10-19-11 Correspondence to Agent

FD

West-Barnette, Shayla

From: West-Barnette, Shayla
Sent: Wednesday, October 19, 2011 11:53 AM
To: 'john@aibmr.com'
Subject: Reviewer Comments for GRN 399 (subject: Bacillus coagulans strain GBI-30, 6086)

Attachments: Reviewer Comments for GRN 399_Final.pdf; B coagulans Citation.pdf

Good Morning Dr. Endres,

Per our teleconference yesterday, I am attaching to this message a list of the reviewers' comments for GRN 399, and a PDF referencing the published case report that Dr. Merker mentioned. Please note that Dr. Merker would also like information about whether *B. coagulans* strain GBI-30, 6086 contains any proteins with homology to known protein toxins--we did not discuss this issue with you yesterday. If you have questions about how to provide this information, please let us know and we will gladly provide you with guidance.

We would appreciate receiving your responses to the reviewers' questions by close of business on November 2, 2011. A signed document on company letterhead is preferred. If you will not be able to respond by this date or if you need clarification, feel free to contact me. Thank you.

Regards,

Shayla

Reviewer nments for GRN 399

B coagulans Citation.pdf (21 K...

Reviewer Comments for GRN 399_Final (Attachment to 10-19-11 Correspondence to Agent)

Reviewer Comments for GRN 399

Administrative

1) Under FDA's fortification policy (21 CFR 104.20), the addition of *B. coagulans* strain GBI-30, 6086 to alcoholic beverages, fats and oils, sugar, and sugar substitutes may raise issues. Please withdraw these intended uses. *Please note that on page 20 of the notice, sugar substitutes are listed among the intended food uses, while on page 4, sugar substitutes are not listed among the intended food uses.*

2) Given the standard of identity for milk (21 CFR Part 131), please note that the addition of *B. coagulans* strain GBI-30, 6086 to such products may present naming/labeling issues.

Chemistry

3) Please discuss whether the materials used during the manufacturing process are food grade and/or meet appropriate regulations. In particular, please discuss the components of the fermentation medium and describe the biological sources of these components.

4) Please discuss how contamination is controlled during the manufacturing process.

Microbiology

5) Please provide information about antibiotic resistance in *B. coagulans* strain GBI-30, 6086. If so, please discuss whether the resistances are transmissible to other microbes.

6) Please provide information about the sensitivity of *B. coagulans* strain GBI-30, 6086 to clinically-used antibiotics.

7) Please provide information about whether *B. coagulans* strain GBI-30, 6086 is pathogenic. We are aware of at least one published case report in which *B. coagulans* is implicated as a pathogen (please see the attached PDF). Please discuss such case reports and discuss why these reports do not raise concerns with respect to the intended uses of *B. coagulans* strain GBI-30, 6086.

8) Please provide information about whether *B. coagulans* strain GBI-30, 6086 contains proteins that have homology to known protein toxins.

9) Please discuss whether *B. coagulans* strain GBI-30, 6086 is expected to survive the intended conditions of use. For example, would the organism survive heating/cooking of baked goods, coffee and tea, pastas, soups and soup mixes? Also, please discuss whether spores are expected to be present in the foods to which the organism is added.

One page has been removed in accordance with copyright laws. The removed abstract is:

Banerjee, “Bacillus infections in patients with cancer”, Arch Intern Med. 1988 Aug;148(8):1769-74. PubMed Abstract

11-1-11 Correspondence to Agent

FD

West-Barnette, Shayla

From: West-Barnette, Shayla
Sent: Tuesday, November 01, 2011 12:04 PM
To: 'John Endres'
Subject: Teleconference and Additional Questions Regarding GRN 399

Hi Mr. Endres,

We'd be glad to talk with you. Can you send me about three possible dates that will work for you?

Also, the chemistry reviewer has additional questions, which are shown below:

Regarding the "Manufacturing and Production" section found on page 6 of the notice:
(1) Please state the identity of the raw materials used during processing (where appropriate)
(2) Please discuss the growth conditions of the culture media during fermentation
(3) Please discuss how fermentation conditions are controlled (e.g. time, pH, temp, etc.), and what measures are used to ensure contamination is controlled.

Shayla

From: johnaibmr@gmail.com [mailto:johnaibmr@gmail.com] **On Behalf Of** John Endres
Sent: Tuesday, November 01, 2011 11:45 AM
To: West-Barnette, Shayla
Subject: GRN 399

Dear Shayla,

Would it be possible to have a very brief discussion by phone with you and Dr. Merker regarding our responses before we submit them? Bob said I needed to organize that with you.

Kind Regards,
John

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We make them better

John R. Endres, ND
Chief Scientific Officer
4117 S. Meridian
Puyallup, WA 98373
Ph. (253) 286-2888
Fx. (253) 286-2451
john@aibmr.com
http://www.aibmr.com

11-16-11 Correspondence from Agent
15

West-Barnette, Shayla

From: johnaibmr@gmail.com on behalf of John Endres [john@aibmr.com]
Sent: Tuesday, November 15, 2011 7:20 PM
To: West-Barnette, Shayla
Subject: GRN 399: Response to FDA questions
Attachments: GANEDEN-Response to FDA GRAS questions 15Nov2011.pdf

Dear Dr. West-Barnette,

Please find attached our response to your questions regarding GRN #399. Please confirm receipt at your convenience.

