Synbiotics in Health and Disease

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Annu. Rev. Food Sci. Technol. 2011. 2:373–93

First published online as a Review in Advance on December 21, 2010

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev-food-022510-133739

1941-1413/11/0410-0373$20.00

Keywords

prebiotic, probiotic, cancer, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gut flora

Abstract

The synbiotic concept was first introduced, along with prebiotics, as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare" (Gibson & Roberfroid 1995). Since, there have been many in vitro and in vivo studies focusing on the application of prebiotics, firstly in health and gradually in disease states. Only recently have studies on synbiotics started to emerge with the main focus being on applications against disease. Here, we review the current literature, with the main focus on in vivo human studies.

INTRODUCTION

Only over the past 30 years has the intrinsic role of diet in the development of diseases such as cardiovascular disease and cancer been realized. In the same time frame, it has become apparent that the colon is one of the most metabolically active organs of the human body, harboring an extremely complex microbial ecosystem that does not only act as a barrier against infection but also plays an active role in salvaging energy from nondigestible food ingredients that human enzymes cannot affect. Studies have accumulated on the colonic microbiota, in particular with the use of newly developed molecular methodologies, and this has greatly aided the identification of targets that could improve human well being.

For the better part of the past three decades, two main avenues of gut microbiota manipulation have been used: probiotics and prebiotics. The concept of synbiotics was first defined in 1995 as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare" (Gibson & Roberfroid 1995). The definition is intrinsically bound to that of both prebiotics and probiotics.

Probiotics

The protective nature of certain microorganisms, in particular lactic acid bacteria, contained in fermented foods and drinks has a long history. Humans have been consuming live bacterial cultures for centuries in the form of fermented milk without any knowledge of the active ingredients or how they work. Probiotics are defined as "live microbial food supplements that beneficially affect the host by improving the intestinal microbial balance" (Fuller 1991).

It is difficult to identify with certainty the first time the term probiotic was used, but it is believed that one of the earliest citations was by Vergin (1954), suggesting that the intestinal microbial balance may be upset following antibiotic use and that it could be restored by a diet of probiotics, including fermented foods. The term was reintroduced in 1965 by Lilly & Stillwell who defined probiotics as "substances produced by microorganisms which promote the growth of other microorganisms," the antonym of antibiotics (Lilly & Stillwel 1965). The definition was further refined approximately 10 years later by Parker (1974), who defined probiotics as "organisms and substances which contribute to intestinal microbial balance," which is closer to the designation proposed by Fuller in 1991. In the past few years, several attempts have been made to improve on Fuller's definition of a probiotic to a more general form that includes beneficial effects of probiotic microorganisms in other sites apart from the colon, such as the urinary tract, or address the issue of adequate bacteria levels to mediate a specific health outcome. For example, Havenaar & Huis In't Veld (1992) defined probiotic as "a preparation or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host." Salminen (1996) defined it as "a live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host." A current widely accepted definition is from WHO (2002): "Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host."

The main criteria to be met by a microorganism to be characterized as probiotic are (Dunne et al. 2001, Tannock 1998):

- Although certain commercially available probiotics are not of human origin, it is believed that if a probiotic is isolated from the human gastrointestinal (GI) tract it is safer for human consumption and may be more effective within the intestinal ecosystem.
- GRAS (generally regarded as safe) status is granted by the FDA to food/food components that have been proven to be safe for human consumption through scientific procedures or through experience based on common use in food, as based on a substantial history of consumption by a significant number of individuals. Bifidobacteria and lactobacilli have a long history of safe consumption without harmful effects on human health.
- Probiotics must be capable of being prepared on a large scale and in a viable manner. It is also very important to be viable and active in the specific delivery vehicle.
- Probiotics have to be resistant to gastric acidity and bile acid toxicity. A low gastric pH is one of the primary host defense mechanisms against ingested microorganisms, including probiotics.
- Probiotics must adhere to human intestinal cells and intestinal mucins. This improves persistence and multiplication in the intestine and may promote competitive exclusion of potential pathogens from mucosal surfaces.
- Probiotics must produce antimicrobial substances against gut pathogens for the restoration of a healthy microflora composition.
- Probiotics must be safe in food and during clinical use, even in immuno-compromised individuals.
- Probiotics must have their efficacy and safety proven in randomized, double-blind placebo-controlled human studies.

