PROBIOTICS, PREBIOTICS, SYNBIOTICS A SCIENTIFIC REVIEW

W.L. Armbrister, PhD.

Probiotic Introduction

In 2017, the Probiotics market's predicted growth was 45.64 billion with a forecasted increase to 64.02 billion USD by 2022. Consumer awareness regarding the benefits of probiotics, increasing demand for animal feed enriched with nutrients, and the associated demand for probiotic dietary supplements appears to be driving the increase and forecasted increase in the Probiotics market (Newswire, 2017).

So,what is all the excitement and fuss about Probiotics? Interestingly, the probiotic concept is not new when you consider that Eile Metchnikoff, the Nobel laureate introduced the idea of administering bacteria to yield a positive health effect over a century ago to the scientific community (Metchnikoff, 1908).

What are Probiotics?

Let's take a trip through the scientific literature to answer some questions about probiotics.

Probiotic Definition

The Food and Agriculture Organization and the World Health Organization define probiotics as products containing viable microorganisms or 'live organisms' with an adequate amount of probiotic bacteria to exert a positive effect (WHO, 2006). To be functional in foods and dietary supplements, probiotics must possess the following abilities: 1. Resist the breakdown by gastric juices in the stomach and 2. Continue to grow in the presence of bile. The probiotic bacteria that meet these requirements are Gram positive and fall in two main genera, *Lactobacillus* and *Bifidobacterium* (Holzapel, Haberer et al., 1998; Klein, Pacj et al., 1998).

The International Scientific Association for Probiotics and Prebiotics (ISAPP) provide additional clarity to the definition for probiotics which includes the following key points: 1. Probiotics must be alive in the product and/dietary supplement when they are ingested. 2. The health benefits of the probiotic in the intended person/animal/host must be confirmed in a controlled study/evaluation. 3. The genus, species, and strain level of the bacteria or bacteria combination must be defined. 4. The probiotic must be safe at the level and use it is intended to be used (Gibson, Hutkins et al., 2017).

Viability (live)

With viability of the bacteria/microbe a key factor, the requirement for foods and dietary supplements must carry through until the end of shelf life. Dietary supplements have a low water activity and have shelf lives of up to two years with ambient storage conditions. Unlike dietary supplements, foods containing probiotics typically have a high water activity, often require refrigeration, and have very short shelf life (often weeks/months vs. years). The dosage level of the bacteria needs to be sufficient to deliver the intended benefit at the beginning and the end of the shelf life under ambient storage conditions (Ouwehand, 2015).

Dose

The level/dose of the bacteria/microbe is significant to the performance of the product, dependent on the population, and likely dependent on the strain and/or combination of probiotic strains. The monograph for Health Canada indicates at least 10^7 (10,000,000) colony-forming units for a general probiotic claim (Natural Health Products, 2015). A common rule of thumb for the dose is 10^9 (1,000,000,000) CFU, but the probiotic strain and/or combination of strains, product form, expected storage conditions of the product form, target population, and desired health benefits are key elements dictating the dosage (Ouwehand, 2015).

Health Benefits

Probiotic products are intended to help reduce the risk for gastrointestinal disturbances and/or assist in maintaining optimal gastrointestinal health. The majority of probiotics are marketed as foods and/or dietary supplements. Probiotics have been extensively studied and explored throughout the world; however, the effects of probiotics are species and strain specific. The numerous human and animal studies across the various species and strains of probiotics point to improvements in intestinal homeostasis, diarrhea, constipation, strengthening of the intestinal barrier integrity, and maintenance of normal intestinal pH (Kumar, Nagpal et al., 2016).

A key health benefit suggested from a meta-analysis conducted on the data of 11 clinical trials and 464 subjects with 13 treatment effects was reduced intestinal transit time in adults (ITT) with short-term (10-28 days) probiotic supplementation. The effects also are strongly impacted by the presence or absence of constipation, the age of the subject, and the specific probiotic strain (Miller & Ouwehand, 2013).