Kind Regards,
John

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John R. Endres, ND
Chief Scientific Officer
4117 S. Meridian
Puyallup, WA 98373
Ph. (253) 286-2888
Fx. (253) 286-2451
john@aibmr.com
http://www.aibmr.com

GANEDEN - Response to FDA Questions 15Nov2011 (Attachment to 11-[redacted]-41 Correspondence from Agent)

15

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Shayla West-Barnette, Ph.D.
Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Food and Drug Administration
(240) 402-1262
Shayla.WestBarnette@fda.hhs.gov

15 November 2011

Re: GRN #399

Response to questions from the preliminary FDA review (questions received October 19, 2011).

Administrative

1) Under FDA's fortification policy (21 CFR 104.20), the addition of *B. coagulans* strain GBI-30, 6086 to alcoholic beverages, fats and oils, sugar, and sugar substitutes may raise issues. Please withdraw these intended uses. *Please note that on page 20 of the notice, sugar substitutes are listed among the intended food uses, while on page 4, sugar substitutes are not listed among the intended food uses.*

Response: The notifier would like to remove the following categories of food from the list of foods in GRN 399 to which *B. coagulans* strain GBI-30, 6086 will be added: (1) Alcoholic beverages, (2) fats and oils. The notifier does intend to use the ingredient as an additive to sugar, and sugar substitutes and understands the potential labeling concerns. The final products marketed for sale to consumers will be labeled to comply with all applicable regulations. As stated in Volume 70 of the Federal Register, page 36024, and Volume 59 page 387, FDA's fortification policy is intended to provide a consistent set of guidelines to be followed when nutrients are added to foods. To preserve a balance of nutrients in the diet, manufacturers who elect to fortify foods are urged to utilize these principles. However, the fortification policy does not have the force of a regulation. The policy does not prohibit the addition of nutrients to any foods, as long as the proposed use of the additive is safe. Based upon the determination of safety, the addition of *B. coagulans* strain GBI-30, 6086 would be safe for addition to sugar and sugar substitutes as a food ingredient. The sugar and sugar substitutes to which the strain will be added will be clearly labeled and will follow all labeling requirements, and will marketed in such a way that consumers are well-aware that the product contains the ingredient.

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2) Given the standard of identity for milk (21 CFR Part 131), please note that the addition of *B. coagulans* strain GBI-30, 6086 to such products may present naming/labeling issues.

Response: The notifier understands that the addition of *B. coagulans* strain GBI-30, 6086 to such products may present naming/labeling issues. Food products containing *B. coagulans* strain GBI-30, 6086 will be labeled in such a way that they comply with all required labeling regulations.

Chemistry

3) Please discuss whether the materials used during the manufacturing process are food grade and/or meet appropriate regulations. In particular, please discuss the components of the fermentation medium and describe the biological sources of these components. Additional question from FDA on November 3rd: Please state the identity of the raw materials used during processing where appropriate, and if any are potential allergens.

Response: All materials used during the manufacturing process of *B. coagulans* strain GBI-30, 6086 are food grade and meet appropriate regulations. They are commercially available and are purchased from approved vendors. The fermentation medium contains ingredients that are commonly used for such purposes. Two of the ingredients used in manufacturing may have allergenic potential for certain individuals. One contains soy and the other contains milk. The finished products will be labeled to comply with the appropriate regulations.

4) Please discuss how contamination is controlled during the manufacturing process. Additional questions from FDA on November 3rd, 2011: Please discuss the growth conditions of the culture media during fermentation. Please discuss how fermentation conditions are controlled (e.g. time, pH, temperature, etc), and what measures are used to ensure contamination is controlled (post fermentation as well).

Response: Careful steps are taken throughout the manufacturing process to control for contamination. Periodical testing of raw materials (including water) is performed to ensure that they meet specifications for contaminants. Heat sterilization of all ingredients is done prior to inoculation of the fermentation tanks with *B. coagulans* strain GBI-30, 6086. The tanks and culture media are completely sterilized (121°–130°C for 30 minutes) prior to inoculation. Culture media, and culture media with inoculum samples are taken, incubated and monitored for growth. If any deviation from strict standard operating procedures occurs, the tanks would be closely monitored for contaminants. The cultures are grown under completely aseptic conditions. The pH is controlled with ammonium hydroxide or sodium hydroxide. The temperature is controlled

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automatically using a jacketed water system at a range of 37°–45°C. All steps in the process are documented and records are maintained and reviewed.

Most importantly, each batch of the finished product is tested for microbiological and heavy metal contaminants, as listed in the specifications section of the notification, prior to release of the batch. The contaminants tested for include: yeast, total coliforms, *E. coli, Staphylococci, Salmonella, P. aeruginosa,* arsenic, cadmium, lead and mercury. The product batch is not released until the sample passes specifications and a certificate of analysis is issued. If a batch of *B. coagulans* strain GBI-30 were to not meet the specifications, the entire batch would be rejected according to cGMP and internal quality control standard operating procedures.

