BEYOND the DAIRY CASE

New Developments in Shelf-Stable Probiotic Foods

Ganeden, Inc.
Probiotics are increasingly appearing in functional foods and beverages, giving consumers a wide variety of choices in addition to yogurt and traditional supplement formats. Probiotics provide a wide range of digestive and immune benefits, but not all probiotics are able to survive food processing and digestion in order to deliver health benefits to the consumer. For this reason, spore-forming probiotics have become of recent interest due to their high resistance to manufacturing, shelf life, and digestive challenges. One spore-forming strain in particular, GanedenBC30® (*Bacillus coagulans* GBI-30, 6086), is a documented safe FDA GRAS microorganism that has been shown in over 20 peer-reviewed clinical trials to reduce symptoms of irritable bowel syndrome, gas, and rheumatoid arthritis and bolster immune response against viral challenges. Because GanedenBC30 survives heat and pressure used in food processing, it is effective at dosages much lower than commonly used probiotics, providing a cost-effective option for formulating probiotic food products. GanedenBC30 has been formulated into over 60 foods, including shelf stable products like tea, muffins, protein powders, pasta, and granola bars. As probiotic and functional food markets continue to grow, GanedenBC30 is will be found in several new food and beverage launches, helping the benefits of probiotics to reach beyond the dairy case.
INTRODUCTION

More than ever, consumers are making food choices based on their desire to improve health and fitness. This is evidenced by the fact that the most successful new product launches of 2009 were foods positioned as healthful (Sloan, 2010), along with an increase in functional food sales despite the recession in 2009 (NBJ, 2010). Consumers want high value from the foods they eat and the food industry has responded with a plethora of functional foods. Functional foods provide health benefits beyond the normal nutritive value of the food (Clydesdale, 2004) and incorporate a wide variety of ingredients, ranging from whole grains and antioxidants to components that aid in digestive health, such as probiotics.

Within the last decade, probiotics have risen beyond specialty and niche markets to become a mainstream ingredient (Stephens, 2009). It is well documented that several probiotic strains provide digestive and immune health benefits, although consumers also purchase probiotics for overall good health (Stephens, 2009). The International Food Information Council (2007) reports that consumers are starting to grasp the relationship between probiotics and digestive health, while Sloan (2010) says consumers are more likely to relate fiber with digestive health over probiotics, indicating there is still room for growth within the probiotics category. Regardless, the probiotic market in the United States has grown at an 8.9% compound annual growth rate (CAGR) between 2003 and 2008 and is expected to grow at 6.2% CAGR through 2013 (Tallon, 2009).

Yogurt is one of the original sources of probiotics and continues to remain a popular probiotic product today, with one in five consumers purchasing yogurt enriched with prebiotics or probiotics (Sloan, 2010). In fact, 40% of all probiotic product launches from 2002 to 2008 were yogurt products, with 60% of new probiotic product launches in 2006 through 2009 being in the dairy category (Tallon, 2009). However, recent advances in the field of probiotics over the last decade have afforded the formulation of a wide variety of foods with probiotics, such as cereal, bars, and other shelf stable products.

Would you prefer to consume probiotics as a supplement or in a food as part of your normal diet?

<table>
<thead>
<tr>
<th>FOOD</th>
<th>SUPPLEMENT</th>
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<td>59%</td>
<td>40%</td>
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Source: Newhope360.com consumer survey, January 2012