Genera/Species - Benefits

Among probiotics, the *Lactobacillius* and *Bifidobacterium* have numerous studies associated with their genera and are the most commonly studied genera. Although not as well studied and utilized, additional species/strains have been studied for their potential health benefits as probiotics (Table 1) (Fijan, 2014).

Table 1 Probiotic Species					
Probiotic Genera					
	Lactobacillus	*Bifidobacterium*	*Lactococcus*	*Enteroccus*	Others
	L. acidophilus	*B. bifidum*	*Lactococcus lactis subsp. Lactis*	*E faecalis*	
Species	*L. rhamnosus*	*B. animalis*	*Lactococcus lactis subsp. Cremoris*	*E faecium*	*Streptococcus thermophilus*
	L. casei	*B. breve*			*Propionibacterium freudenreichii*
	L. gasseri	*B. infantis*			*Pediococcus acidilactici*
	L. reuteri	*B. longum*			*Saccharomyces cerevisiae*
	L. bulgaricus	*B. lactis*			*Saccharomyces boulardii*
	L. plantarum	*B. adolascentis*			*Leuoconostoc mesenteteroides*
	L. salivarus				*E. coli strain nissle*
	L. johnsonli				
	L. fermentum				
	L. helveticus				

L. acidophilus - Benefits

The probiotic genera, *lactobacillus*, accounted for the largest probiotic market share in 2016 (Newswire, 2017). *Lactobacillus acidophilus* has been shown to have a beneficial effect on lactose digestion in the digestive tract as an adjunct for milk (Kim & Gillard, 1983). Additionally, research has shown *lactobacillus acidophilus* creates an environment in the gastrointestinal tract that defends against foodborne pathogens and spoilage organisms. An enhanced immune response of the host has been reported among the following organisms including: *B. longum, L. acidophilus, L. casei subsp. Rhamnosum,* and *Lactobacillus helveticus* (Isoluri, Rautava et al., 2002). Lower serum cholesterol levels were observed in rats fed with milk containing L. acidophilus and notably Gilliland et al showed that dietary elevation of plasma cholesterolemia in humans can be prevented through the introduction of a bile-resistant and cholesterol-assimilating strain of *L. acidophilus* (Grunewald, 1982; Gillard, Nelson et al., 1985).

B.bifidobacterium longum – Benefits

The probiotic genera, *bifidobacterium longum* has been shown along with lactobacillus acidophilus to improve the immune system when administered as a whey probiotic beverage. The findings of this research suggest that the immune system improvements are related to improvements in plasma lipid profile and the antioxidant power of the probiotics (Lollo, de Moura et al., 2013). The *Bifidobacterium longum* BB536 also has been reported to have various physiological effects including lower harmful bacteria levels and an improved gastrointestinal environment (Yaeshima, Takahashi et al., 1997; Odamaki, Sugahara et al., 2012).

PREBIOTICS

Prebiotic - Introduction

According to the Grand View Research Inc., the prebiotics market is expected to achieve USD 7.11 billion by 2024. Fiber-rich products are thriving and fueling the demand for prebiotics. The emphasis on improved health and well-being is expected to propel the demand over the next eight years. The extensively-studied prebiotic, inulin, accounted for 40% of the 2015 global prebiotics market in terms of revenue. Dietary supplements containing prebiotics are projected to drive market demand over the next several years (PR_Newswire, 2016).

Prebiotic – Overview

Prebiotics are defined as non-digestible (by the host) food ingredients having a positive or beneficial effect on the intestinal tract via their metabolism. There are three factors that each prebiotic ingredient should demonstrate to be classified as a prebiotic: 1) Resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; 2) Fermentation by the microflora of the intestines; 3) Selective stimulation and/or activity of intestinal bacteria contributing to positive health and well-being (Gibson, Probert et al., 2004).