The idea of introducing microbial strains that will potentially benefit the host and improve well being is now widely accepted; however, ingested microorganisms must survive host physiological barriers that common pathogens encounter. As such, they have to survive passage though the stomach and small intestine and upon reaching the small intestine and colon, they must compete with well-established commensal flora.

Prebiotics

The concept of prebiotics is much more recent and was first introduced in 1995 by Gibson & Roberfroid as an alternative approach that would overcome the survivability issues of probiotics during storage, distribution, and GI passage. Prebiotics were defined "as nondigestible dietary ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health" (Gibson & Roberfroid 1995).

Since they were first introduced, prebiotics have been heavily researched and constant attempts to redefine them include the following:

> In 2004, Gibson and coworkers proposed an updated definition that excludes nondigestibility and broadens the target organisms as the GI microflora: "A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microflora that confers benefits upon host well being and health."

In 2007, the new version of the definition focused again on the digestive ecosystem microbiota, but this time the focus was the formulation of the prebiotic ingredient to include whole foods and dietary supplements: "A prebiotic is a nonviable food component, ingredient, or supplement that

selectively modulates the microbiota of the digestive ecosystem, thus conferring benefits upon host well being and health" (Roberfroid 2007).

In 2008, a further attempt was made to exclude the nondigestibility aspect and expand the target site being defined as the microbota to include skin, oral, and vaginal prebiotics: "A prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota" (Pineiro et al. 2008).

The currently accepted definition remains closer to the 1995 concept. Mention is made on dietary prebiotics as opposed to other potential applications of vaginal or skin prebiotics, and the target organ is once again the GI microbiota: "A dietary prebiotic is a selectively fermented ingredient that results in specific changes in the composition and/or activity of the GI microbiota thus conferring benefit(s) upon host health" (Gibson et al. 2010).

Based on the current definition, criteria that have to be fulfilled for a dietary ingredient to be characterized as a prebiotic are as follows:

- Fermentability can be demonstrated in vitro in fecal batch culture experiments simulating the pH and temperature conditions of selected regions of the human colon. Substrates that stimulate bacterial growth can be further evaluated in more complex in vitro continuous culture models, established to simulate the transit of luminal contents through the proximal, transverse, and distal parts of the colon as well as varying pH and temperature conditions therein. Promising substrates should be further evaluated in double-blind, placebo-controlled randomized human studies to confirm the observed in vitro effect.
- The main attribute of a prebiotic is as a selective substrate for one or more beneficial bacteria commensal to the GI tract, which are stimulated to grow and/or are metabolically activated and consequently move the colonic microbiota of the host toward a healthier composition. In order to confirm selectivity of a prebiotic, it is of utmost importance to accurately monitor the changes in the fecal microbiota during prebiotic supplementation both in vitro and in vivo. Although both criteria are important for a dietary ingredient to be characterized as a prebiotic, selectivity is the most important and difficult to fulfill.

Nondigestibility of prebiotic ingredients has been excluded from later definitions. However, to elicit an effect on the target site, the prebiotic must be either nondigestible or partially digestible to reach targets that are lower in the GI tract.

Currently, only three dietary ingredients have achieved prebiotic status in the European Union and fulfill all the above-presented criteria: fructooligosaccharides (FOSs) (and inulin), galactooligosaccharides (GOSs), and lactulose. They have been studied extensively in vitro and in vivo in human studies, with the main drawback of this approach being selectivity. It is difficult to ensure that a nondigestible oligosaccharide will be fermented only by bacteria beneficial to the host and also that the products of fermentation will not be used to promote the growth and/or activity of potential pathogens.

SYNBIOTICS

Based on the evolution of the probiotic and prebiotic terms, a synbiotic should consist of probiotics, which are live microorganisms present in adequate amounts to confer a health benefit to the host, and prebiotics, nonviable food components, ingredients, or supplements that selectively modulate the microbiota of the digestive ecosystems, thus conferring benefits upon host well being and health as well as improving the survival and implantation of the probiotic in the GI tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria that improve host welfare.

In addition to the conditions for a product to be defined as a prebiotic and probiotic, a further condition must be fulfilled for synbiotics: The prebiotic selectively supports the growth of the probiotic component.

Since the first introduction of this concept in 1995, synbiotics have not been redefined. Although their definition has evolved as a result of the changes in the definitions of probiotics and prebiotics, a more rigorous framework is required for synbiotics as well.