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PROBIOTICS AND HEALTH

Probiotics are defined by the Food and Agriculture Organization and World Health Organization as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (2002). The probiotic concept was first introduced by Nobel Laureate Elie Metchnikoff, who hypothesized that fermented dairy products, rich in lactic acid cultures, improved longevity (Metchnikof, 1908). Since the early 20th century, hundreds of research and clinical trials have been conducted on interactions between lactic acid bacteria and the digestive tract (Kailasapathy and Chin, 2000). The most commonly studied genera of probiotics include Lactobacillus, Bifidobacterium, and Streptococcus (Sanders et al., 2007), with Lactobacillus and Bifidobacterium strains among the most common probiotics to appear in food products. However, recent research has been conducted to evaluate the use of spore-forming bacteria (e.g. Bacillus coagulans) as probiotics because of their high resistance to heat, drying, and freezing processes (Duc et al., 2004; Sanders et al., 2003). Bacillus coagulans GBI-30, 6086, sold under the brand name GanedenBC30® (Ganeden, Mayfield Heights, OH), is one such spore-former that has been documented in over 20 published clinical trials and has appeared in more than 60 probiotic food product launches.
The health benefits of probiotics often fall under digestive benefits (e.g., alleviation of irritable bowel syndrome (IBS), diarrhea, constipation, gas, or lactose intolerance) and immune health benefits, although other documented benefits include lowering cholesterol, preventing dental caries, and helping with issues such as allergies and vaginal infections (Sanders et al., 2007). In the last several years, there have been reports of yogurts made with probiotics reducing the duration of upper respiratory infections (Guillemard et al., 2010) and improving liver function (Higashikawa et al., 2010). *Bacillus coagulans* (originally classified as Lactobacillus sporogenes; De Vecchi and Drago, 2006), is one probiotic that has been the subject of recent clinical trials and has been shown to help with a variety of digestive symptoms. Meta-analyses reviewed by Doron et al. (2008) showed that of several probiotics investigated, *Bacillus coagulans* as well as Lactobacillus GG and Saccharomyces boulardii, were most effective in preventing antibiotic-associated diarrhea. Similarly, Astegiano et al. (2006) studied the effects of a probiotic mixture containing *Bacillus coagulans* on IBS symptoms and observed significant reductions in abdominal pain (62%, $P < 0.0001$), abdominal distention (58%, $P < 0.0001$), and alternation between constipation and diarrhea (62%, $P = 0.01$) as compared with baseline values.

*Bacillus coagulans* also has been reported to be helpful in the treatment of patients with diarrhea predominant IBS. Dolin (2009) reported that patients treated with GanedenBC30 (*Bacillus coagulans* GBI-30, 6086) had significantly less bowel movements per day ($P = 0.042$) as compared to the placebo group. GanedenBC30 also was evaluated as a treatment for IBS by Hun (2009) and was found to significantly improve abdominal pain and bloating scores as compared to baseline values. Kalman et al. (2009) demonstrated further digestive health benefits of GanedenBC30, reporting that the administration of *Bacillus coagulans* GBI-30, 6086 to patients with gas symptoms significantly reduced Gastrointestinal Symptom Rating Scale (GSRS) abdominal pain subscores ($P = 0.046$) and GSRS total scores ($P = 0.048$) when measured against the placebo group. This research suggests that *Bacillus coagulans* GBI-30, 6086 provides relief for discomforting symptoms in adults after meal consumption. GanedenBC30 also can provide relief for lactose intolerant consumers, as Maathuis et al. (2010) showed that *Bacillus coagulans* GBI-30, 6086 digests lactose, fructose, and milk protein in an in vitro stomach and small intestine model.

In addition to digestive health benefits, *Bacillus coagulans* also has been shown to improve immune system response. Given that the digestive and immune systems of the human body are closely linked, as 70% of the immune system is comprised of gut-associated lymphoid tissue (Vighi et al., 2008), it is no surprise that probiotics have been shown to have beneficial impact on both digestive and immune function. Baron (2009) demonstrated that *Bacillus coagulans* GBI-30, 6086 enhanced T-cell response to adenovirus and the H3N2 Texas strain of influenza A via the increase of TNF-α, suggesting that GanedenBC30 can improve immune defenses upon exposure to viruses. A second study was conducted to further investigate the effects of *Bacillus coagulans* GBI-30, 6086 on immune markers, which showed increased levels for several other cytokines including IL-6, IL-8, and INF-γ, as well as CD3CD69 cells (Kimmel et al., 2010).

Several probiotic strains provide digestive and immune health benefits, although consumers also purchase probiotics for overall good health.

![Figure 1. Credible Craving bar formulated with GanedenBC30 (*Bacillus coagulans* GBI-30, 6086).](image)
Since GanedenBC30 is effective in improving immunity against outside factors, therapeutic use of *Bacillus coagulans* GBI-30, 6086 also could benefit those with autoimmune disorders. Mandel et al. (2010) reported significant improvement in pain scale scores for rheumatoid arthritis patients after taking supplements containing *Bacillus coagulans* GBI-30, 6086 for 60 days. C-reactive protein levels, an inflammation biomarker, also were reduced as a result of *Bacillus coagulans* GBI-30, 6086 administration (Mandel et al., 2010).