Most of the ingredients/compounds that are accepted in industry as prebiotics are mainly carbohydrates, polysaccharides or oligosaccharides. Some of the compounds that should be considered “prebiotic” based on how they meet the prebiotic criteria include: inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), isomalto-oligosaccharides, lactulose, lactosucrose, soybean oligosaccharides, and xylo-oligosaccharides (Rastall & Maitin, 2002). Interestingly, fruits and vegetables like chicory, artichokes, garlic, onions, bananas, and leeks comprise the main sources of naturally occurring prebiotics (Macfarlane & Cummings, 1999).

Prebiotic - Benefits

Prebiotics have recently received more awareness and their potential health benefits have been noted (Fig. 1). Prebiotics can serve as protectants of probiotic cultures found in foods i.e. dietary supplement powder. Often, these probiotic cultures are supplied in a dried form (encapsulated, spray-dried, or freeze-dried). In this form, probiotic cultures require protection to prevent activation and ensure stabilization during storage, and prebiotics are well-suited for this application (Ross et al, 2005). Prebiotics also are utilized to protect different strains of Lactobacillus and Bifidobacterium from gastric and intestinal juices. Serving as the gastric armor for the probiotics, prebiotics also serve as an energy source once they reach the colon (Chen, Chen et al., 2005; Okuro, Thomazini et al., 2013).

Figure 1

Prebiotic - Inulin

Inulin is a well-studied probiotic composed of a β-2,1-linked fructosyl backbone with a terminal glucose moiety. Although inulin is present in a wide variety of plants and vegetables, commercially produced inulin is extracted from chicory roots. Because the β-2,1-bonds of the inulin are not susceptible to breakdown through hydrolysis in the gastrointestinal tract, the inulin arrives in the colon intact. Once, the inulin arrives in the colon, it is fermented and completely broken down. Inulin is classified as a dietary fiber and meets the definition of a dietary fiber in almost every country worldwide. Through the extensive study of inulin, improvement in bowel function has been well supported (Hond, B. et al., 2000; Dahl, Wright et al., 2014). Additionally, inulin has been linked to improvements in mineral absorption, gut health and the influence of appetite and energy regulation (Abrams, Hawthorne et al., 2007) (Cani, Joly et al., 2006).

Emergent Prebiotic - Iso-malto-oligosaccharide

Iso-malto-oligosaccharides are manufactured from starch which is treated enzymatically with a mixture of α-amylase, α-glucosidase, and pullulanase. The degree of polymerization (DP) of the polysaccharide for the starch is up to 5. Because of its structure, a large portion of the Iso-malto-oligosaccharide reaches the colon, and the remainder is broken down by intestinal enzymes, which results in a rise in blood glucose levels (Oku & Nakamura, 2003). Benefits shown with human clinical studies include improvements in bowel function as well as decreases in total cholesterol levels (Chen, Lu et al., 2001; Yen, Tseng et al., 2011).

SYNBIOTICS

Overview

A symbiotic product combines one or more probiotic microorganisms with a prebiotic fiber.

PROBIOTIC + PREBIOTIC = SYNBIOTIC

The benefits associated with synbiotics is the optimization of bacterial survival and persistence in the gastrointestinal tract (Furrie, Macfarlane et al., 2005). The development of appropriately selected probiotics and prebiotics may enhance the beneficial effects of both synergistically (Bielecka, Biedrzycka et al., 2002; Holzapfel & Schillinger, 2002). The concept of synbiotics offers both opportunities for optimal benefits for foods and dietary supplements as well as stability for the product (Ross, Desmond et al., 2005).

REFERENCES

Abrams, S.A., Hawthorne, K.M., Aliu, O., Hicks, P., Chen, Z. & Greiffin, I. (2007) An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. *Journal of Nutrition*, **137**, 2208-2212.

Bielecka, M., Biedrzycka, E. & Majkowska, A. (2002) Selection of probiotics and prebiotics for symbiotic and confirmation of their in vivo effectivelness. *Food Research International*, **35**(2-3), 125-131.