Microbiology

5) Please provide information about antibiotic resistance in *B. coagulans* strain GBI-30, 6086. If so, please discuss whether the resistances are transmissible to other microbes.

6) Please provide information about the sensitivity of *B. coagulans* strain GBI-30, 6086 to clinically-used antibiotics.

Response: The responses to questions #5 and #6 have been combined due to their relatedness. *B. coagulans* strain GBI-30, 6086 was tested for sensitivity and resistance to various antibiotics by Covance laboratories. The results indicate that the strain is sensitive to many common clinically-used antibiotics, and the results are shown in the table below. There is nothing in the literature to suggest that *B. coagulans* can transmit antibiotic resistance to other bacterial species.

Antibiotic	MIC (mg/L)
Ampicillin	0.125
Chloramphenicol	0.250
Ciprofloxacin	0.030
Clindamycin	0.125
Erythromycin	0.125
Gentamicin	0.031
Kanamycin	Resistant at highest concentration
Linezolid	0.060
Neomycin	2.000
Rifampicin	0.016
Streptomycin	Resistant at highest concentration

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Tetracycline	0.250
Trimethoprim	0.063
Vancomycin	0.063
Virginiamycin	0.016

7) Please provide information about whether *B. coagulans* strain GBI-30, 6086 is pathogenic. We are aware of at least one published case report in which *B. coagulans* is implicated as a pathogen. Please discuss such case reports and discuss why these reports do not raise concerns with respect to the intended uses of *B. coagulans* strain GBI-30, 6086.

8) Please provide information about whether *B. coagulans* strain GBI-30, 6086 contains proteins that have homology to known protein toxins.

Response: The answers to questions #7 and #8 have been combined due to their relatedness.

B. coagulans strain GBI-30, 6086 does not contain homology to any known protein toxins. Brad Goodner PhD, Professor of Biology at Hiram College, used PCR amplification to look for any evidence of genes related to haemolysin BL and non-haemolytic enterotoxin in the Ganeden *B. coagulans* strain GBI-30 (unpublished data). Haemolysin BL and non-haemolytic enterotoxin are known toxins found in *pathogenic* strains of the Bacillus genus. There was no evidence for the presence of genes encoding these toxins in *B. coagulans* strain GBI-30, 6086. Likewise, comparative genomic analyses using the publically available 36D1 *Bacillus coagulans* genome by the same professor showed no evidence of genes encoding for known virulence determinants (unpublished data).

There is a single reported case in the literature of the isolation of *Bacillus coagulans* in a hospitalized cancer patient diagnosed with bacteremia {Banerjee, 1988 #63566}. The study reports 24 cases of infection with Bacillus species in hospitalized cancer patients over an eight-year period at the University of Maryland Cancer Center in Baltimore. Specific details regarding the single patient with a *B. coagulans* infection were not well described. *B. coagulans* was responsible for only one of the 24 episodes of Bacillus bacteremias reported in the study. The patients with Bacillus infection had been diagnosed with lymphoma, leukemia, or breast cancer, and most had recently received chemotherapy. Twelve of the patients had *"definite infections"* (defined as "clinical signs of infection; at least two positive blood cultures drawn within a 24-hour period from separate sites positive for the same species, or one positive blood culture with a microbiologically documented source of infection"), while the other 12 only had *"possible infections"* (defined as "isolation of a Bacillus species from one blood culture without a microbiologically documented source of infection in a patient who is febrile"). The authors do not

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specify as to whether the *B. coagulans* episode was a "definite infection" or a "possible infection". Some of the patients had a Hickman catheter in place for more than three months. It is unclear in the report whether this was the case for the *B. coagulans* episode. Infection resulting from catheterization in a hospital setting is a common complication. None of the cases reported were suggested to be due to oral consumption of probiotic bacteria. Several patients had concomitant infections with other bacterial species.

Any bacteria could cause opportunistic infections under certain circumstances. Since this is a single case report (and the only published study we are aware of with regard to infection), and for the reporting problems discussed above, and since it concerned a hospitalized cancer patient, there is no concern for pathogenicity from oral consumption of *B. coagulans* strain GBI-30 for the general population.

Lastly, FDAs Medwatch was searched for the reporting of any serious adverse events using the search terms: *Bacillus coagulans* and *Lactobacillus sporongenes*. No FDA recalls were found or reported. Furthermore, additional searches for information available in the public domain reveal no reports of serious adverse events from oral consumption of *Bacillus coagulans* or *B. coagulans* strain GBI-30, 6086.

9) Please discuss whether *B. coagulans* strain GBI-30, 6086 is expected to survive the intended conditions of use. For example, would the organism survive heating/cooking of baked goods, coffee and tea, pastas, soups and soup mixes? Also, please discuss whether spores are expected to be present in the foods to which the organism is added.

Response: As a spore former, *B. coagulans* strain GBI-30, 6086, is able to survive more harsh environmental conditions than non-spore-forming probiotic bacteria. The raw material added to food typically, *B. coagulans* strain GBI-30, 6086, contains chiefly spores. Internal studies have shown that *B. coagulans* strain GBI-30, 6086 survives manufacturing conditions such as baking, freezing, boiling and extrusion. In addition, it has been shown to survive the acidic condition of the stomach.

