GRAS Notice (GRN) No. 1079 Part 2 - Amendments

From:	Joel Villareal
To:	Hice, Stephanie
Cc:	Jim Lassiter; Brandon M. Griffin; Kenneth Cairns; Kent Phan; Livia Consedine
Subject:	FW: [EXTERNAL] Re: GRN 001079 - Questions for Notifier
Date:	Saturday, April 15, 2023 10:22:00 PM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png image007.png image009.png II965.1-CBI.2.1.pdf

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Dear Dr. Hice,

Upon additional review of the documents that were sent as attachments to the previous response identified in II965.1-CBI.2, there was an inclusion of documents received from the Sponsor that were incorrectly demarked as "Confidential" and were uploaded in that previous response.

The attached response to this has been amended accordingly to remove "Confidential" citation in the attached documents. Therefore, please disregard the prior notification and please accept the interim response with the amended report II965.1-CBI.2.1 for your review. This will be followed by the subsequent response in the next email with the remaining items requested for the Agency for this submission.

Respectfully,

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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2510-2521 and is legally privileged.

From: Joel Villareal <joel@rejimus.com>
Date: Friday, April 14, 2023 at 6:09 PM
To: Stephanie.Hice@fda.hhs.gov <Stephanie.Hice@fda.hhs.gov>
Cc: Jim Lassiter <jim@rejimus.com>, Brandon M. Griffin <brandon@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>, Kent Phan <kent@rejimus.com>, Livia Consedine <livia@rejimus.com>
Subject: FW: [EXTERNAL] Re: GRN 001079 - Questions for Notifier

Dear Dr. Hice,

In response to the document "2023-03-15 GRN 1079 – Questions for Notifier" for the request for more information for GRN 001079 (Bifidobacterium longum CBT BG7) and in accordance with the below correspondence, attached you will find responses to the questions/comments (II965.1-CBI.2) with the respective attachments included therein.

Please note that there are still five (5) questions that will require additional time to gather/verify information and documentation from the Sponsor. These additional information and documents will be provided to the agency for review once we have received them and we anticipate this information to be provided by Friday, 4/21/23. Please let us know if this suffices for this response.

Thank you for sending your initial feedback and if there any other questions/concerns, please let us know.

Kind Regards.

Joel Villareal | Regulatory Manager
Quality Development Services
joel@rejimus.com

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From: Jim Lassiter <jim@rejimus.com>
Date: Monday, April 3, 2023 at 1:50 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Cc: Brandon M. Griffin <brandon@rejimus.com>, Joel Villareal <joel@rejimus.com>, Kenneth Cairns <kenneth@rejimus.com>
Subject: Re: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

After careful conferring with colleagues assigned aspects of completion – we wish to avail ourselves of your kindness in allowing for complete delivery of the materials by the end of NEXT week. We will forward each individually as they are completed and reviewed. Thank you again for your assistance and efforts.

Respectfully,

--

Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Monday, April 3, 2023 at 12:17 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

Thank you for providing an update.

You mention in your email that the responses to the questions for GRN 001078, 001080, 001081, and 001082 are intended to be delivered over the course of the next week (with the responses to the questions for GRN 001079 to be issued shortly). Do you anticipate that you'll transmit each of the amendments to us by Friday, April 7, 2023? Or, are you referring to the end of next week?

Thank you in advance for your clarification.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
stephanie.hice@fda.hhs.gov

Pronouns: They-Them-Their (what is this?)

From: Jim Lassiter <jim@rejimus.com>
Sent: Monday, April 3, 2023 12:58 PM
To: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Subject: [EXTERNAL] Re: GRN 001078 - Questions for Notifier

Dr. Hice:

Please excuse the delay in providing updates and requests concerning this filing as we are actively working to address each of the requests for each of the submissions. We are preparing the responses to the inquiries posted and will issue the GRN 001079 shortly. The inquires posed to the notices 001078, 001080, 001081 and 001082 are also intended to be delivered promptly thereafter over the course of the next week as they are completed.

The majority of the requests have resulted in inquires and clarifications common across the

submissions needing input from the Sponsor of the notifications to address the last of the issues fully. We are working to address those succinctly with each update to follow.

Your continued patience in this matter is sincerely appreciated.

Respectfully,
--
Jim C. Lassiter | COO
jim@rejimus.com

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From: Hice, Stephanie <Stephanie.Hice@fda.hhs.gov>
Date: Friday, March 31, 2023 at 11:39 AM
To: Jim Lassiter <jim@rejimus.com>
Subject: RE: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

I wanted to follow-up to my March 15, 2023, email to see if you intended to provide responses to our questions for GRN 001078 soon? We typically request from a response within **10 business days**. If you are unable to complete the response within that time frame, you may contact me to discuss further options.

Thank you for your attention to our comments.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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Pronouns: They-Them-Their (what is this?)

From: Hice, Stephanie
Sent: Wednesday, March 15, 2023 12:03 PM
To: Jim Lassiter <jim@rejimus.com>
Subject: GRN 001078 - Questions for Notifier

Dear Mr. Lassiter,

During our review of GRAS Notice No. 001078, we noted questions that need to be addressed and are attached to this email.

We respectfully request a response within **10 business days**. If you are unable to complete the response within that time frame, please contact me to discuss further options. Please do not include any confidential information in your response.

If you have questions or need further clarification, please feel free to contact me. Thank you in advance for your attention to our comments.

Sincerely,

Stiffy Hice

Stephanie (Stiffy) Hice, Ph.D. (they/them/their)
Regulatory Review Scientist & Microbiology Reviewer

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4/15/2023

Stephanie Hice, PhD
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RE: Response to FDA Questions/Comments Regarding GRN 001079
II965.1-CBI.2.1

Dear Dr. Hice,

REJIMUS, INC. received your email dated 3/15/23 regarding additional FDA questions/comments to GRN 001079. This is the first response to address the majority of the questions presented. Additional documentation from the Sponsor has been requested and a follow-up response will be necessary and is expected to be provided to you by 4/21/23 to address the identified questions surrounding the intended use levels and the overall safety conclusion.

Should you have any questions or concerns with this additional information or have additional requests based on the information provided so far, please let us know, and we will be sure to address that promptly for the Agency.

Sincerely,

Jim Lassiter, President/COO
REJIMUS, INC.
jim@rejimus.com

TABLE OF CONTENTS

FDA QUESTIONS/COMMENTS REGARDING GRN 001079

Question 1

On page 7, the notifier states "… originally isolated from human feces or fermented food is identified as *Bifidobacterium longum* and has been uniquely characterized as a distinct strain known as CBT BG7 by means of genomic typing". For the administrative record, please clarify whether *B. longum* strain KCTC 12200BP (*B. longum* strain "CBT BG7") was originally isolated from human feces or human food.

Response

In general, Bifidobacteria and lactic acid bacteria were isolated from human feces or fermented foods. However, B. longum BG7 (KCTC 12200BP) was "originally isolated from feces of healthy breast-fed infant." (Kwon et al. 2015)

Attachment II965.1-CBI.2.1-A1

Question 2

On page 7, the notifier states "The gram staining morphology of Bifidobacterium can vary as long, slender rods, in clusters, pairs or even independently", however, does not describe the morphology of *B. longum* strain KCTC 12200BP. For the administrative record, please provide a brief description of the morphology of *B. longum* strain KCTC 12200BP.

Response

B. longum strain KCTC 12200BP is a gram-positive non-spore forming rod. The morphology of the colony is a circular shape with raised convex and smooth surface.

Question 3

For the administrative record, please provide a brief description of *B. longum* strain KCTC 12200BP including phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites), and whether this poses a safety concern. For example, on page 16, the notifier states, "*Bifidobacterium longum* CBT BG7 is not known to secrete any exotoxins or any other substances that are classified as harmful to humans" but does not describe how this was confirmed.

Response

Bifidobacterium longum CBT BG7 is a lactic acid bacterium (LAB). LAB produce bacteriocins, small peptides 3-6 kDA in size that help protect against pathogenic invasion (Savadogo et al. 2006, Toure et al. 2003). Most bacteriocins produced by LAB are membrane active compounds that increase permeability of the cytoplasmic membrane and show a spectrum of bactericidal activity that falls within two broad groups as shown in the Table 1 below (Savadogo et al. 2006). Characteristics of bacteriocins produced by Bifidobacterium spp. are shown in Table 2 below. Therefore, the phenotypic characteristics of B. longum strain KCTC 12200BP do not pose a safety concern.