Cani, P.D., Joly, E., Horsmans, Y. & Delzenne, N.M. (2006) Oligofructose promotes saiety in healthy human: a pilot study. *European Journal of Clinical Nutrition*, **60**(5), 567-572.

Chen, H.L., Lu, Y.H., Lin, J.J. & Ko, L.Y. (2001) Effects of isomalto-oligosaccharides on bowel functions and indicators of nutritional status in constipated elderly men. *Journal of the American College of Nutrition*, **20**, 22-49.

Chen, K., Chen, M.J., Liu, J.R., Lin, C.W. & Chiu, H.Y. (2005) Opitimization of incorporated prebiotics as coating material for probiotic microencapsulization. *Journal of Food Science*, **70**, M260-M266.

Dahl, W.J., Wright, A.R., Specht, G.J., Christman, M., Mathews, A. & Meyer, D. (2014) Consuming foods with added oligfructose improves stool frequency; a randomized trial in healthy young adults. *Journal of Nutritional Science*, **3**, 1-8.

Fijan, S. (2014) Microorganisms with claimed probiotic properties: an overview of recent litreature. *Journal of Evironmental Research in Public Health*, 11(5), 4745-4767.

Furrie, E., Macfarlane, S., Kennedy, A., Cummings, J.H., Walsh, S.V., O'neail, D.A. & Macfarlane, G.T. (2005) Sumbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resultion of inflammation in patients with active ulcerative colitis: a randomized controlled pilot study. *Gut*, **54**(2), 242-249.

Gibson, G., Hutkins, R., Sanders, M.E., Prescott, S., Reimer, R., Salminen, S., Scott, K., Stanton, C., Swanson, K., Cani, P., Verbeke, K. & Reid, G. (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology*, **14**(6), 491-502.

Gibson, G., Probert, H., Loo, J., Rastall, R. & Roberfroid, M. (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, **17**(2), 259-275.

Gillard, S.E., Nelson, C.R. & Maxwell, C. (1985) Assimilation of cholesterol by Lactobacillus acidophilus. *Applied Environmental Microbiology*, **49**, 377-381.

Grunewald, K.K. (1982) Serum cholesterol levels in rats fed skim milk fermented by Lactobacillus adiophilus. *Journal of Food Science*, **47**, 2078-2079.

Holzapel, W.H., Haberer, P., Snel, J., Schillinger, U. & Huis in't Veld, J.H.J. (1998) Overview of gut flora and probiotics. *International Journal of Food Microbiology*, **41**, 85-101.

Holzapfel, W. & Schillinger, U. (2002) Introduction to pre- and probiotics. *Food Research International*, **35**(2-3), 109-116.

Hond, E.D., B., G. & Ghoos, Y. (2000) Effect of high performance chicory inulin on constipation. *Nutrition Research*, **20**(5), 731-736.

Isoluri, E., Rautava, S., Kalliomaki, M., Kirjavainen, P. & Salminen, S. (2002) Role of probiotics in food hypersensitivity. *Current Opinion in Allergy and Clinical Immunology*, **2**(3), 263-271.

Kim, H.S. & Gillard, S.E. (1983) Lactobacillus acidophilus as a dietary adjunct to milk to aid lactose digestion in humans. *Journal of Dairy Science*, **66**(5), 959-966.

Klein, G., Pacj, A., Bonneparte, C. & Rueter, G. (1998) Toxonomy and physiology of lactic acid baceria. *International Journal of Food Microbiology*, **41**(2), 103-125.

Kumar, M., Nagpal, R., Hemalatha, R., Yadav, H. & Marotta, F. (2016). *Chapter 6 - Probiotics and Prebiotics for Promoting Health: Through Gut Microbiota A2 - Watson, Ronald Ross.* In Chapter 6 - Probiotics and Prebiotics for Promoting Health: Through Gut Microbiota A2 - Watson, Ronald Ross Ed Preedy, V.R. pp. 75-85. Academic Press.

