Ganeden Biotech presents

The Probiotic That Can Take It
According to the World Health Organization (WHO) probiotics are: “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” Probiotics have been seen to exhibit a variety of effects: They may be used to prevent and treat antibiotic-associated diarrhea and acute infectious diarrhea; they also may be effective in relieving symptoms of Irritable Bowel Syndrome and Inflammatory Bowel Disease.

Other documented effects include such overlapping mechanisms as the regulation of intestinal microbial homeostasis, the stimulation of local and systemic immune responses, the prevention of pathogens infecting the mucosa, the stabilization or maintenance of the gastrointestinal barrier function, the inhibition of procarcinogenic enzymatic activity, and the competition for limited nutrients.

However, probiotic effects appear to be strain specific. Regardless of the strain and its potential effect, probiotics intended for the gastro-intestinal tract must survive processing, shelf-life and gastric acidity in order to reach the intestinal tract, colonize the host epithelium, and exhibit a beneficial effect.

What Makes GanedenBC<sup>30</sup> Different?

GanedenBC<sup>30</sup> (Bacillus coagulans GBI-30, 6086) is a strain of lactic-acid producing bacteria, with self affirmed GRAS (generally recognized as safe) status, that can remain viable through processing, shelf-life and the low pH of stomach acid.

Unlike other probiotic strains used in food and beverages, GanedenBC<sup>30</sup> is a spore-forming probiotic bacterium, meaning that inside the bacterial cell is a hardened structure, or spore, which is analogous to a seed. This spore protects the cell’s genetic material from the heat and pressure of manufacturing processes, challenges of shelf life as well as the acid and bile to which it is exposed during digestive transit. The viable spore then is able to germinate and produce new vegetative cells once it is safely inside the small intestine. Other “traditional” probiotic organisms are not able to form these protective spores, making them vulnerable to heat, pressure, shelf life variables and the acid and bile challenges of the digestive system.

Survival and Germination of Spore

A recent study evaluating the survival and metabolic activity of GanedenBC<sup>30</sup> (Bacillus coagulans, GBI-30, 6086) in an in vitro gut model developed by the Dutch research company, TNO, showed conclusively that GanedenBC<sup>30</sup> survives and germinates in the small bowel, even under the least conducive circumstances. The study clearly demonstrates the potential of GanedenBC<sup>30</sup> to aid in carbohydrates and protein digestion.
Safety

- GanedenBC™ is considered safe for chronic human consumption by panel of scientists and awarded self-affirmed GRAS (Generally Recognized As Safe) status.
- Safety assessment of GanedenBC™ was reviewed by an independent panel of food safety and toxicology experts and published in Food and Chemical Toxicology.¹¹
- Bacterial reverse mutation assays showed GanedenBC™ has no mutagenic effects.
- Micronucleus assays in mice showed GanedenBC™ demonstrated no signs of toxicity.

Safety assessment of a proprietary preparation of a novel Probiotic, Bacillus coagulans, as a food ingredient

Food and Chemical Toxicology

J.R. Endres, A. Clewell, K.A. Jade, A.G. Schauss — AIBMR Life Sciences Inc., 4117 South Meridian, Puyallup WA 98373, USA, T. Farber — ToxaChemica, International, 11430 Strand Drive, Rockville MD 20852, USA, J. Hauswirth — Van Gemert and Hauswirth, LLC, 3222 Green Forest Ct., Ellicott City MD 21042, USA

Abstract

It has been demonstrated that some strains of Bacillus coagulans can survive extremes of heat, acidity of the stomach, and bile acids, to which commonly consumed probiotics are susceptible. A toxicological safety assessment was performed on a proprietary preparation of B.coagulans—GanedenBC™—a novel probiotic. Seven toxicological studies were conducted and included: in vitro bacterial reverse mutation assay; in vitro chromosomal aberration assay; micronucleus assay in mice; acute and 90 day subchronic repeated oral toxicity studies were conducted in Wistar Crl:(WI) BR rats; acute eye and skin irritation studies were conducted in rabbits.

The results of this toxicological safety assessment indicate that GanedenBC™ & B.coagulans does not demonstrate mutagenic, clastogenic, or genotoxic effects. Furthermore, the results of the acute and 90-day subchronic oral toxicity studies in rats resulted in the conclusion of a NOAEL greater than 1000 mg/kg per day. Since the concentration of the cell mass used in the 90-day study was 1.36 x 10⁹ CFUs/g, this corresponds to 95.2 x 10⁹ CFUs for a 70 kg human and since the suggested human dose is in the range of 100 x 10³ to 3 x 10⁶ CFUs, this gives a safety factor ranging from 3173 to 95,200 times. Based upon scientific procedures and supported by history of use, GanedenBC™ is considered safe for chronic human consumption.

QPS STATUS

Bacillus coagulans has been added by the European Food Safety Authority (EFSA) to their Qualified Presumption of Safety (QPS) list.⁴

Clinical Evidence

Bacillus coagulans Significantly Improved Abdominal Pain and Bloating in Patients with IBS

Postgraduate Medicine, Vol. 121, Issue 2, March 2009

Larysa Hun, MD, FAAP

Abstract

Background: Symptoms of irritable bowel syndrome (IBS) can have a profound impact on emotional health and quality of life, and current treatments are sometimes unsatisfactory for patients facing this lifelong disease. Probiotics, which can normalize gastrointestinal microflora, may alleviate symptoms of IBS.