Table 1: Antimicrobial peptides produced by lactic acid bacteria (Savadogo et al. 2006).

Group I: Modified bacteriocins (the lantibiotics)		Group II: Unmodified bacteriocins	
Type A	**Type B**	**One peptide bacteriocins**	**Two peptide bacteriocins**
Nisin	NK [a]	Pediocin-like bacteriocins [b]:	Lactococcin G
Lactocin S		Pediocin PA1, Leucocin A,	Lactacin F
Lacticin 481		Sakacin P, Curvacin A,	Plantaricin E/F
Carnocin UI 49		Mesentericin Y105,	Plantaricin J/K
Cytolysin		Carnobacteriocin BM1, Carnobacteriocin B2,	Lactobin A
		Enterocin A, Piscicolin 126,	Plantaricin S[c]
		Bavaricin MN, Piscicocin V1a	Pediocin L50[d]
			Thermophilin 13
		Nonpediocin- like bacteriocins:	
		Lactococcin A and B, Crispacin A, Divergicin 750, Lactococcin 972, AS-48[e], Enterocin B, Carnobacteriocin A	

[a] Not known: lantibiotics of type B produced by lactic acid bacteria are presently not known
[b] References for the pediocin like bacteriocins are: Pediocin PA1 (Henderson et al.,1992 ; Marug et al., 1992), leucocin A (Hastings et al., 1991), sakacin P (Tichaczek et al., 1992), curvacin A (Tichaczek et al., 1992 ; Holck et al., 1992), mesentericin Y105 (Hechard et al., 1992), carnobacterioin BM1 and B2 (Quadri et al., 1994), enterocin A (Aymerich et al., 1996), piscicolin 126 (Jack et al. , 1996), bavaricin MN (Kaiser , Montville ,1996), piscicocin V1a (20).
[c] Reference for plantaricin S: (Tichaczek et al., 1993).
[d] originally published as a modified ine peptide bacteriocin (Cintas et al. , 1995), but recent results indicate that is an unmodified two-peptide bacteriocin (Cintas et al.unpublished results)
[e] As-48 is a cyclic antimicrobial peptide produced by *Enterococcus faecalis* (Martinez-Bueno et al. , 1994).

Table 2: Bacteriocins from *Bifidobacterium* spp. and their main characteristics (Martinez et al. 2013).

Bacteriocin	Species and strain	Mol. wt. (kDa)	Heat range stability	pH range stability	Production phase	Optimal production	Inhibitory spectrum	Reference
Bifidin	*B. bifidum* NCDC 1452	(–)	(100 °C–30 min)	4.8–5.5	After 48 h	pH: 4.8	Gram-positive and Gram-negative bacteria	Anand et al. (1984, 1985)
Bifidocin B	*B. bifidum* NCFB 1454	3.3	(121 °C–15 min)	2–12	(12–18 h)	37 °C, pH 5.0–6.0	*Bacillus cereus, Enterococcus faecalis, Listeria monocytogenes, Pediococcus acidolactici, Streptococcus faecalis*, etc.	Yildirim and Johnson (1998); Yildirim et al. (1999)
Bifilong	*B. longum*	120	(100 °C–30 min)	2.5–5.0	(–)	(–)	Gram-positive and Gram-negative bacteria	Kang et al. (1989)
Bifilact Bb-46	*B. longum* Bb-46	25–127	(121 °C–15 min)	4–7	(–)	(–)	*Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, E. coli*	Saleh and El-Sayed (2004)
Bifilact Bb-12	*B. lactis* Bb-12	25–89	Unstable for high temperatures	4–7	(–)	(–)	*Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, E. coli*	Saleh and El-Sayed (2004)
Thermophilicin B67	*B. thermophilum* RBL67	5–6	(100 °C–5 min)	2–10	24 h	pH 6 and 40 °C	*Listeria sp., Lactobacillus acidophilus*	von Ah (2006)
Bifidin I	*B. infantis* BCRC 14602	3	(121 °C–15 min)	4–10	18 h	(–)	*LAB* strains, *Staphylococcus, Bacillus, Streptococcus, Salmonella, Shigella, E. coli.*	Cheikhyoussef et al. (2009a, 2010)
Lantibiotic (Bisin)	*B. longum* DJO10A	(–)	(–)	(–)	1–8 h	Auto-induction by crude lantibiotic	*Streptococcus thermophilus* ST403, *Clostridium perfringens, Staphylococcus epidermidis, Bacillus subtilis, Serratia marcescens, E. coli* DH5a.	Lee et al. (2011)

(–): not available.

Attachment(s) II965.1-CBI.2.1-A2 and II965.1-CBI.2.1-A3

Question 4

On page 26, the notifier states "The substance's potential for pathogenicity and acute toxicity tested negative". For the administrative record, please provide a statement affirming that *B. longum* strain KCTC 12200BP is non-pathogenic and non-toxigenic.

Response

Based on the results of the toxicity study, there were no signs of the mortality or adverse effects of the animals at levels of 1 x 10^{11} CFU/kg. In addition, "no virulence genes or pathogenicity islands were detected in the genome" (Kwon et al. 2015). Therefore, it can be affirmed that B. longum strain KCTC 12200BP is non-pathogenic and non-toxigenic.

Attachment II965.1-CBI.2.1-A1

Question 5

In Table 10, the notifier lists "nr" under the EFSA cutoff value for kanamycin (page 19). For the administrative record, please clarify if this stands for "not required".

Response

The notation "nr" in Table 10 refers to "not required" according to EFSA. Owing to the inherent characteristics of Bifidobacterium species regarding aminoglycosides, kanamycin is not required.

Question 6

In Table 1, the notifier lists "w" under "utilized" column for a few of the substrates assessed in the notifier's assessment of *B. longum* strain KCTC 12200BP's fermentative characteristics (page 8). For the administrative record, please clarify what "w" means.

Response

The notation in the table shown "w" is an indication of a "weak" reaction. These individual data points (D-Turanose and 5-Ceto-gluconate) were not considered critical to the overall acceptability of the data and information regarding the safety determinations for this microorganism.

Question 7

For the administrative record, please state whether *B. longum* strain KCTC 12200BP is genetically engineered.

Response

B. longum strain KCTC 12200BP is not genetically engineered. The strain was naturally isolated from human feces (Kwon et al. 2015).

Attachment II965.1-CBI.2.1-A1

Question 8

On pages 8 and 9, the notifier discusses various genotypic analyses performed on *B. longum* strain KCTC 12200BP, including comparisons to six other strains of Bifidobacterium. Table 2 includes the comparisons of these seven Bifidobacteria strains, however, the accompanying legend only lists six of the strains. For

the administrative record, please provide an updated copy of Table 2 with a revised legend that correctly identifies each of the seven strains.

Response

Table 2 has been updated to include the seven strains. B. lactis (DSM 10140) has been included as part of the seven strains.

Percent Identity

Divergence	1	2	3	4	5	6	7
1		99.1	99.1	98.0	92.9	90.8	93.2
2	0.5		98.8	95.8	93.4	90.6	93.7
3	0.9	0.9		96.8	93.4	90.8	93.0
4	3.0	3.1	2.3		92.9	91.4	93.1
5	5.0	5.1	4.6	4.6		89.3	94.1
6	6.7	7.0	6.9	6.3	7.6		90.9
7	4.9	5.0	5.2	5.1	4.9	6.7	

1. *B. longum* (KCTC 12200BP)
2. *B. longum*^{T} (ATCC 15707)
3. *B. infantis*^{T} (ATCC 15697)
4. *B. breve*^{T} (ATCC 15700)
5. *B. bifidum*^{T} (DSM 20456T)
6. *B. lactis*^{T} (DSM 10140)
7. *B. catenulatum*^{T} (KCTC 3221)

Question 9

On page 9, the notifier describes how pulse field gel electrophoresis was performed on *B. longum* strain KCTC 12200BP and *B. longum* strain ATCC 15707, however, does not provide a discussion regarding the results obtained. For the administrative record, please briefly summarize the results from this analysis.

Response

The presented method for pulse field gel electrophoresis in the notification demonstrated that the DNA fragments of B. longum strain KCTC 12200BP are different from the reference B. longum strain ATCC 15707. Therefore, it can be indicated that B. longum strain KCTC 12200BP is a new strain of B. longum species.

Question 10

On page 12, the notifier states “Stock organism is prepared and tested for microbiological contaminants”. Please clarify what microbiological contaminants are analyzed for at this stage.

Response

The stock organism is analyzed for i) aerobic microbial count and ii) total yeast and mold count.