Although still an ongoing subject of research, there are several mechanisms by which probiotics such as GanedenBC30 exert health benefits. Unlike other strains within the Bacillus genus, *Bacillus coagulans* produces lactic acid, specifically the preferred L (+) isomer (Losada and Olleros, 2002). Lactic acid lowers gut pH and creates an unfavorable environment for pathogenic growth. Additionally, *Bacillus coagulans* participates in competitive exclusion via the production of bacteriocin-like compounds such as coagulin (Duc et al., 2004; Hyronimus et al., 1998) and lactosporin (Riazi et al., 2009). Bacillus species are capable of immunomodulation via interaction with gut-associated lymphoid tissue (Duc et al., 2004). For some probiotics, cell wall components and metabolites participate in immune modulation. This was recently demonstrated for GanedenBC30, as Jensen et al. (2010) demonstrated the immune modulating and anti-inflammatory effects of both cell wall and metabolite fractions.

Because of all the interactions that probiotics have with the human body, it is imperative that the safety of probiotic strains intended for human consumption is well established and documented. Such safety data should prove candidate probiotic strains do not possess any toxic or pathogenic properties and do not confer antibiotic resistance to the consumer (Sanders et al., 2007). GanedenBC30 was the first strain of *Bacillus coagulans* for which safety data was published in a peer-reviewed journal (Endres et al., 2009) and was found to be generally recognized as safe (GRAS) status by an independent scientific panel (PR Newswire, 2009a). In 2012 GanedenBC30 became the first spore forming probiotic to receive a letter of no objection from the FDA after reviewing the GRAS dossier relating to the patented strain.

**PROCESSING, SHELF LIFE, AND DIGESTION OF PROBIOTICS**

In order to deliver health benefits, probiotic bacteria must overcome several challenges present in food processing. Furthermore, ensuring that probiotics remain viable throughout shelf life is a formidable challenge (Sanders et al., 2007). Although probiotics are prevalent in dairy products, many of the most commonly used strains do not have the ability to survive high heat processes used in food manufacturing and cannot survive during extended storage at room temperature.

Most probiotic foods currently on the market are dairy foods, since products like yogurt and kefir have been considered traditional probiotic carriers (Ranadheera et al., 2010). Temperatures used during the manufacture of these products are selected to allow for optimum growth of traditional yogurt cultures (e.g. 42°C), while the refrigerated storage of the finished product stops the fermentation but allows the bacteria to survive. While common strains of probiotic bacteria (e.g. lactic acid bacteria) survive mild temperatures used during fermentation, they are inactivated by greater temperatures used in food processes such as baking, pasteurization, and extrusion. Spore-forming probiotics prove advantageous for use in foods requiring harsh processing conditions because spores afford protection.
against high heat and pressure. A calcium-dipicolinic acid-peptidoglycan complex is formed during sporulation of *Bacillus coagulans* (Losada and Olleros, 2002), which provides a natural encapsulation and allows it to survive conditions that otherwise devastate the viability of non-spore-forming probiotic bacteria. Hence, *Bacillus coagulans* is stable during production and storage, ensuring survivability during shelf life at room temperature or refrigerated conditions (De Vecchi and Drago, 2006).

Many challenges against probiotic survival in foods also exist for probiotic supplements, such as high pressure and maintaining viability during shelf life. A recent report by ConsumerLab.com showed varied results of probiotic survivability among several supplements. Most products tested contained various strains of *Lactobacillus* and *Bifidobacterium* species; some products contained the amount of probiotic claimed on the label while others fell short (Consumerlab.com, 2009). Only one product, Weil Daily Digestive Support Formula (Weil Nutritionals, Vail, AZ), contained a spore-forming probiotic, *Bacillus coagulans* (Consumerlab.com, 2009). Weil Daily Digestive Formula is formulated with GanedenBC30® (*Bacillus coagulans* GBI-30, 6086) and contained the full amount of probiotic claimed on the product packaging (Consumerlab.com, 2009). Because several probiotics fail to survive shelf life in supplements, it is no surprise that there are very few shelf stable probiotic foods available on the market.