Lollo, P.C., de Moura, C.S., Morator, P.N., Cruz, A.G., Castro, W., Betim, C.B., Nisishima, L., Faria, J., Junior, M., Fernandes, C.O. & Amaya-Farfan, J. (2013) Probiotic yogart offers higher immune-protection that probiotic whey beverage. *Food Research International*, **54**, 118-124.

Macfarlane, G.T. & Cummings, J.H. (1999) Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? *British Medical Journal*, **318**, 999-1003.

Metchnikoff, E. (1908). *The Prologation of Life*. G.P. Putman's & Son's, New York, NY.

Miller, L.E. & Ouwehand, A.C. (2013) Probiotic supplementation decreases intestinal transit time: Meta-analysis of randomized controlled trials. *World Journal of Gastroenterology*, **19**(29), 4718-4725.

Natural Health Products. (2015) Probiotics Monograph.

Newswire, P. (2017). *Probiotics Market by application, dietary supplements, animal feed, source, form, end user and region - forcast for 2022*. In Probiotics Market by application, dietary supplements, animal feed, source, form, end user and region - forcast for 2022, New York, NY.

Odamaki, T., Sugahara, H., Yonezawa, S., Yaeshima, T., Iwatsuki, K., Tanabe, S., Tominaga, T., Togashi, H., Benno, Y. & Xioao, J.Z. (2012) Effect of the oral intake of yogart containing Bifidobacterium longum BB536 on the cell numbers of enterotoxigenic Bacteroides fragilis in microbiota. *Anaerobe*, **18**(1), 14-18.

Oku, T. & Nakamura, S. (2003) Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosyl-sucrose, and isomalto-oligosaccharide in helath human subjects. *European Journal of Clinical Nutrition*, **57**(9), 1150-1156.

Okuro, P., Thomazini, M., Balieiro, J., Liberal, R. & Favaro-Trindade, C. (2013). Co- encapsulation of Lactobacillus acidophilus with inulin or polydextrose in solid lipid microparticles provides protection and improves stability.

Ouwehand, A.C. (2015) The role of probiotics in digestive health. *Nutrition and Dietary Supplements*, **7**, 103-109.

PR_Newswire (2016). *Prebiotics market analysis by ingredient (FOS, inulin, GOS, MOS), by application (food & beverges, animal feed, and dietary supplements) and segment forcasts to 2024.* In Prebiotics market analysis by ingredient (FOS, inulin, GOS, MOS), by application (food & beverges, animal feed, and dietary supplements) and segment forcasts to 2024, New York, NY.

Rastall, R. & Maitin, V. (2002) Probiotics and symbiotics: towards the next generation. *Current Opinion in Biotechnology*, **12**, 490-496.

Ross, R.P., Desmond, C., Fitzgerald, G.F. & Stanton, C. (2005) Overcoming the technological hurdles in the development of probiotic foods. *Journal of Applied Microbiology*, **98**, 1410-1417.

WHO (2006). *Probiotics in food: health and nutritional properties and guidelines for evaluation.* In Probiotics in food: health and nutritional properties and guidelines for evaluation pp. 1-51. World Health Organization, Rome, Italy.

Yaeshima, T., Takahashi, S., Matsumoto, N., Ishibashi, N., Hayasawa, H. & Lino, H. (1997) Effect of yogart containing Bifidobacterium longum BB536 on the intestinal environment, fecal characteristics and defecation frequency. *Bioscience of Microflora, Food and Health*, **16**(2), 73-77.

Yen, C.Y., Tseng, Y.H., Kuo, Y.W., Lee, M.C. & Chen, H.L. (2011) Long-term supplementation of isomalto-oligosaccharides improves colonic microflora profile. bowel function, and blood cholesterol levels in constipated elderly people-a placebo-controlled, diet controlled trial. *Nutrition*, **27**, 445-450.