Question 11

For the administrative record, please briefly specify how the purity of *B. longum* strain KCTC 12200BP is ensured during manufacturing, and state whether the fermentation process is conducted in a contained, sterile environment.

Response

Prior to inoculation of the organism into the prepared sterilized medium, the stock of the strain is checked for purity. As a process inspection in the cultivation of the organism, a bacterial morphology under microscopy is performed.

The fermentation process is conducted in a contained, sterile environment. The broth storage tank and its components used in the fermentation process are steam sterilized prior to use. During the fermentation process, the bottom valve of the broth storage tank is opened, and the cultivated broth is transferred to a separator that is cleaned via Clean-in-place (CIP) procedures.

Question 12

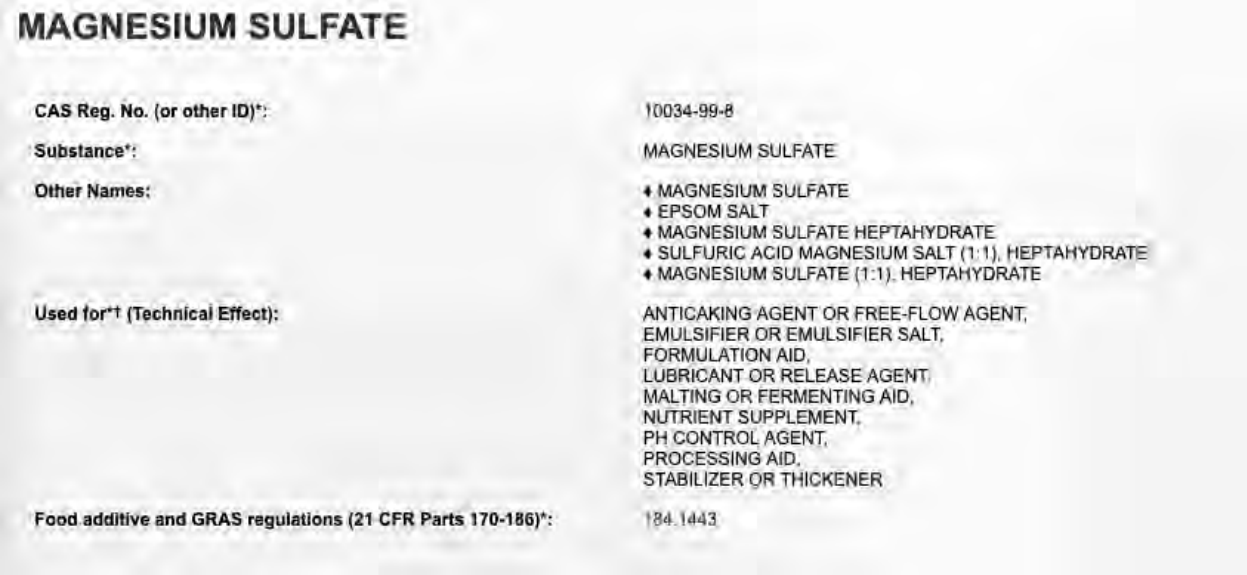
In Table 3, the notifier provides a list of raw materials used during the manufacturing process (page 11). The CAS numbers provided for yeast extract powder, fructose, monobasic potassium phosphate, and corn starch do not appear to correspond to the correct substances. For the administrative record, please provide the correct CAS numbers for these substances. In addition, we note that the correct name for the ingredient designated by CAS No. 10034-99-8 is magnesium sulfate heptahydrate. Please confirm.

Response

The CAS numbers for the following raw materials have been corrected.

Ingredient	CAS No.
Fructose	[57-48-7]
Yeast Extract Powder	[8013-01-2]
Potassium Phosphate, Monobasic	[7778-77-0]

According to the U.S. Food and Drug Administration Substances Added to Food database (screenshot below), Magnesium sulfate has an identified CAS Number of 10034-99-8 as shown in the screenshot below. It is acknowledged that Magnesium sulfate heptahydrate does have the same CAS number.



MAGNESIUM SULFATE

CAS Reg. No. (or other ID)*:	10034-99-8
Substance*:	MAGNESIUM SULFATE
Other Names:	♦ MAGNESIUM SULFATE ♦ EPSOM SALT ♦ MAGNESIUM SULFATE HEPTAHYDRATE ♦ SULFURIC ACID MAGNESIUM SALT (1:1), HEPTAHYDRATE ♦ MAGNESIUM SULFATE (1:1), HEPTAHYDRATE
Used for*† (Technical Effect):	ANTICAKING AGENT OR FREE-FLOW AGENT, EMULSIFIER OR EMULSIFIER SALT, FORMULATION AID, LUBRICANT OR RELEASE AGENT, MALTING OR FERMENTING AID, NUTRIENT SUPPLEMENT, PH CONTROL AGENT, PROCESSING AID, STABILIZER OR THICKENER
Food additive and GRAS regulations (21 CFR Parts 170-186)*:	184.1443

Question 13

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (page 11). The reference provided for L-cysteine monohydrochloride (21 CFR 182.1272) does not correspond to a regulation in the CFR. For the administrative record, please provide a clarified reference for this substance. Further, the references provided for trehalose (FEMA No. 4600), dibasic potassium phosphate (21 CFR 182.1073), monobasic potassium phosphate (21 CFR 175.105), and corn starch (21 CFR 182.70/21 CFR 182.90), either do not appear to be applicable references for these substances based on the intended use or correspond to different substances than those listed in the table. Based on these intended uses, more appropriate references would be GRN 000045, SCOGS Report No. 32 (for both dibasic and monobasic potassium phosphate), and SCOGS Report No. 115, respectively. For the administrative record, please provide a statement of affirmation.

Response

The regulatory references for the following raw materials have been corrected and are affirmed.

Ingredient	Reference
L-cysteine monohydrochloride	21 CFR§184.1272
Trehalose	GRN 000045
Potassium Phosphate, Dibasic	SCOGS Report No. 32
Potassium Phosphate, Monobasic	SCOGS Report No. 32
Corn starch	SCOGS Report No. 115

Question 14

In Table 3, the notifier cites 21 CFR 172.320 to support the regulatory status of the use of L-arginine as a "coating ingredient" (page 11). We note that 21 CFR 172.320 authorizes the use of L-arginine as a nutrient added to food and is not applicable to its use as a coating ingredient. FDA has not evaluated its use as a coating ingredient. Therefore, we respectfully request that you provide a statement stating that you will remove the use of arginine as a coating ingredient.

Response

The formulation of the microorganism delivered in dry form will not use L-arginine as a coating ingredient. The coating system for the microorganism consists of the remaining ingredients shown in the original Table 3.

Coating Ingredient	CAS No.	Reference
Trehalose	[6138-23-4]	GRN 000045
Potassium Phosphate, Dibasic	[7758-11-4]	SCOGS Report No. 32
Potassium Phosphate, Monobasic	[7778-7-0]	SCOGS Report No. 32
Xanthan Gum	[11138-66-2]	21 CFR §172.695
Corn starch	[977050-21-3]	SCOGS Report No. 115
Sodium Carboxymethylcellulose	[9004-32-4]	21 CFR §182.1745
Sodium Chloride	[7647-14-5]	21 CFR §182.1

Question 15

In Table 3 (page 11), the notifier lists the components of the fermentation media, and other raw materials, including soy peptone. Per the Food Allergen Labeling and Consumer Protection Act, soy is one of the major food allergens. Aside from this substance, please state whether any of the remaining raw materials used in the manufacturing process are major allergens or are derived from any of the nine major allergens. For any of the raw materials used that are major allergens or are derived from any of the nine major allergens, please discuss why these materials do not pose a safety concern.

Response

Aside from the noted soy peptone used only in the fermentation medium, the product that is the subject of this GRAS determination does not have any other raw materials used in the manufacturing process that represent any of the major food allergens required to be listed in accordance with the Food Allergen Labeling and Consumer Protection Act, identified as milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans and sesame.

Question 16

In Table 3, the notifier provides a list of raw materials used during the manufacturing process (page 11). For the administrative record, please clarify what "coating ingredient" means in this context.

Response

The inclusion of these materials occurs toward the end of the fermentation process. The intent of the inclusion is to encapsulate the microorganism comprising the finished ingredient for delivery in its dried and final form.

Question 17

Please clarify whether all raw materials used during the manufacturing process are food grade.

Response

All raw materials used during the manufacturing process are food grade. The raw materials used have regulatory statuses that are safe for inclusion in food.