John R. Endres, ND
Chief Scientific Officer

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PUYALLUP, WA 98373

(253) 286-2888 PH
(253) 286-2451

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3-6-12 Correspondence to Agent

FD

West-Barnette, Shayla

From: West-Barnette, Shayla
Sent: Tuesday, March 06, 2012 10:33 AM
To: 'John Endres'
Subject: Re: GRN 399 (subject: Bacillus coagulans strain GBI-30, 6086), Intended Use in Cough Drops

Hello Dr. Endres,

We are aware that cough drops are listed (along with hard candy) as a food category under 21 CFR 170.3(n)(25). However, given the fact that most cough drops are marketed as over-the-counter drugs, we believe it would simplify matters if cough drops were not among the intended uses for *B. coagulans* strain GBI-30, 6086.

Please consider withdrawing the intended use of *B. coagulans* strain GBI-30, 6086 in cough drops. We would appreciate receiving a written response from you regarding Ganeden's decision. Might you be able to respond within a week?

Please let me know if you have any questions.

Regards,

Shayla

3-6-12 Correspondence from Agent

West-Barnette, Shayla

From: johnaibmr@gmail.com on behalf of John Endres [john@aibmr.com]
Sent: Tuesday, March 06, 2012 12:05 PM
To: West-Barnette, Shayla
Subject: Re: GRN 399 (subject: Bacillus coagulans strain GBI-30, 6086), Intended Use in Cough Drops

Dear Dr. West-Barnette,

Thanks very much for your communication. Ganeden Biotech agrees that it would be most prudent to withdraw "cough drops" as listed under 21 CFR 170.3(n)(25) from the intended uses for *B. coagulans* strain GBI-30, 6086 per your email earlier today. We wanted to get this response to you quickly to facilitate your continued actively work on completing this GRAS review. Your efforts are most appreciated.

Kind Regards,
John

John R. Endres, ND
Chief Scientific Officer
4117 S. Meridian
Puyallup, WA 98373
Ph. (253) 286-2888
Fx. (253) 286-2451
john@aibmr.com
http://www.aibmr.com

On Tue, Mar 6, 2012 at 7:33 AM, West-Barnette, Shayla <Shayla.WestBarnette@fda.hhs.gov> wrote:

> Hello Dr. Endres,
>
> We are aware that cough drops are listed (along with hard candy) as a food category under 21 CFR 170.3(n)(25). However, given the fact that most cough drops are marketed as over-the-counter drugs, we believe it would simplify matters if cough drops were not among the intended uses for *B. coagulans* strain GBI-30, 6086.
>
> Please consider withdrawing the intended use of *B. coagulans* strain GBI-30, 6086 in cough drops. We would appreciate receiving a written response from you regarding Ganeden's decision. Might you be able to respond within a week?
>
> Please let me know if you have any questions.
>
> Regards,

Shayla

3-26-12 Correspondence to Agent

FD

West-Barnette, Shayla

From: West-Barnette, Shayla
Sent: Monday, March 26, 2012 3:00 PM
To: 'John Endres'
Subject: Questions Regarding GRN 399 (Subject: Bacillus coagulans strain GBI-30, 6086)

Hello Dr. Endres,

As we discussed in our telephone communication a few moments ago, the review team for GRN 399 has the following additional questions:

1) In the method of manufacture for *B. coagulans* strain GBI-30, 6086, Ganeden describes the fermentation of the strain. In the amendment dated November 15, 2011, the notifier states that the raw material added to food contains chiefly spores. Please discuss how the *B. coagulans* strain GBI-30, 6086 culture begins with vegetative cells and ends with a composition that is mainly comprised of spores.

2) On page 21 of the notice, Ganeden states that *B. coagulans* strain GBI-30, 6086 is currently added to foods at levels of 10^8 to 2×10^9 cfu/serv. Please describe which foods *B. coagulans* strain GBI-30, 6086 is currently added to, whether these foods are included in the intended uses described in the notice, and whether this affects the estimated daily intake of *B. coagulans* strain GBI-30, 6086.

We would appreciate your responses to these questions. If you need clarification or if I may be of further assistance, please feel free to contact me.

Regards,

Shayla

3-27-12 Correspondence from Agent

M

West-Barnette, Shayla

From: johnaibmr@gmail.com on behalf of John Endres [john@aibmr.com]
Sent: Tuesday, March 27, 2012 4:31 PM
To: West-Barnette, Shayla
Subject: GRN #399: Ganeden Bacillus coagulans: Response to 2 questions from 3/26/2012
Attachments: GANEDEN-Response to FDA GRAS questions 27Mar2012.pdf

Dear Dr. West-Barnette:

Please find attached as a pdf document our response to the questions you sent by email and that we discussed by phone yesterday, 3/26/2012. We wanted to get this response to you as quickly as possible in order to facilitate the completion of your review of GRN #399

Kind Regards,
John

John R. Endres, ND
Chief Scientific Officer
4117 S. Meridian
Puyallup, WA 98373
Ph. (253) 286-2888
Fx. (253) 286-2451
john@albmr.com
http://www.albmr.com

GANEDEN - Response to FDA GRAS questions 27Mar2012 (Attachment to 3-27-12 Correspondence from Agent)

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Shayla West-Barnette, Ph.D.
Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Food and Drug Administration
(240) 402-1262
Shayla.WestBarnette@fda.hhs.gov

27 March 2012

Re: GRN #399

Response to questions from the secondary FDA review (questions received March 26, 2012).