Objective: This preliminary controlled study was conducted to evaluate the effects of the probiotic Bacillus coagulans GBI-30, 6086 on IBS symptoms.

Methods: This was a randomized, double-blind, parallel-group, placebo-controlled clinical trial involving 44 subjects who received either placebo or GBI-30, 6086 once a day for 8 weeks. Self-assessments of the severity of IBS symptoms (abdominal pain and bloating) were recorded every day for 8 weeks. Because baseline values were significantly different between the 2 study groups, within-group analysis was conducted.

Results: Improvements from baseline abdominal pain and bloating scores in the GBI-30, 6086 group were statistically significant for all 7 weekly comparisons (P < 0.01). In the placebo group, only changes in abdominal pain scores at weeks 6 and 8 achieved statistical significance (P < 0.05). No treatment-related adverse events or serious adverse events were reported during the 8-week study period.

Conclusions: Preliminary data suggest that the patented B.coagulans GBI-30, 6086 probiotic may be a safe and effective option for the relief of abdominal pain and bloating for patients with IBS. Larger, extended trials are needed to verify these results.

A Patented Strain of Bacillus coagulans Increased Immune Response to Viral Challenge

Postgraduate Medicine, Vol. 121, Issue 2, March 2009

Mira Baron, MD

Abstract

Background: Viral respiratory tract infection is the most common illness among humans. Probiotics have been known to enhance the immune system and, therefore, may represent a significant therapeutic advancement for treating viral respiratory tract infections.

Objective: A controlled study was conducted to evaluate the effects of the patented GanedenBC™ probiotic (Bacillus coagulans GBI-30, 6086, marketed as Sustenex®[Ganeden Biotech, Inc., Mayfield Heights, OH]) on the immune system when exposed to adenovirus and influenza in otherwise healthy adults.

Concluded→

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Concluded→
### Methods:
Ten healthy men and women (average age, 44 years) were instructed to consume 1 capsule of GanedenBC³⁰ with water once a day for 30 days. At baseline and after completion of the 30-day treatment, blood levels of cytokines were measured in vitro after T-cell exposure to adenovirus and influenza A. Each participant served as his/her own control with baseline blood draw.

### Results:
The use of GanedenBC³⁰ significantly increased T-cell production of TNF-α in response to adenovirus exposure (P = 0.027) and influenza A (H3N2 Texas strain) exposure (P = 0.004), but it did not have a significant effect on the response to other strains of influenza. No serious adverse events were reported throughout the study.

### Conclusion:
The patented GanedenBC³⁰ probiotic may be a safe and effective therapeutic option for enhancing T-cell response to certain viral respiratory tract infections.

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**Genome Sequencing**

Ganeden Biotech is currently involved in a project to fully sequence the genome of Bacillus coagulans GBI-30, 6086, the organism sold under the trademark name, GanedenBC³⁰. The sequencing was done at the University of Florida's Interdisciplinary Center for Biotechnology Research on an ABI SOLID sequencer.

The resulting genomic data is being analyzed at Hiram College's Center for Deciphering Life's Languages with the assistance of DNAStar products. The assembly of contigs is ongoing and Ganeden hopes to have a rough draft of the genome completed in the fall of 2009. When the assembly is complete, the researchers plan to publish the results and use the knowledge to better characterize opportunities and applications for GanedenBC³⁰.

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**GanedenBC³⁰— a Lactic Acid Producing Bacteria**

The role of lactic acid producing bacteria in intestinal microecology has been the subject of extensive research. Such bacteria have been proven beneficial for human health. They constitute an important element of the healthy digestive tract and are commonly found in a variety of food and nutrition products. Lactic acid producing bacteria have been shown to be effective in improving the immune system and supporting digestive health. There are two optical isomers of lactic acid, D(-) and L(+). Optical isomers share the same structure but their molecules differ in spatial arrangement. This minute difference is a determining characteristic of a molecule which has profound impact in its recognition and action. Some literature suggests that for lactic acid producing bacteria to be efficacious and safe the bacteria must produce optically pure L+ lactic acid. In fact, D- lactic acid has been shown to be harmful as a toxic bacterial metabolite. GanedenBC³⁰ produces only the L+ lactic acid isomer, making it the choice probiotic strain in terms of lactic acid production.

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**Stability During Manufacturing**

The unique, spore-forming nature of GanedenBC³⁰ allows it to survive a diverse range of food & beverage manufacturing processes. GanedenBC³⁰ is able to withstand high heat and pressure in commonly used parameters, ranging from milk pasteurization to boiling water for steeping tea; and from microwaving hot cereal to baking cookies and muffins. The same spore forming nature of GanedenBC³⁰ also enables the probiotic to survive in shelf stable products, assuring the probiotic cells are still viable at the end of shelf life. The distinctive composition of GanedenBC³⁰ provides the food industry with the opportunity to reach beyond the dairy and supplement shelves and overcome the challenges of incorporating probiotics into other food categories.