Question 18

Figure 3 includes an "enzymatic modification" in the flow chart for the manufacturing process as the first step, however, this step is not described in any detail in the notice (page 12). Table 3 does not specify what type of enzyme, or its source (page 11). Please clarify the following:

a. the identity of the enzyme(s) used in the stated "enzymatic modification" step, including the enzyme commission number(s)

b. the intended use of the enzyme(s) during the manufacturing process

c. the source of the enzyme(s) (e.g., microbial-derived)

d. if the enzyme is produced by a microorganism, please provide clarification regarding the strain's phenotype (i.e., pathogenicity, toxigenicity), and genotype (i.e., genetically engineered)

e. how the notifier ensures that the enzyme(s) is inactivated and/or removed from the final product

Response

a. *The enzyme used in the enzymatic modification step is a protease (Alcalase) with the enzyme commission number 3.4.21.62.*

b. The intended use of the enzyme during the manufacturing process is for protein hydrolysis.

c. The source of the enzyme is from the microorganism, Bacillus licheniformis.

d. The microorganism, Bacillus licheniformis, where the enzyme is produced is a non-pathogenic strain and is not genetically engineered. In addition, protease enzymes using the non-pathogenic strain of Bacillus licheniformis are considered GRAS according to 21 CFR§184.1027 "Mixed carbohydrase and protease enzyme product."

e. After fermentation is complete, all components of the fermentation media, including the enzyme, are removed from the strain through the separator.

Question 19

In Table 4, the notifier lists a specification for appearance as "light yellow powder", however, the results from the batch analyses are "light brown powder" (page 13). For the administrative record, please clarify this discrepancy.

Response

The submitted specification in Table 4 shows a correct result for Appearance as "Light yellow powder." The description included on page 13 was a typographical error and should be identified consistently as "light yellow powder" consistent with the attached is the Certificate of Analysis for the three non-consecutive batches.

Attachment II965-CBI.2.1-A4

Question 20

The method for measuring viable cell count is listed on page 13 as USP <2022>. We note that this method is intended to be used to measure the absence of *Clostridium* species, *Escherichia coli*, *Salmonella* species, and/or *Staphylococcus aureus* in dietary supplements. Please clarify this discrepancy.

Response

The method referenced in the GRAS notification was misidentified. As a clarification, the viable cell count is performed as an in-house method. The method for viable cell count is attached.

Attachment: II965.1-CBI.2-A5

Question 21

The method for measuring coliforms is listed on page 13 as USP <2023>, we note that this is not a USP method, but rather refers to "Microbiological Attributes of Nonsterile Nutritional and Dietary Supplements". Please provide the correct method used to analyze for the presence of coliforms.

Response

Coliforms are tested according to Korean FDA Food Code VIII. Food Analytical Method, 4.7 Coliforms.

Question 22

In Table 4, the notifier lists specifications for microorganisms, including coliforms, but does not provide specifications for other common, notable foodborne pathogen analyses, such as Salmonella serovars (page 13). For the administrative record, please clarify if further analysis is performed to identify the genera or species of any presumptive positive result from analysis of coliforms. If further analysis is not performed, please describe why analysis for coliforms is sufficient. Additionally, please briefly describe how contamination is controlled during the manufacturing process.

Response

Microbiological testing such as E. coli, S. aureus, Salmonella, L. monocytogenes is performed and meets specifications as shown in the Certificate of Analysis for each presented batch. Testing of presumptive positive coliform results are further conducted to confirm the genus and species of any presumptive coliforms identified during the initial testing.

The contamination control program utilized during the manufacturing process includes the testing for contamination of stock organism(s), and all equipment used in the fermentation as well as the manufacturing processes, which are conducted through controlled cleaning programs. The finished ingredient testing is performed to verify purity and potency in accordance with the approved specification.

Attachment II965-CBI.2.1-A4

Question 23

The notifier does not provide specifications for heavy metals (Table 4, page 13). We note that we typically request that, at a minimum, a limit for lead be included in the specifications for fermentation-derived ingredients. Please include a limit for lead in the specifications for *B. longum* strain KCTC 12200BP and provide analytical results from a minimum of three non-consecutive batches to demonstrate that the ingredient can be manufactured that to meet this specification limit. Please note that the limit for lead should be as low as possible and be reflective of the results of the batch analyses. In addition, please specify the analytical method that is used to test for lead.

Response

Heavy metals are being performed as identified in the Certificate of Analysis. These include results for Lead, Arsenic, Cadmium, and Mercury in three non-consecutive batches. The limit for Lead is $\leq$ 1.0 mg/kg. Attached is the Certificate of Analysis of the three non-consecutive batches. The analytical method used for testing for lead is through ICP performed under Korean FDA Food Code, VIII. Food Analytical Method, 9.1 Heavy Metal.

Attachment: II965.1-CBI.2.1-A4

Question 24

Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications (including lead) have been validated for that particular purpose.

Response

All analytical methods used in the testing of the batches (including lead) have been validated for their respective purposes.

Question 25

On page 14, the notifier states that *B. longum* strain KCTC 12200BP is intended to be added to dairy products at concentrations needed to provide at least 10^{11} CFU per serving. According to the stability

study (Table 5, page 13), the survival rate decreases ~30% during 12-months of storage. Considering the loss during storage, please provide narrative how the notifier ensures that 1×10^{11} CFU per serving remains viable over the product shelf life.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels. The response to this question will be addressed in the follow-up response.

Question 26

Please provide food subcategories included in the estimation of consumption of "dairy products" in Table 7 (page 15). In addition, please specify a serving size for each food subcategory and provide the reference that was used as the basis for determining the serving size.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels of each of the food sub-categories. The response to this question will be addressed in the follow-up response.

Question 27

Please clarify what population is represented by "all users" in the dietary exposure estimate (Table 7, page 15). If the dietary exposure estimate is not for the U.S. population aged 2 years and older, please provide mean and 90th percentile eaters-only dietary exposure estimates for U.S. population aged 2 years and older.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and the appropriate dietary exposure. The response to this question will be addressed in the follow-up response.

Question 28

On page 15, the notifier states, "three daily servings would result in a cumulative exposure of 2.68×10^{11} CFU per day ($8.94 \times 10^{10} \times 3$)". Further, the notifier states, "the recommended levels of the cumulative exposure of 2.68×10^{11} CFU per day and the cumulative exposure at an estimated 90th percentile of 5.55×10^{11} CFU per day". Please note that the cumulative dietary exposure should consider background sources, and all current and proposed uses of *B. longum* strain KCTC 12200BP. For the administrative record, please confirm that the term "cumulative" was incorrectly used in the statements mentioned above.

Further, on page 15 the notifier states, "The estimated 90th percentile of consumers of dairy products at this level of recommended consumption adjusted for the findings of the per capita data". We consider that the data in Table 7 represents estimates for "users" (eaters) only, i.e., individuals consuming the proposed dairy products at least once during the survey period. Please note that "per capita" estimates would include eaters and non-eaters. For the administrative record, please confirm that the estimates in Table 7 are for the eaters-only population and explain what is meant by "the findings of the per capita data".

Response

Currently, B. longum strain KCTC 12200BP is considered a novel ingredient in food and there are no current uses of this strain. As dairy products are the only proposed food, the dietary exposure of the ingredient is only based on the dairy products only. Therefore, the term "cumulative" was inappropriately used.

The estimates used in the Table 7 is confirmed as eaters-only population. Therefore, the appropriate term should be "findings from the eaters-only population" and not "findings of the per capita data."

Question 29

Please provide an updated literature search that discusses the safety of *B. longum*, including the safety of Bifidobacteria, this strain, or closely related strains, as applicable. Please do not limit your discussion solely to studies in human populations and include a discussion on pathogenicity and toxigenicity. Further, any reports of bacteremia, or foodborne illness involving Bifidobacteria, should also be discussed. For example, but not limited to, please see:

- Esaiassen, E., Hjerde, E., Cavanagh, J. P., Simonsen, G. S., and Klingenberg, C. (2017). Bifidobacterium bacteremia: clinical characteristics and a genomic approach to assess pathogenicity. Journal of Clinical Microbiology, 55, 2234- 2248. doi: 10.1128/JCM.00150-17

- Ha, G. Y., Yang, C. H., Kim, H., and Chong, Y. (1999). Case of sepsis caused by *Bifidobacterium longum*. Journal of Clinical Microbiology, 37(4), 1227-1228. doi: 10.1128/JCM.37.4.1227-1228.1999

Please include the date (month and year) the literature search was performed and discuss whether there are any publications that may be contradictory to a GRAS conclusion.