FDA Question from 3.26.2012:

1) In the method of manufacture for *B. coagulans* strain GBI-30, 6086, Ganeden describes the fermentation of the strain. In the amendment dated November 15, 2011, the notifier states that the raw material added to food contains chiefly spores. Please discuss how the *B. coagulans* strain GBI-30, 6086 culture begins with vegetative cells and ends with a composition that is mainly comprised of spores.

From the amendment dated 11.15.2012 regarding this question:

> 9) Please discuss whether *B. coagulans* strain GBI-30, 6086 is expected to survive the intended conditions of use. For example, would the organism survive heating/cooking of baked goods, coffee and tea, pastas, soups and soup mixes? Also, please discuss whether spores are expected to be present in the foods to which the organism is added.
>
> **Response**: As a spore former, *B. coagulans* strain GBI-30, 6086, is able to survive more harsh environmental conditions than non-spore-forming probiotic bacteria. The raw material added to food typically, *B. coagulans* strain GBI-30, 6086, contains chiefly spores. Internal studies have shown that B. coagulans strain GBI-30, 6086 survives manufacturing conditions such as baking, freezing, boiling and extrusion. In addition, it has been shown to survive the acidic condition of the stomach.

Response to question 1 from 3.26.2012:

The manufacturing flowchart on page 6 of the FDA GRAS Notification dated 8/11/2011 (GRN #399) outlines the major steps in the production of *Bacillus coagulans* GBI-30, 6086. The end step under the section labeled as *Growth of Culture under Controlled Conditions* is one where the flow of nutrients is discontinued. Vegetative cells of spore forming bacteria such as *Bacillus coagulans* GBI-30, 6086 form spores when the nutrients necessary for growth are restricted. The concept of starvation and the

4117 South Meridian
Puyallup, WA 98373

(253) 286-2888 ph
(253) 286-2451

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formation of spores for bacteria like *Bacillus coagulans* GBI-30, 6086 is well established in the field of microbiology.

FDA Question from 3.26.2012:

2) On page 21 of the notice, Ganeden states that *B. coagulans* strain GBI-30, 6086 is currently added to foods at levels of 10^8 to 2 x 10^9 cfu/serv. Please describe which foods *B. coagulans* strain GBI-30, 6086 is currently added to, whether these foods are included in the intended uses described in the notice, and whether this affects the estimated daily intake of *B. coagulans* strain GBI-30, 6086.

Response to question 2 from 3.26.2012:

All of the foods to which *Bacillus coagulans* GBI-30, 6086 is currently added are contained in the list on page 20 of the FDA GRAS Notification dated 8/11/2012 (GRN #399) derived from 21CFR§170.3(n). This does not affect the estimated daily intake of *Bacillus coagulans* GBI-30, 6086 as it is described on page 21 of the notification (*see below*)

> *Bacillus coagulans* GBI-30, 6086 is currently added to foods at a level of 100 X 10^6 to 2 x 10^9 CFUs per serving. The acceptable daily intake (ADI) concluded from the GRAS self-declaration is 93.8 x 10^9 CFUs (the derivation of this number can be found in the 2011 publication of the one-year repeated dose toxicological study by *Endres and colleagues*). At the current and intended addition level per serving to foods, between 46.9 and 938 servings of food per day would have to be consumed to exceed the *Bacillus coagulans* GBI-30, 6086 ADI.
>
> According to the *USDA Nutrition Insights*, a publication of the USDA Center for Nutrition Policy and Promotion, October 2000,[10] males aged 51 or older consume the greatest servings of food per day. They consume 18.2 servings of food per day from the following categories: grains, fruits, vegetables, milk, meat and other (fats, oils, sweets). Therefore, even if *Bacillus coagulans* GBI-30, 6086 were added to every category of food outlined above, at current and intended addition levels, the ADI would not be exceeded. Therefore, based upon the greatest estimate of servings of food consumed per day in the US and the higher addition level of *Bacillus coagulans* GBI-30, 6086 per serving, the maximum estimated daily intake (EDI) is 36.4 x 10^9 CFUs per day, which is significantly less than the ADI derived from the NOAEL from the 1-year chronic reproduction toxicology study described above.

To reiterate, the maximum EDI (described above) is 36.4 x 10^9 CFUs per day, whereas the ADI concluded from the GRAS self-declaration is 93.8 x 10^9 CFUs resulting in a large margin of safety.

John R. Endres, ND
Chief Scientific Officer

4117 South Meridian
Puyallup, WA 98373

(253) 286-2888 ph
(253) 286-2451

www.aibmr.com

4-12-12 Corespondence to Agent

FD

West-Barnette, Shayla

From: West-Barnette, Shayla
Sent: Thursday, April 12, 2012 4:03 PM
To: 'John Endres'
Subject: Reviewer Questions for GRN 399 (Subject: B. coagulans strain GBI-30, 6086)

Attachments: Reviewer Questions for GRN 399 for 4-12-12 Teleconference Final.pdf; The Use of Bacterial Spore Formers as Probiotics.pdf

Hello Dr. Endres,

Thank you for speaking with us via teleconference a few minutes ago about our review questions for GRN 399. We appreciate your willingness to address the issues that were put forth.