While surviving food manufacturing and shelf life are sizeable challenges for probiotics, strains must also survive against low pH, enzymes, and bile salts present in the digestive tract (Kailasapathy, 2002). The acidic conditions of the stomach inactivate several lactic acid bacteria strains. As Ding and Shah (2009) observed, there were losses of six or more logs (99.9999% loss) for ten *Lactobacillus* and *Bifidobacterium* probiotic strains subjected to a pH of 2 for 120 minutes. Although yogurt is a commonly used delivery vehicle for probiotics, similar results were reported for Dannon® Activia® yogurt subjected to pH conditions simulating digestion (ConsumerReports.org, 2006). In addition to low pH, bile salts are known to inhibit growth of Gram-positive bacteria, including *Bifidobacterium* and *Lactobacillus* species (Ding and Shah, 2009). However, *Bacillus coagulans*, unlike other commonly used probiotics, is not inactivated by bile salts (Hyronimus et al., 2000). Furthermore, *Bacillus coagulans* spores are resistant to heat, pH, and other adverse conditions (De Vecchi and Drago, 2006; Losada and Olleros, 2002), affording survivability throughout manufacturing, shelf life, and digestion of probiotic foods.

Because the most commonly used probiotic strains in the *Lactobacillus* and *Bifidobacterium* genera have limited survivability during food manufacturing, shelf life, and digestion, microencapsulation has been investigated as a means of improving stability of such probiotic strains. Microencapsulation techniques studied in the literature include spray drying, extrusion, phase separation, and electrostatic methods and use proteins, gums, starches, and other polysaccharides as encapsulants (Anal and Singh, 2007; Kailasapathy, 2002). Such methods can yield viable probiotics for survival in frozen, refrigerated, and fermented dairy products (Anal and Singh, 2007; Kailasapathy, 2002), but does not necessarily allow probiotics to survive in shelf stable items or survive

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Figure 3. Suja Sunrise Probiotic Organic Juice formulated with GanedenBC30® (*Bacillus coagulans* GBI-30, 6086).

Top 5 Food Categories of Digestive and Immune Launches in Food

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<thead>
<tr>
<th>2007</th>
<th>2008</th>
<th>2009</th>
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<td>Milk</td>
<td>Cookies</td>
<td>Tea</td>
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<td>206</td>
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Source: Consumer Insight: The Future of Functional Food and Beverages, October 2011
any manufacturing processes that utilize heat and pressure. Anal and Singh (2007) reported that no microencapsulation methods have resulted in successful shelf stable probiotics. Weinbreck et al. (2010) recently reported an unsuccessful attempt at encapsulating Lactobacillus GG with either whey protein or palm oil for addition to shelf stable infant formula powder. Other reports on microencapsulation focus on enhancing stability against digestive challenges. Ding and Shah (2009) studied the use of various gums as microencapsulants, which for most strains, resulted in a loss of 3 log CFU/mL (99.9% of cells) upon exposure to low pH and bile as compared to 6 log CFU/mL with no encapsulation. Spores of *Bacillus coagulans* are not as easily affected by low pH conditions in the stomach as are vegetative probiotic cells (Hong et al., 2005).

When using probiotics as a food ingredient, it is important to add a clinically relevant dosage that has been proven to exert health benefits (Sanders et al., 2007). Many probiotic supplements contain anywhere from 1 to 30 billion probiotic cells and often contain multiple strains (Consumerlab.com, 2009). However, not all probiotic strains provide the same health benefits and not all probiotic strains survive conditions inherent in food manufacturing (Sanders et al., 2007), placing even greater importance on the selection of viable probiotics for food formulations. Multiple peer-reviewed, clinical trials provide evidence that GanedenBC30 has a beneficial impact on both digestive and immune health. Hun (2009) showed efficacy of GanedenBC30 in alleviating IBS symptoms at a dosage of 800 million CFU/day. This dosage is much lower than other probiotic strains, which allows for small inclusion rates with no impact on sensory attributes of food products. Furthermore, only small overages are needed when using GanedenBC30 to ensure that an efficacious dose is present in the food product at the end of shelf life. Other common probiotic strains require large overages due to substantial losses throughout food processing and digestion. Formulation with small dosages that still provide health benefits helps promote cost savings for both manufacturers and consumers. Therefore GanedenBC30 is a suitable, cost-effective option for probiotic food products intended to provide digestive and immune benefits or improve overall health.