Response

A PubMed and Google Scholar search was performed for "*Bifidobacterium longum*", and "CBT BG7" to determine if there are any adverse events in a human populations or animal studies. Published studies are summarized below.

Reference	Study Title	Subjects	Dose	Duration	Summary of Safety
Schellekens et al. (2021)	*Bifidobacterium longum* counters the effects of obesity: Partial successful translation from rodent to human	Healthy overweight adults and mice	Mice were administered 2 x 10^8 CFU/mL of *Bifidobacterium longum* APC1472 and humans took a daily dose of 1 x 10^{10} CFU.	12 weeks	No mortality was observed in the mice subjects. There were seven adverse events; however, 6 of the adverse events were from the placebo group. The adverse event from the treatment group is constipation.
Jiang et al. (2021)	Strain-Specific effects of *Bifidobacterium longum* on hypercholesterolemic and potential mechanisms	Sprague-Dawley rats	Six groups were administered 10^9 CFU/mL of different *B. longum* strains (HC-CCFM 1077, HC-I3, HC-J3, ad HC-B3)	28 days	No mortality was notated in the study.
Takeda et al. (2023)	Usefulness of *Bifidobacterium longum* BB536 in Elderly Individuals With Chronic Constipation: A Randomized Controlled Trial	80 older adults with constipation	5 x 10^{10} CFU of *B. longum* BB536	Once daily for 4 weeks	No mortalities were notated. One case of diarrhea in both the treatment and placebo group.

Reference	Study Title	Subjects	Dose	Duration	Summary of Safety
Mitelmao et al. (2022)	The effect of probiotics on functional constipation in adults: A randomized, double-blind controlled trial	132 Adults with constipation	Two treatment groups: 3 x 10^9 CFU of *L. acidophilus* 02, *B. bifidum* 01, *L. rhamnosus* 04 8 x 10^9 CFU of *B. longum* 03, *B. lactis* 01, *L. casei* 03, *B. animalis* THT, *L. acidophilus* 02, *B. bifidum* 01, *L. rhamnosus* 04	Once daily for 30 days	One adverse event was observed in one of the groups (abdominal pain), but the author deemed the adverse event as not serious.
Tremblay et al. (2021)	Safety and effect of a low- and high-dose multi strain probiotic supplement on a microbiota in a general adult population: a randomized, double-blind, placebo-controlled study	69 healthy adults	5 billion or 25 billion CFU of *L. helveticus* R0052, *L. rhamnosus* R0011, *Pediococcus acidilactiti* R1001, *B. longum ssp. longum* BB536, *L. casei* R0215, *L. plantarum* R1012, *B. breve* R0070, *Lactococcus lactis ssp. lactis* R1058	Daily for 28 days	No adverse events were observed.
Karyana et al. (2022)	The efficacy of probiotics supplementation of the lipid profiles of obese adolescents: a randomized trial	58 obsese adolescents	1.25 x 10^9 CFU of 5 strains containing *Bifidobacterium longum*	8 weeks	No adverse effects were observed.

Esaiassen et al. (2017) discusses the frequency and causes for bacteremia by Bifidobacterium species. A review of the publication shows that Bifidobacterium longum is the most frequent species that caused

bacteremia. However, the authors specifies that these cases of bacteremia occur mainly in patients who were immunocompromised, had a known medical condition, or a gastrointestinal tract condition. Ha et al. (1999) presented a case of sepsis in a human male where the isolated organism was identified as Bifidobacterium longum. The author concluded that this case of sepsis was introduced externally (i.e., "improperly sterilized acupuncture needles or from the colon via minute perforations caused by those needles"). Boyle et al. (2006) presented a review publication on what may cause bacteremia. However, the author mentions "all cases of bacteremia or fungemia gave occurred in patients with underlying immune compromise, chronic disease, or debilitation, and no reports have described sepsis related to probiotic use in otherwise healthy persons." Therefore, these publications conclude that food-borne illness, such as bacteremia, are typically caused by medical or external causes.

Owing to the results of the updated literature search performed on April 2022 and additional publication on the pathogenicity and toxigenicity as well as no significant adverse effects of B. longum, none of the published studies is contradictory with the GRAS conclusion.

Attachment(s) II965.1-CBI.2.1-A6, II965.1-CBI.2.1-A7, II965.1-CBI.2.1-A8, II965.1-CBI.2.1-A9, II965.1-CBI.2.1-A10, II965.1-CBI.2.1-A11, II965.1-CBI.2.1-A12, II965.1-CBI.2.1-A13, II965.1-CBI.2.1-A14

Question 30

Tables 8 and 9, the notifier lists several GRAS notices, where the subject of the notice was a strain of *B. longum* or Bifidobacteria, that have been submitted to FDA and have received "no questions" letters (page 18). We evaluated GRNs 000049, 000950, 000952, 000985, 001002, and 001003, and responded in letters respectively dated March 19, 2002, March 1, 2021, March 17, 2021, December 21, 2021, July 22, 2022, and April 26, 2022, stating that we had no questions at the time regarding the notifiers' GRAS conclusions. For the administrative record, please briefly discuss these GRNs in the context of the notifier's safety conclusion.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and confirm the safety conclusion. The response to this question will be addressed in the follow-up response.

Question 31

In Table 9, the notifier lists the substance associated with GRN 000813 as "Bifidobacterium bifidum BORI", however, the substance associated with this GRAS notice is *B. longum* BORI. For the administrative record, please provide a statement of acknowledging this (page 18).

Response

It is acknowledged that the substance associated with GRN 000813 should be identified as B. longum BORI.

Question 32

On page 26, the notifier states "The applicable GRAS notices, referenced in Table 8 and Table 9 within Part 6 of this notice, incorporate myriad studies demonstrating the safety of ingestion of substances closely related to *Bifidobacterium longum* CBT BG7" but does not identify or summarize the relevant information from each GRAS notice. As each GRAS notice stands on its own, for the administrative record, please briefly summarize the information incorporated by reference from the GRAS notices listed in Tables 8 and 9.

Response

Table 8 and 9 has been updated to include a summary of each of the listed GRAS notices:

Table 8. *GRAS notices containing Bifidobacterium longum receiving reply from FDA that it had no questions (GRAS Notices Inventory Database).*

GRAS No.	Date of Closure	Substance	Intended Use	Amount
877	12/26/19	*Bifidobacterium longum* BB536	Infant formula	1 x 10^8 CFU per gram of product
813	6/21/19	*Bifidobacterium longum* BORI	Powdered non-exempt term infant formula	Up to 10^8 CFU per gram of powdered formula.
			Fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form at up to 10^9 CFU per serving.	Up to 10^9 CFU per serving.
758	8/20/18	*Lactobacillus helveticus* strain R0052, *Bifidobacterium longum* subsp. *infantis* strain R0033,	Powdered infant formulas	5 x 10^7 CFU per gram of product

GRAS No.	Date of Closure	Substance	Intended Use	Amount
		and *Bifidobacterium bifidum* strain R0071		
268	7/08/09	*Bifidobacterium longum* strain BB536	Breads/baked goods, cereals, dairy products/dairy-based foods and dairy substitutes, fruit products, candy, chewing gum, cocoa powder, condiment sauces, flavored beverage syrups, fruit flavored powder beverage mixes, gelatin desserts, gravies, margarine, peanut and other nut butter/spreads, snack foods, weaning foods Milk based powdered infant formula	$1x10^{10}$ colony forming units (cfu) per serving $1x10^{10}$ cfu per gram of infant formula powder

Table 9. *GRAS notices of Bifidobacterium organisms of species other than longum receiving reply from FDA of no questions (GRAS Notices Inventory Database)*

GRAS No.	Date of Closure	Substance	Intended Use	Amount
872	12/09/19	*Bifidobacterium animalis* subsp. *lactis* UABIa-12	Foods generally, excluding infant formula and foods under the authority of USDA	10^9 to 10^{11} CFU per serving
856	12/09/19	*Bifidobacterium animalis* subsp. *lactis* strain BB012	Conventional foods for use by the general population, excluding foods subject to regulation by the USDA	5×10^{11} CFU per serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
855	2/5/20	*Bifidobacterium animalis* subsp. *lactis* strain R0421	Exempt powdered milk-based infant formula intended for healthy term infants	5 x 10^9 CFU/800 ml of formula as prepared.
814	6/25/19	*Bifidobacterium bifidum* BGN4	Powdered non-exempt term infant formula Fermented milk; includes buttermilk and kefir; flavored milk beverages mixes, dried milk powder; imitation milk; yogurt; baby cereals and foods, powder form; meal replacement powder and nutrition drink mix powder; and sugar substitute, powder form	10^8 CFU per gram of powdered formula Up to 10^9 CFU per serving
455	9/30/13	*Bifidobacterium breve* M-16V	Exempt term powdered amino acid-based formulas	Up to 10^8 CFU per gram of infant formula powder
454	9/27/13	*Bifidobacterium breve* M-16V	Non-exempt powdered term infant formulas (milk- or soy-based) and exempt powdered term infant formula containing partially hydrolyzed milk or soy proteins	Up to 10^8 colony forming units per gram of infant formula powder