I have attached to this message the list of questions that were discussed during the teleconference. I have also attached a PDF copy of the 2005 article by Cutting et. al, which discusses the safety of sporeforming bacteria as food ingredients.

Please review the attached list of questions and let us know your expected timeframe for responding. Also, feel free to let me know if you need additional clarification.

Regards,

Shayla

Reviewer Questions for GRN 399...

The Use of Bacterial Spore For...

Reviewer Questions for GRN 399 for 4-12-12 Teleconference Final

Reviewer Questions/Comments for GRN 399

1) In an amendment dated November 15, 2011, Ganeden states that the raw material that will be added to food contains chiefly *B. coagulans* strain GBI-30, 6086 spores. Please provide the relative proportions of spores and vegetative cells in the *B. coagulans* strain GBI-30, 6086 food ingredient.

2) Please provide data and/or information about the persistence of *B. coagulans* strain GBI-30, 6086 in the gut. Please discuss whether the administration of *B. coagulans* strain GBI-30, 6086 spores would result in differences in persistence compared to administration of vegetative cells.

3) We are aware of published information about the use of bacterial spore formers as food ingredients (please see the attached article by Cutting et al. (2005)). Please discuss the relevance of this and other related published articles to the safety and general recognition of safety of *B. coagulans* strain GBI-30, 6086.

4) We are aware that Ganeden has marketed *B. coagulans* strain GBI-30, 6086 as a dietary supplement. Please provide information relating to the chronic use of *B. coagulans* strain GBI-30, 6086 as a dietary supplement, including serving levels and exposure levels and any reports from consumers or medical professionals on any adverse effects.

5) In its notice, Ganeden provided published human studies describing immune stimulation by *B. coagulans* strain GBI-30, 6086. Please discuss any additional information relating to any long term effects either found or anticipated from chronic immune stimulation by *B. coagulans* strain GBI-30, 6086 in humans.

6) On page 22 of its notice, Ganeden provides the GRAS conclusion reached by its GRAS panel. Please provide the entire GRAS panel report and supporting documentation that was considered by the GRAS panel.

ATTACHMENT

1) Cutting, S. et al. The use of bacterial spore formers as probiotics. *FEMS Microbiology Reviews.* 2005. 29:813-835.

-5-15-12 Correspondence from Agent

West-Barnette, Shayla

From: johnaibmr@gmail.com on behalf of John Endres [john@aibmr.com]
Sent: Tuesday, May 15, 2012 3:12 PM
To: West-Barnette, Shayla; Merker, Robert I
Subject: GANEDEN GRN #399
Attachments: Ganeden FDA Response 15May2012.pdf; Adami (1999) 10071861.pdf; Cutting (2011) 21315976.pdf; Endres (2009) 19248815.pdf; Endres (2011) 21338652.pdf; Honda 2011.pdf; Hong (2005) 16102604.pdf; Hyronimus (2000) 11078170.pdf

HI Dr. West-Barnette and Dr. Merker:

Please find attached our response to the most recent questions from FDA for GRN #399 (received 4/12/2012. Please also find attached copies of the references as pdf's for your convenience.

Kind Regards,
John

We don't make natural products
We make them better

John R. Endres, ND
Chief Scientific Officer
4117 S. Meridian
Puyallup, WA 98373
Ph. (253) 286-2888
Fx. (253) 286-2451
john@aibmr.com
http://www.aibmr.com

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Ganeden FDA Response 15 May 2012 (Attachment to 5-16-12 Correspondence from Agent)

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Shayla West-Barnette, Ph.D.
Consumer Safety Officer
Center for Food Safety and Applied Nutrition
Food and Drug Administration
(240) 402-1262
Shayla.WestBarnette@fda.hhs.gov

15 May 2012

Re: GRN #399

Response to questions from the third FDA review (questions received April 12, 2012).

(1) **Question:** In an amendment dated November 15, 2011, Ganeden states that the raw material that will be added to food contains chiefly *B. coagulans* strain GBI-30, 6086 spores. Please provide the relative proportions of spores and vegetative cells in the *B. coagulans* strain GBI-30, 6086 food ingredient.

Response: The last four batches of Ganeden *B. coagulans* strain GBI-30, 6086 produced in 2012 were analyzed for the relative proportions of spores and vegetative cells. The results below indicate that the finished product is virtually 100% spores and that this is very consistent from batch to batch.

***B. coagulans* strain GBI-30, 6086**			
Batch	Vegetative cells (CFU/g)	Spores (CFU/g)	% Spores
A	4,000	17,400,000,000	99.999977%
B	10,200	15,000,000,000	99.999932%
C	15,300	20,000,000,000	99.999924%
D	2,850	19,000,000,000	99.999985%

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(253) 286-2451

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(2) Question: Please provide data and/or information about the persistence of *B. coagulans* strain GBI-30, 6086 in the gut. Please discuss whether the administration of *B. coagulans* strain GBI-30, 6086 spores would result in differences in persistence compared to administration of vegetative cells.