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**GanedenBC³⁰ Survivability in Various Manufacturing Applications**

<table>
<thead>
<tr>
<th>Item Tested</th>
<th>Parameters</th>
<th>AVG % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled water and steeped a tea bag containing GanedenBC³⁰</td>
<td>for 4 minutes. A cell count was performed on the hot tea.</td>
<td>84%</td>
</tr>
<tr>
<td>Added GanedenBC³⁰ to the muffin mix/batter. Baked muffins at 375°F for 30 minutes.</td>
<td>A cell count was performed after cooling.</td>
<td>87%</td>
</tr>
<tr>
<td>GanedenBC³⁰ was combined with milk. It was processed at 177°F for 22 seconds, chilled and packed immediately.</td>
<td>A cell count was performed after 21 days.</td>
<td>86%</td>
</tr>
<tr>
<td>GanedenBC³⁰ was mixed with product at 185°F and run through high pressure extrusion equipment. The material was cooled to room temperature and a cell count was performed.</td>
<td></td>
<td>94%</td>
</tr>
<tr>
<td>One serving of oatmeal was mixed with GanedenBC³⁰, mixed with water and heated in a microwave for 1 minute and 30 seconds. A cell count was performed on the hot cereal.</td>
<td></td>
<td>87%</td>
</tr>
<tr>
<td>Dry granola mix was combined with GanedenBC³⁰. The dry blend was mixed with wet ingredients at 130°F and rolled into a uniform consistency before entering a cooling tunnel. A cell count was performed after product was stored for 2 weeks at room temperature.</td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>Added GanedenBC³⁰ to a batch of cookies. Baked cookies at 480°F for 5 minutes. A cell count was performed after cooling.</td>
<td></td>
<td>98%</td>
</tr>
</tbody>
</table>
Ideal Applications for GanedenBC<sup>30</sup>

GanedenBC<sup>30</sup> performs remarkably well in a variety of applications. GanedenBC<sup>30</sup> is an ideal ingredient which can be easily added to dry blended products including beverage, soup, and baking mixes, and also functions well in formed products such as granola, protein, and meal replacement bars. Stick packs containing GanedenBC<sup>30</sup> that can be mixed with a water bottle. In any application that has a mixing step, GanedenBC<sup>30</sup> can easily be added to create a functional food.

Frequently Asked Questions

What is the specific probiotic strain in GanedenBC<sup>30</sup>?

Bacillus coagulans GBI-30, 6086.

Does GanedenBC<sup>30</sup> survive stomach acids?

Due to its spore-forming nature, GanedenBC<sup>30</sup> is able to withstand the acidic environment of the stomach to arrive alive and then colonize the intestinal tract.

What are the health benefits of GanedenBC<sup>30</sup>?

In clinical studies, GanedenBC<sup>30</sup> has been shown to improve abdominal pain and bloating in IBS patients and increase immune response to viral challenges.

Is GanedenBC<sup>30</sup> Safe?

An independent panel of scientists awarded GanedenBC<sup>30</sup> self-affirmed GRAS (Generally Recognized As Safe) status after an extensive evaluation of research studies and toxicology data. Bacillus coagulans has been added by the European Food Safety Authority (EFSA) to their Qualified Presumption of Safety (QPS) list and has been used in hundreds of millions of servings of products without a single reported adverse effect.

Does GanedenBC<sup>30</sup> require any added ingredients to facilitate its growth in the intestines?

No. GanedenBC<sup>30</sup> is extremely hearty and can utilize a variety of ingested foods consumed throughout the day. The addition of prebiotics does not harm GanedenBC<sup>30</sup> in any way.

Is GanedenBC<sup>30</sup> easy to formulate with?

Yes. GanedenBC<sup>30</sup> can withstand almost any manufacturing process, including baking, boiling, freezing, drying and high pressure.

Microbiological Background

Brand Name: GanedenBC<sup>30</sup>
Genus: Bacillus
Species: B. coagulans
Strain: Bacillus coagulans GBI-30, 6086

Bacillus coagulans was first isolated and described in 1933 and was elaborated in the fifth edition of Bergey's Manual of Determinative Bacteriology. It was initially considered to be a spore-forming Lactobacillus. Since Bacillus coagulans exhibits characteristics typical of both genera Lactobacillus and Bacillus, its taxonomic position between the families Lactobacillaceae and Bacillaceae was often debated. However, in the seventh edition of Bergy's, Bacillus coagulans was finally transferred to the genus Bacillus. DNA-based technology was used in distinguishing between the two species of bacteria which are morphologically similar and possess similar physiological and biochemical characteristics.

• Bacillus coagulans is a Gram-positive spore-forming rod 0.9u by 3.0u to 5.0u in size, aerobic to microaerophilic.
• On activation of spore formation in the acidic environment of the stomach, this organism can germinate and proliferate in the intestine, producing the favored L (+) optical isomer of lactic acid.
• Bacillus coagulans provides a biotherapeutic platform for several potentially useful applications.
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