GRAS No.	Date of Closure	Substance	Intended Use	Amount
453	9/27/13	*Bifidobacterium breve* M-16V	baked goods, breakfast cereals, fruit juices and nectars, fruit ices, vegetable juices, milk-based drinks and powders, dairy product analogs, frozen dairy desserts, processed cheese, imitation cheese, cheese spreads, butter-type products, snack foods, gelatin, pudding, fillings, meal replacements, snack bars, nut and peanut spreads, hard and soft candies, cocoa-type powder, and condiment sauces at levels	Up to 5 x 10^9 colony forming units per serving
445	4/10/13	*Bifidobacterium animalis* subsp. *lactis* strains HN019, Bi-07, BI-04 and B420	Ready-to-eat breakfast cereals, bars, cheeses, milk drinks and milk products, bottled water and teas, fruit juices, fruit nectars, fruit 'ades' and fruit drinks, chewing gum, and confections	Maximum level of 2 x 10^{11} colony forming units per serving

GRAS No.	Date of Closure	Substance	Intended Use	Amount
377	9/29/11	*Bifidobacterium animalis* subsp. *lactis* strain Bf-6	Intended foods include: dairy foods such as fluid milks, yogurt, milk-based desserts and gravies and cheeses; dry seeds, nuts, and nut butters; grain products such as flour, yeast breads, quickbreads, cakes, cookies, pies, pastries, crackers, pancakes, waffles, French toast, crepes, pasta, cooked and ready-to-eat cereals, grain mixtures, and meat substitutes; fruits and fruit beverages; dark-green vegetables, olives, pickles, relishes, and vegetable soups; salad dressings; sugars and sugar substitutes, syrups, honey, molasses, jellies, jams, preserves, gelatin desserts, ices, and popsicles, candies, and chewing gum; and carbonated soft drinks, sports drinks, energy drinks, and water	Maximum level of 10^{11} colony forming units (cfu) per serving.

Question 33

The notifier lists the intended use of *B. longum* strain KCTC 12200BP as up to 10^{11} CFU/serving in dairy products. FDA has evaluated and issued "no questions" letters to seven previous GRAS notices, where the subject of the notice was a strain of *B. longum* with various intended uses. The highest intended use level evaluated was up to 10^{10} CFU/serving. For the administrative record, please briefly discuss the 1-log increase in use level in the context of the notifier's safety conclusion.

Response

In Progress

Additional information has been requested to the Sponsor to verify the serving size/intended levels and confirm the safety conclusion. The response to this question will be addressed in the follow-up response.

Question 34

On page 19, the notifier states "While the conclusion of general recognition of safety (GRAS) is based upon scientific procedures, there is a history of use of *Bifidobacterium longum* CBT BG7 in foreign countries and in multiple food products" but does not provide a summary of these food products. For the administrative record, please provide a brief summary of these food products.

Response

Below is a table of food products that contain Bifidobacterium longum CBT BG7 in foreign countries.

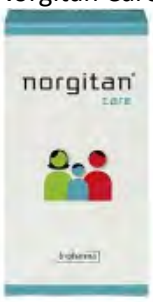
Product	**Availability**	**Ingredients**	**Amount per Serving**
DUOLAC® Care 	Singapore https://www.watsons.com.sg/duolac-care-60s/p/BP_66142	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *B. lactis* BL3 *L. rhamnosus* LR5 *B. bifidum* BF3	1.12×10^9 CFU 2.19×10^9 CFU 2.19×10^9 CFU 2.19×10^9 CFU 2.00×10^9 CFU 1.91×10^9 CFU 1.25×10^{10} Total CFU / Tablet
DUOLAC® Gold 	Korea https://www.ebay.com/itm/Duolac-Gold-Probiotics-Adult-30-days-Dual-Coated-Lactic-Acid-Bacteria-Triplets-/231644172196	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3 *B. bifidum* BF3	1.60×10^9 CFU 1.76×10^9 CFU 1.76×10^9 CFU 1.60×10^9 CFU 1.75×10^9 CFU 1.53×10^9 CFU 1.0×10^{10} Total CFU / Stick

Product	**Availability**	**Ingredients**	**Amount per Serving**
DUOLAC® Kidlac 	Vietnam http://www.quanglong.vn/ChiTietSanPham.aspx?MaSanPham=3 Myanmar https://karunamyanmar.com/product/kidlac/ https://karunamyanmar.com/product/kidlac/	*B. longum* BG7 *L. acidophilus* LA1 *B. breve* BR3 *E. faecium* EF4	2.00×10^7 CFU 2.00×10^7 CFU 2.00×10^7 CFU 4.40×10^8 CFU 5.10×10^8 Total CFU / Stick
DUOLAC® Daily Vitality 	Singapore Korea https://www.ebay.co.uk/itm/Duolac-Daily-Vitality-Probiotics-Capsule-30-days-Bifidus-Family-Constipation-/232069794895 Finland https://www.apteekkituotteet.fi/Duolac-Daily-Vitality	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3 *B. bifidum* BF3	1.12×10^9 CFU 1.23×10^9 CFU 1.23×10^9 CFU 1.12×10^9 CFU 1.23×10^9 CFU 1.07×10^9 CFU 7.0×10^9 Total CFU / Capsule
DUOLAC® Normal Immune 	Denmark https://www.duolac.dk/products/duolacc-normal-immunforsvar-2/ Finland https://www.apteekkituotteet.fi/Duolac-Normal-Immune	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3	5.09×10^8 CFU 7.12×10^8 CFU 6.61×10^8 CFU 5.09×10^8 CFU 6.10×10^8 CFU 3.0×10^9 Total CFU / Tablet
DUOLAC® Yam Yam 	Korea https://www.ebay.com/itm/Duolac-Yam-Yam-Probiotics-Chews-40-days-Dual-Coated-Bifidus-Triplets-Kid-Child-/232069760518	*B. longum* BG7 *B. breve* BR3 *E. faecium* EF4 *L plantarum* LP3	1.25×10^9 CFU 1.25×10^9 CFU 3.75×10^9 CFU 1.25×10^9 CFU 7.5×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
DUOLAC® Daglig Vitalitet 	Denmark https://www.duolac.dk/products/duolac-daglig-vitalitet/	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *B. lactis* BL3 *L. rhamnosus* LR5 *B. bifidum* BF3	1.12 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.23 x 10^9 CFU 1.12 x 10^9 CFU 1.07 x 10^9 CFU 7.0 x 10^9 Total CFU / Capsule
DUOLAC® Daglig Børn 	Denmark https://www.duolac.dk/products/duolac-daglig-boern/	*B. longum* BG7 *B. breve* BR3 *S. thermophilus* ST3 *L. plantarum* LP3	3.83 x 10^8 CFU 3.83 x 10^8 CFU 1.15 x 10^9 CFU 3.83 x 10^8 CFU 2.3 x 10^9 Total CFU / Tablet
DUOLAC® Balance Baby 	Korea https://www.ebay.com/itm/Duolac-Baby-Probiotics-Powder-30-days-Dual-Coated-Bifidus-Triplets-Kid-Child-/232069774531	*B. longum* BG7 *B. breve* BR3 *L. rhamnosus* LR5 *L. plantarum* LP3 *B. infantis* BT1 *B. bifidum* BF3	7.56 x 10^8 CFU 7.56 x 10^8 CFU 9.89 x 10^8 CFU 9.89 x 10^8 CFU 7.56 x 10^8 CFU 7.56 x 10^8 CFU 5.0 x 10^9 Total CFU / Stick
DUOLAC® Duo-D Drops 	Denmark https://www.duolac.dk/products/duolac-duo-d-draaber/	*B. longum* BG7 *B. breve* BR3 *B. bifidum* BF3 *B. infantis* BT1	1.25 x 10^8 CFU 1.25 x 10^8 CFU 1.25 x 10^8 CFU 1.25 x 10^8 CFU 5.0 x 10^8 Total CFU / 6 Drops