Response: *B. coagulans* strain GBI-30, 6086 is consumed as an ingredient in food as virtually 100% spores. Vegetative cells of *B. coagulans* were shown by Hyronimus, et al. to be very sensitive to low pH with no vegetative cells surviving either a 2.5 pH or 3.0 pH environment in vitro. While vegetative cells of *B. coagulans* were found to be resistant to oxgall bile by the same authors, it is expected that none of the vegetative cells in *B. coagulans* strain GBI-30, 6086 would survive exposure to the acidic environment of the stomach (Hyronimus et al. 2000).

Honda, et al. demonstrated in a continuous culture fermentation system (mimicking the condition of the ascending colon in humans) that *B. coagulans* strain GBI-30, 6086 spores are able to germinate. One female and three male donors 25–39 years of age who had not taken prebiotics, probiotics or antibiotics provided fecal samples for the study. Two continuous culture systems were run in parallel for each donor. After inoculation with the fecal matter from the donors and a 12 h culture period, the medium feed was started at a rate of 10 mLh^{-1}. At 108 h, and every 24 h for four consecutive days, *B. coagulans* strain GBI-30, 6086 was added to the probiotic treated vessel. Samples from both vessels were collected at 192 h, 204 h, 228 h, 252 h following cessation of the addition of *B. coagulans* strain GBI-30, 6086 at 180 hrs. 16S rRNA analysis confirmed that the major morphotype of the *B. coagulans* strain GBI-30, 6086 treated systems was most closely related to *B. coagulans* (GenBank accession AB271752). The authors suggest that the increase in the colony counts indicates that *B. coagulans* strain GBI-30, 6086 was able to germinate in this model and persist for 72 hrs after cessation of the addition of *B. coagulans* strain GBI-30, 6086 to this continuous culture system (Honda et al. 2011).

B. coagulans is not known to adhere to or colonize the gastrointestinal tract (GIT). Adami, et al. conducted a study to investigate changes in certain bacterial groups in piglet feces. The study consisted of three groups of piglets (n=93) that received either a diet with no additives, a diet supplemented with *B. coagulans*, or a diet supplemented with an antibiotic—each from birth. With respect to the diet supplemented with *B. coagulans*, the authors concluded that *B. coagulans* is transient and does not adhere to the intestinal epithelium. The feces of the probiotic treated group were found to be free of *B. coagulans* one week following discontinuation of administration of the probiotic (Adami et al. 1999).

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In conclusion, the two studies presented above demonstrate that *B. coagulans* strain GBI-30, 6086 does germinate in a continuous culture fermentation system (mimicking the condition of the ascending colon in humans) and is found in this model to survive for 72 h following cessation of the addition of this particular strain of *B. coagulans*. The piglet study by Adami, et al. demonstrated in a relevant in vivo animal model that *B. coagulans* transiently occupies the gastrointestinal tract for no more than seven days following discontinuation of supplementation. While it is not exactly known how long *B. coagulans* strain GBI-30, 6086 occupies the GIT in humans after discontinuation of its consumption, the piglet model suggests it is transient and is likely less than seven days.

(3) **Question:** We are aware of published information about the use of bacterial spore formers as food ingredients (please see the attached article by Cutting et al. (2005)). Please discuss the relevance of this and other related published articles to the safety and general recognition of safety of *B. coagulans* strain GBI-30, 6086.

Response: The focus of this response will be to address the safety of specifically *B. coagulans* strain GBI-30, 6086 as an ingredient added to food. *B. coagulans* strain GBI-30, 6086 has been the subject of a thorough safety assessment that appear in the public domain as two publications (Endres et al. 2009; Endres et al. 2011). Further and more relevant evidence for the safe consumption of *B. coagulans* strain GBI-30, 6086 is evidenced from a long history of human exposure. More than one billion servings of *B. coagulans* strain GBI-30, 6086 have been consumed over the course of nine years (2003–present). To date, there have been no reportable adverse events.

While we very much respect the question regarding the general safety of spore-formers as ingredients in foods, due to significant differences between these various organism, it is our opinion that a broad discussion of the safe use of these other bacteria is somewhat irrelevant to the safe use of *B. coagulans* strain GBI-30, 6086 that is the subject of this GRAS notification because the data supporting the safe use of this particular ingredient is very strong. Previous to these responses the number of servings sold and the period of time over which the servings were sold had not been presented.

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However, Cutting (2011) and Hong, et al. (2005) do point to the safe use of *B. coagulans* and other Bacillus species commonly used in probiotic supplements or as ingredients in food as all "appear to show no indicators of adverse effects". Hong, et al. also suggest that every product must be investigated on a case by case basis, which is one of the primary reasons that we present the data that pertains specifically to *B. coagulans* strain GBI-30, 6086 consumption (Hong et al. 2005; Cutting 2011).

(4) Question: We are aware that Ganeden has marketed *B. coagulans* strain GBI-30, 6086 as a dietary supplement. Please provide information relating to the chronic use of *B. coagulans* strain GBI-30, 6086 as a dietary supplement, including serving levels and exposure levels and any reports from consumers or medical professionals on any adverse effects.