**RECENT INNOVATIONS IN PROBIOTIC FOODS**

Most probiotic foods currently available on the market are dairy foods, which is not surprising since dairy products were the first reported to have beneficial health benefits as a result of fermentation. Several brands of spoonable probiotic yogurts are commercially available, such as Activia® (Dannon Company, White Plains, NY) and Yoplus® (General Mills, Minneapolis, MN), both of which include Bifidobacterium strains. Drinkable fermented dairy beverages also are formulated with probiotics, including Yakult (Yakult USA, Torrance, CA), which is formulated with Lactobacillus casei shirota. Lifeway Foods (Morton Grove, IL) has launched a more convenient probiotic dairy product in the form of kefir probiotic wellness bars. Both Attune® candy bars (Attune Foods, Mill Valley, CA) and Goodbelly® probiotic fruit drinks (NextFoods, Boulder, CO) take a step away from dairy products, but still require refrigeration. Very few shelf stable probiotic foods formulated with lactic acid probiotic bacteria are on the market, the most notable of which may be Kashi® Vive cereal (Kashi Company, La Jolla, CA) that includes a strain of Lactobacillus acidophilus.

Due to the advent of spore-forming probiotic bacteria like GanedenBC30, new probiotic shelf stable food products are being developed in convenient, portable formats that do not require refrigeration. PC Brands (Solana Beach, CA)
launched the first granola bar formulated with GanedenBC30 (Figure 1), being among the first products to truly bring portability and convenience to probiotic products. In 2009, Heartland Sweeteners (Carmel, IN) introduced Nevella® with probiotics, a sucralose-based sweetener formulated with GanedenBC30 (Figure 2). Nevella provides consumers with a probiotic food ingredient to use as a sugar substitute for many purposes such as sweetening coffee and baking. Because GanedenBC30 withstands the high temperatures of both baking and boiling water used to make tea and coffee, recent probiotic food offerings have expanded to include Isabella’s® Healthy Bakery Activate Muffins (Cuyahoga Falls, OH) and Bigelow® Lemon Ginger Herb Plus tea (R.C. Bigelow, Fairfield, CT) (Figures 3 and 4, respectively).

While perhaps the most notable advantage of GanedenBC30 is its ability to be used in shelf stable products, it also is suitable for inclusion in traditional dairy product formulations like Yovation® probiotic frozen yogurt line (Pierre’s Ice Cream Company, Cleveland, OH) (Figure 5). One of the most recently launched products formulated with GanedenBC30 is fluid milk produced by Guernsey Farms Dairy (Northville, MI) and enriched with the probiotic (PR Newswire, 2010). However, it is the spore-forming property and unique survivability of GanedenBC30 that has allowed its addition into multiple shelf stable foods including pasta (Racconto® Essentials Digestive Health Pasta, Melrose Park, IL) and oatmeal (PC Brands, Solana Beach, CA). GanedenBC30 has extended into the food service industry, as Red Mango frozen yogurt stores (Dallas, TX) recently developed a line of probiotic iced teas containing Bacillus coagulans GBI-30, 6086 and has incorporated the same probiotic into their frozen yogurt products (PR Newswire, 2009b).

CONCLUSIONS

Probiotics have been studied in numerous clinical research trials and are proven to benefit digestive and immune health. However, probiotics must surmount key challenges, including manufacturing processes, shelf life, and digestion, in order to provide health benefits. GanedenBC30 (Bacillus coagulans GBI-30, 6086) is a spore-forming probiotic that survives against heat and pressure used in food processing, remains viable throughout shelf life at room temperature and refrigerated conditions, and survives digestive challenges without need for microencapsulation. GanedenBC30 has been shown in several clinical trials to relieve symptoms of IBS, gas, and rheumatoid arthritis and provide immune benefits against viruses. While GanedenBC30 is found in dairy products, the most common vehicle for probiotics, the high viability of GanedenBC30 has allowed for formulation and launch of several shelf stable food products including granola bars, muffins, and pasta. As the probiotic food segment and functional food market continue to grow, several new foods are likely to include this probiotic in the future.
REFERENCES