Product	**Availability**	**Ingredients**	**Amount per Serving**
Lactobex® Strong 	Latvia http://www.lactobex.lt	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3 *B. bifidum* BF3	1.12×10^9 CFU 1.23×10^9 CFU 1.23×10^9 CFU 1.12×10^9 CFU 1.23×10^9 CFU 1.07×10^9 CFU 7.0×10^9 Total CFU / Capsule
Lactobex® 	Latvia http://www.lactobex.lt	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *B. lactis* BL3 *L. rhamnosus* LR5	2.0×10^8 CFU 2.0×10^8 CFU 2.0×10^8 CFU 2.0×10^8 CFU 2.0×10^8 CFU 1.0×10^9 Total CFU / Stick
Lactobex® Baby 	2011 Latvia http://www.lactobex.lt	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3	3.30×10^8 CFU 3.30×10^8 CFU 3.40×10^8 CFU 1.0×10^9 Total CFU / Stick
NBL Probiotic Optima 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/yetiskin/nbl-probiotic-optima/	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *E. faecium* EF4 *L. plantarum* LP3 *B. lactis* BL3	7.7×10^7 CFU 1.5×10^8 CFU 2.3×10^8 CFU 5.7×10^8 CFU 2.3×10^8 CFU 2.3×10^8 CFU 1.5×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
NBL Probiotic Kids 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/cocuk/nbl-probiotic-kids/	*B. longum* BG7 *B. breve* BR3 *S. thermophilus* ST3 *L. plantarum* LP3	3.83×10^8 CFU 3.83×10^8 CFU 1.15×10^9 CFU 3.83×10^8 CFU 2.3×10^9 Total CFU / Tablet
NBL Probiotic Gold 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/yetiskin/nbl-probiotic-gold/	*B. longum* BG7 *L. acidophilus* LA1 *L. rhamnosus* LR5 *E. faecium* EF3 *B. bifidum* BF3	4.26×10^8 CFU 4.26×10^8 CFU 4.26×10^8 CFU 8.16×10^8 CFU 4.26×10^8 CFU 2.5×10^9 Total CFU / Stick
NBL Probiotic D3 Drop 	Turkey https://www.nblprobiotic.com/nbl-probiotic-ailesi/cocuk/nbl-probiotic-drop/	*B. longum* BG7 *B. breve* BR3 *B. bifidum* BF3 *B. infantis* BT1	1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU 1.25×10^8 CFU 5.0×10^8 Total CFU / 6 Drops
PRODUO Stop 	Spain http://produo.es/familia-produo-tratamiento-flora-bacteriana-intestinal/produo-stop-alteraciones-microbiota/	*B. longum* BG7 *L. acidophilus* LA1 *L. rhamnosus* LR5 *E. faecium* EF3 *B. bifidum* BF3	4.26×10^8 CFU 4.26×10^8 CFU 4.26×10^8 CFU 8.16×10^8 CFU 4.26×10^8 CFU 2.5×10^9 Total CFU / Sachet

Product	Availability	Ingredients	Amount per Serving
PRODUO Flora 	Spain http://produo.es/familia-produo-tratamiento-flora-bacteriana-intestinal/produo-flora-tratamiento-microflora-intestinal/	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3	5.09×10^8 CFU 7.12×10^8 CFU 6.61×10^8 CFU 5.09×10^8 CFU 6.10×10^8 CFU 3.0×10^9 Total CFU / Tablet
PRODUO Daily Care 	Spain http://produo.es/familia-produo-tratamiento-flora-bacteriana-intestinal/produo-daily-care-flora-bacteriana/	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3 *B. bifidum* BF3	1.12×10^9 CFU 1.23×10^9 CFU 1.23×10^9 CFU 1.12×10^9 CFU 1.23×10^9 CFU 1.07×10^9 CFU 7.0×10^9 Total CFU / Capsule
PRODUO Daily Kids 	Spain https://www.farmaciaevacontreras.com/producto/produo-daily-kids/	*B. longum* BG7 *B. breve* BR3 *S. thermophilus* ST3 *L. plantarum* LP3	3.83×10^8 CFU 3.83×10^8 CFU 1.15×10^9 CFU 3.83×10^8 CFU 2.3×10^9 Total CFU / Tablet
Norgitan Care 	Belgium http://www.apomed.be/2555849-norgitan-care-5-souches-bacteries-vivantes-coloniser-votre-intestin-5425014928174-b-pharma.html	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3	5.09×10^8 CFU 7.12×10^8 CFU 6.61×10^8 CFU 5.09×10^8 CFU 6.10×10^8 CFU 3.0×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
Lacto-B™ 	Indonesia https://www.tokopedia.com/onlinemedika/lacto-b-sachet-untuk-mencegah-diare-pada-bayi	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3	3.30×10^8 CFU 3.30×10^8 CFU 3.40×10^8 CFU 1.0×10^9 Total CFU / Stick
LIPROLAC 	Indonesia https://www.kalbestore.com/liprolac-vanilla-powder.html	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. bifidum* BF3	8.50×10^7 CFU 2.00×10^8 CFU 6.80×10^8 CFU 2.00×10^8 CFU 8.50×10^7 CFU 1.25×10^9 Total CFU / Sachet
Floradicol7 	Belgium http://www.bioradix.be/nl/producten/floradicol.html	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. plantarum* LP3 *L. rhamnosus* LR5 *B. lactis* BL3 *B. breve* BR3	3.44×10^8 CFU 5.74×10^8 CFU 2.30×10^9 CFU 2.30×10^9 CFU 5.74×10^8 CFU 5.74×10^8 CFU 3.44×10^8 CFU 7.0×10^9 Total CFU / Capsule
Lucovitaal® Probiotica 	Netherlands https://www.lucovitaal.nl/probiotica-tabletten.html	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *E. faecium* EF4 *L. plantarum* LP3 *B. breve* BR3	3.94×10^7 CFU 1.57×10^8 CFU 4.70×10^7 CFU 1.93×10^8 CFU 4.70×10^8 CFU 9.41×10^7 CFU 1.0×10^9 Total CFU / Tablet

Product	Availability	Ingredients	Amount per Serving
Phital® Probiotica Plus 	Netherlands https://www.phital.nl/producten/probiotica/probiotica-plus-duolac	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3	5.10×10^8 CFU 7.14×10^8 CFU 6.63×10^8 CFU 5.10×10^8 CFU 6.12×10^8 CFU 3.0×10^9 Total CFU / Stick
Nutriforte Lactoghurt 	Malaysia http://www.nutriforte.com.my/Lactoghurt+Probiotics_20_1.htm	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. lactis* BL3	3.67×10^8 CFU 3.91×10^8 CFU 4.89×10^8 CFU 3.67×10^8 CFU 4.89×10^8 CFU 2.1×10^9 Total CFU / Tablet
Lacclean Gold Lab 	Vietnam https://www.alibaba.com/product-detail/LACCLEAN-GOLD-LAB-health-food_246152457.html	*B. longum* BG7 *L. acidophilus* LA1 *S. thermophilus* ST3 *L. rhamnosus* LR5 *B. bifidum* BF3	8.50×10^7 CFU 2.00×10^8 CFU 6.80×10^8 CFU 2.00×10^8 CFU 8.50×10^7 CFU 1.25×10^9 Total CFU / Sachet

Conclusion

We sincerely appreciate this opportunity to clarify the additional questions submitted so far as part of this review and we look forward to a positive assessment of these responses and the notification itself. Should the agency have any additional questions or requests on the above responses or the prior responses, please let us know at your earliest convenience and we will do everything we can to address those promptly. We look forward to completing the follow up response to the Agency addressing the remaining items that are identified herein as "in progress" promptly with final inputs from the Sponsor.