Response: Ganeden Biotech has sold more than one billion servings of *B. coagulans* strain GBI-30, 6086 since 2003 as either dietary supplements or as an ingredient in conventional foods. To date, there have no reportable adverse events. The serving size is 100 X 10^6 to 2 x 10^9 CFUs per serving.

(5) Question: In its notice, Ganeden provided published human studies describing immune stimulation by *B. coagulans* strain GBI-30, 6086. Please discuss any additional information relating to any long-term effects either found or anticipated from chronic immune stimulation by *B. coagulans* strain GBI-30, 6086 in humans.

Response: As stated above, Ganeden Biotech has sold more than one billion servings as dietary supplements and as an ingredient in food since 2003. To date, there have been no reportable adverse events. Given this safe history of human exposure for an extended period of time, we opine that there is nothing to suggest any physiologically relevant chronic perturbation to the immune system affecting human health from consumption of *B. coagulans* strain GBI-30, 6086 either as a dietary supplement or as an ingredient in food. It is our opinion that the history of safe use by humans is very good evidence of its safe use. As stated in the notification and above, *B. coagulans* strain GBI-30, 6086 has also been the subject of a thorough safety assessment using animal models of toxicology in rats for as long as a year without any signs of toxicity (either in general or with reproduction). These studies are published and have been freely available for downloading from PubMed since 2009. The corresponding author has not received any inquiries regarding the conclusions from these two studies and, thus, taken together with the history of human exposure, it is our opinion that general recognition of safety has been satisfied.

We also are aware that several commonly consumed foods, nutrients, vitamins, and minerals in the US may have an effect of immune system modulation, however, the detailed mechanisms of action with regard to chronic exposure in such a complex system

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remain yet to be elucidated. Possibly, the best evidence for safety in this respect for consumption of *B. coagulans* strain GBI-30, 6086 is the lack of any evidence to suggest such a concern and likely no more concern than other potentially immune modulating substances that are commonly and frequently consumed.

(6) **Question:** On page 22 of its notice, Ganeden provides the GRAS conclusion reached by its GRAS panel. Please provide the entire GRAS panel report and supporting documentation that was considered by the GRAS panel.

Response: There is nothing from the comprehensive safety assessment that the GRAS panel reviewed that has any bearing whatsoever on safety of the intended use of *B. coagulans* strain GBI-30, 6086 that the FDA did not see. Some proprietary manufacturing information was not included in the GRAS notification to FDA because it was considered confidential to Ganeden Biotech and they do not wish to have this information in the public domain.

Adami A and Cavazzoni V. Occurrence of selected bacterial groups in the faeces of piglets fed with Bacillus coagulans as probiotic. *J Basic Microbiol.* 1999; 39: 3-9.

Cutting SM. Bacillus probiotics. *Food Microbiol.* 2011; 28: 214-220.

Endres JR, Clewell A, et al. Safety assessment of a proprietary preparation of a novel Probiotic, Bacillus coagulans, as a food ingredient. *Food Chem Toxicol.* 2009; 47: 1231-1238.

Endres JR, Qureshi I, et al. One-year chronic oral toxicity with combined reproduction toxicity study of a novel probiotic, Bacillus coagulans, as a food ingredient. *Food Chem Toxicol.* 2011; 49: 1174-1182.

Honda H, Gibson GR, et al. Use of a continuous culture fermentation system to investigate the effect of GanedenBC30 (Bacillus coagulans GBI-30, 6086) supplementation on pathogen survival in the human gut microbiota. *Anaerobe.* 2011; 17: 36-42.

Hong HA, Duc le H, et al. The use of bacterial spore formers as probiotics. *FEMS Microbiol Rev.* 2005; 29: 813-835.

Hyronimus B, Le Marrec C, et al. Acid and bile tolerance of spore-forming lactic acid bacteria. *Int J Food Microbiol.* 2000; 61: 193-197.

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(253) 286-2451

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68 pages have been removed in accordance with copyright laws. The removed reference citations are:

Annunciata Adami “Occurrence of selected bacterial groups in the faeces of piglets fed with Bacillus coagulans as probiotic” J. Basic Microbiol. 39 (1999) 1, 3 -9

Cutting, “Bacillus probiotics”, Food Microbiology 28 (2011) 214-220

Endres, “Safety assessment of a proprietary preparation of a novel Probiotic, Bacillus coagulans, as a food ingredient”, Food and Chemical Toxicology 47 (2009) 1231-1238

Endres, “One-year chronic oral toxicity with combined reproduction toxicity study of a novel probiotic, Bacillus coagulans, as a food ingredient”, Food and Chemical Toxicology 49 (2011) 1174-1182

Honda, “Impact Of Ganedenbc" (Bacillus Coagulans Gb1-30, 6086) on Population Dynamics of the aHuman Gut Microbiota in Continuous Culture Fermentation System”, International Journal of Probiotics and Prebiotks Vol. 6, No. 1, pp. 65 -72, 2011 ISSN 1555.1431

Huynh A. Hong, “The use of bacterial spore formers as probiotics”, FEMS Microbiology Reviews 29 (2005) 813-835

B. Hyronimus, “Acid and bile tolerance of spore-forming lactic acid bacteria”, International Journal of Food Microbiology 61 (2000) 193-197