much.

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

Analytical Report

Sample Code	502-2018-00025077	Report date	22-May-2018
Certificate No.	AR-18-SU-025007-01		

BIFIDO.CO.,LTD.

Our reference:	502-2018-00025077/ AR-18-SU-025007-01
Client Sample Code:	BAFC120623
Sample described as:	Bifidobacterium bifidum BGN4
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
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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For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

END OF REPORT

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 101, Jialingjiang Road, SND
Suzhou 215000
Jiangsu Province, P.R.China

Phone +86 400 828 5088
Fax
www.eurofins.cn

Analytical Report

Sample Code	502-2018-00025078	Report date	22-May-2018
Certificate No.	AR-18-SU-025008-01		

BIFIDO.CO.,LTD.

Our reference:	502-2018-00025078/ AR-18-SU-025008-01
Client Sample Code:	B4PBM121221
Sample described as:	Bifidobacterium bifidum BGN4
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	230g
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

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Suzhou 215000
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Analytical Report

Sample Code	502-2018-00025079	Report date	22-May-2018
Certificate No.	AR-18-SU-025009-01		

BIFIDO.CO.,LTD.

Our reference:	502-2018-00025079/ AR-18-SU-025009-01
Client Sample Code:	B4PC180328
Sample described as:	Bifidobacterium bifidum BGN4
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	

SIGNATUR

(b) (6)

Shine Xie
Food Chemistry Manager

EXPLANATORY NOTE

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< LOQ: Below Limit of Quantification
N/A means Not applicable
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