Attachments

II965.1-CBI.2.1-A1	Kwon S-K, Kwak M-J, Seo J-G, Chung MJ, Kim JF. Complete genome sequence of *Bifidobacterium longum* KCTC 12200BP, a probiotic strain promoting the intestinal health. *Journal of Biotechnology* 214 (2015) 169-170.
II965.1-CBI.2.1-A2	Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Bacteriocins and lactic acid bacteria- a minireview. *African Journal of Biotechnology* Vol. 5(9), pp. 678-683, 2 May 2006.
II965.1-CBI.2.1-A3	Martinez FAC, Balciunas EM, Converti A, Cotter PD, de Souza Oliveira RP (2013). Bacteriocin production by *Bifidobacterium* spp. A review. *Biotechnology Advances* 31 (2013) 482-488.
II965.1-CBI.2.1-A4	Certificate of Analysis
II965.1-CBI.2.1-A5	In-house analytical method for Viable Cell Count
II965.1-CBI.2.1-A6	Schellekens H, Torres-Fuentes C, van de Wouw M, Long-Smith CM, Mitchell A, Strain C, Berding K, Bastiaanssen TFS, Rea K, Golubeva AV, Arboleya S, Verpaalen M, Pusceddu MM, Murphy A, Fouhy F, Murphy K, Ross P, Roy BL, Stanton C, Dinan TG, Cryan JF (2021). *Bifidobacterium longum* counters the effects of obesity: Partial successful translation from rodent to human. *EBioMedicine* 63 (2021) 103176.
II965.1-CBI.2.1-A7	Jiang J, Wu C, Zhang C, Zhang Q, Yu L, Zhao J, Zhang H, Narbad A, Chen W, Zhai Q (2021). Strain-specific effects of *Bifidobacterium longum* on hypercholesterolemic rats and potential mechanisms. *Int. J. Mol. Sci.* 2021, 22, 1305.
II965.1-CBI.2.1-A8	Takeda T, Asaoka D, Nojiri S, Yanagisawa N, Nishizaki Y, Osada T, Koido S, Nagahara A, Katsumata N, Odamaki T, Xiao J-Z, Ohkusa T, Sato N (2022). Usefulness of *Bifidobacterium longum* BB536 in elderly individuals with chronic constipation: a randomized controlled trial. *Am J Gastroenterol*. 2023; 118:561-568.

II965.1-CBI.2.1-A9	Mitelmao FCR, Hackel K, de Cassia Bergamaschi C, Gerenutti M, Silva MT, Balcao VM, Vila MMDC (2022). The effect of probiotics on functional constipation in adults: A randomized, double-blind controlled trial. *Medicine* 2022; 101:43(e31185).
II965.1-CBI.2.1-A10	Tremblay A, Fatani A, Ford AL, Piano A, Nagulesapillai V, Auger J, MacPherson CW, Christman MC, Tompkins TA, Dahl WJ (2021). Safety and effect of a low- and high-dose multi-strain probiotic supplement on microbiota in a general adult population: a randomized, double-blind, placebo-controlled study. *Journal of Dietary Supplements,* 18:3, 227-247.
II965.1-CBI.2.1-A11	Karyana PG, Apsari NLS, Artana WD, Suarta K, Yantie PVK, Nesaa NNM, Putra GNS, Soetjiningsih (2022). The efficacy of probiotics supplementation of the lipid profiles of obese adolescents: a randomized trial. *Bali MedJ* 2022, Volume 11, Number
II965.1-CBI.2.1-A12	Esaissen E, Hjerde E, Cavanagh JP, Simonsen GS, Klingenberg C, Norwegian Study Group on Invasive Bifidobacterial Infections (2017). *Bifidobacterium* bacteremia: Clinical characteristics and a genomic approach to assess pathogenicity. *J Clin Microbiol* 55:2234-2248.
II965.1-CBI.2.1-A13	Ha GY, Yang CH, Kim H, Chong Y (1999). Case of sepsis caused by *Bifidobacterium longum. Journal of Clinical Microbiology,* Apr. 1999, p. 1227-1228.
II965.1-CBI.2.1-A14	Boyle RJ, Robins-Browne RM, Tang MLK (2006). Probiotic use in clinical practice: what are the risks? *Am J Clin Nutr* 2006; 83:1256-64.

One-hundred and twenty-nine pages have been removed in accordance with copyright laws. The removed reference citations can be found at in the attachments list after the conclusion section of the notifier response.

The following 2 attachments remain:

- Attachment II934.2-CBI.7-A4 Certificate of Analysis
- Attachment II934.2-CBI.7-A5 in-house analytical method for Viable Cell Count

Attachment
II965.1-CBI.2.1-A4

CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium longum***

Place of Production: KOREA

Batch(Lot) No. : BG7 25R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 11 Apr. 2017

Exp. Date: 10 Apr. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light yellow powder	Light yellow powder
Initial viable cell	$\geq 5.0 \times 10^{10}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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Manufacturer : 397, Aegibong-ro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea
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CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium longum***

Place of Production: KOREA

Batch(Lot) No. : BG7 58R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 02 Aug. 2017

Exp. Date: 01 Aug. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light yellow powder	Light yellow powder
Initial viable cell	≥ 5.0×10^{10} CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g	Passes test
L. monocytogene	Absent in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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CELL BIOTECH

Certificate of Analysis

Product Name : ***Bifidobacterium longum***

Place of Production: KOREA

Batch(Lot) No. : BG7 67R

Issued Date: 24 Oct. 2018

Net Weight : 10kg(10kg × 1ea)

Mfg. Date: 12 Sep. 2017

Exp. Date: 11 Sep. 2018

Manufacturing origin country: KOREA
Shipping Origin country: KOREA

ITEMS	SPECIFICATION	RESULTS
Appearance	Light yellow powder	Light yellow powder
Initial viable cell	$\geq 5.0 \times 10^{10}$ CFU/g	Passes test
Coliforms	Absent	Passes test
Yeast & Mold	≤ 10 CFU/g	Passes test
E. coli	Absent in 1g	Passes test
S. aureus	Absent in 1g	Passes test
Salmonella	Absent in 25g Absent	Passes test
L. monocytogene	in 10g	Passes test
Lead (Pb)	≤ 1.0 mg/kg	Passes test
Cadmium (Cd)	≤ 0.3 mg/kg	Passes test
Mercury (Hg)	≤ 0.1 mg/kg	Passes test
Arsenic (As)	≤ 0.1 mg/kg	Passes test

Remark : *Be kept in an airtight container and stored at a temperature not exceeding 5 ℃.*

Director, Head of Quality Management Division

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Attachment
II965.1-CBI.2.1-A5

Analytical Method of Viable Cell Count

Materials :

1. The diluent (Buffered peptone water)

Composition	g/L
Peptone	10
Sodium chloride	5
Disodium phosphate	3.5
Monopotassium phosphate	1.5
Tween 80	0.5
Sterilized water	979.5
pH	6.8~7.0

* Adjust pH with 0.1N NaOH

Method:

1. Dissolve precisely 1 g of the specimen in 15 mL falcon tube filled with 9 mL of the sterilized diluent (pH: 6.8 ~ 7.0)
2. Auto-vortex for 20 min. using tube adaptor at room temperature to remove the coating materials completely. If the tube adaptor is not equipped, semiauto-vortex for 20 min. in a pattern of 2-minute-vortexing-and-3-minute-resting.

   * Vortex or vortexing of the followings means semiauto-vortex or semiauto-vortexing.
3. Prepare approx. 10 glass tubes containing 9 mL of the diluent respectively. And perform the first serial dilution with a 1 in 10 (1:9) dilution method.
4. After diluting the first glass tube, vortex 3 min. and check the bacterial cells by microscope (×1,000). If the bacteria are not released completely, repeat this procedure.
5. Vortex the first glass tube for 10 sec. and continue serial dilution with a 1 in 10 (1:9) dilution method until the expected final dilution, at which 30 colonies are formed in the final culture plate. The operation between the two tubes must be done within one minute.

Dilution factor	Vortex for
10^{-1}	20 min
10^{-2}	3 min
10^{-3}	1 min
10^{-4}	30 sec
10^{-5}~	15 sec

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6. Select the last 3 tubes and vortex one tube for 10 sec. and put 1.0 mL of the diluted solution into the sterilized culture plate (Petri-dish). Pour about 20 mL of the readymade culture media (MRS or BL) carefully into the plate, cap it with the plate cover and shake the plate smoothly (clockwise 5 times and then counterclockwise 5 times). Mark the dilution ratio on the plate cover. Perform the same procedure for the other 2 tubes.
   * MRS agar for Lactobacillus, Lactococus, Enterococcus and Streptococcus species
   * BL agar for Bifidobacterium species or for total viable cell count.
   * CBT uses MRS agar and BL agar manufactured by Difco.
7. Leave the plates at room temp. until the media become hard. And then incubate the culture plate at 37℃ for 72 hrs in an aerobic incubator (for MRS agar) or for 72 hrs in an anaerobic incubator (for BL agar).
8. Select the plate at which 30~300 colonies are formed and calculate viable cells inversely using the following formula.

Formula: Viable cells (cfu/g) = Colony number × Dilution Factor

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