The word synbiotic is derived from the Greek "συν" and "βίος," which literally translate to together and life. The use of the two words together also implies synergy.

Based on the current definition, two types of synbiotic approaches exist:

- Complementary, whereby the probiotic is chosen based on specific desired beneficial effects on the host, and the prebiotic is independently chosen to selectively increase concentrations of the beneficial microbiota components. The prebiotic may promote growth and activity of the probiotic, but only indirectly as part of its target range.
- Synergistic, whereby the probiotic is again chosen based on specific beneficial effects on the host, but the prebiotic is chosen to specifically stimulate growth and activity of the selected probiotic. Here, the prebiotic is selected to have a higher affinity for the probiotic and is chosen to improve its survival and growth in the host. It may also increase the levels of beneficial host GI microbiota, but the primary target is the ingested probiotic.

Both approaches may, directly or indirectly, comply with the synbiotic definition. However, it is the synergistic approach that is most relevant with the current synbiotic definition. The two approaches have different implications. For example, the complementary approach targets separately the host well being with a probiotic and a prebiotic. Because of this, each component must be administered in such a dose as to elicit a desirable effect via the vehicle of administration. The relatively high doses of prebiotic (commonly more than 6 g d^{-1} for adults) required to mediate an effect on the gut microflora will, in most cases, exclude the encapsulation option. With a synergistic approach, the synbiotic is perceived as a single product, whereby the primary role of the prebiotic is to improve the survivability and implantation of the probiotic. The implication is that the necessary dose of prebiotic may be limited to this effect alone, and as such a smaller dose of the probiotic is required.

By definition, synbiotics are mixtures of probiotics and prebiotics, implying that the efficacy of each component will be established for a synbiotic formulation. **Figure 1** summarizes suggested steps in establishing the efficacy of probiotic and prebiotic components and for a final synbiotic product. Briefly, each food ingredient/microorganism present in the synbiotic formula must be fully characterized using the latest available technologies. The criteria for each group will be established in conjunction with safety determinations for both the probiotic and prebiotic, as well as a combination of the two. The in vitro and in vivo efficacy will have to be examined. Specificity of the prebiotic for selective stimulation of the selected probiotic(s) should also established. Indications on the potential growth of a probiotic on a selected substrate can be obtained through a genomic scan of the glycosidase spectra of the microorganism, which may allow for a rational selection of the potential prebiotics. Growth curve experiments provide information on a prebiotic to achieve the highest growth rates and cell yields for the probiotic. However, such experiments are limited in the degree of information that they can provide, as they do not examine interactions with the commensal microbiota and cannot provide information on the affinity of other microbiota for the selected prebiotic. Further information on the behavior of the synbiotic and mechanism of its action may be provided via pH-controlled fecal batch culture experiments. This methodology allows running different combinations and controls on minimal media, with the test prebiotic as the sole growth substrate using the same fecal inoculum for all tests. The

probiotic and prebiotic components of the formulation should be tested alone, as well as alongside the synbiotic. This type of experiment provides information on whether each of the components can mediate an effect on the fecal microbiota when used alone as well as on whether the synbiotic combination can mediate a superior effect. Using molecular-based methodologies, information on the survivability of the probiotic in a mixed fecal environment over the fermentation period, as well as to the mechanism of synbiotic activity, synergistic, or complementary can be obtained. Synbiotic efficacy must then be established in vivo in double-blind, placebo-controlled randomized human studies. Ideally, a crossover design should be followed, in that volunteers crossover from each probiotic, prebiotic, and synbiotic treatment, and the efficacy on health biomarkers is followed. For human studies, the synbiotic should preferably be administered in a formulation that it is going to be marketed in, as product formulations may influence not only shelf life but also probiotic survivability and potential impacts on the host.

With prebiotics, there has been a gradual progression from in vitro to in vivo studies in healthy human volunteers, followed by testing prebiotic efficacy against diseases such as inflammatory bowel disease (IBD), colon cancer, and irritable bowel syndrome (IBS). For synbiotics, however, a small number of preliminary in vivo studies have been performed to date, and the focus has been almost exclusively on disease management. The level to which studies fulfill the synbiotic criteria varies greatly. As yet, a study has not been published that has investigated all the necessary aspects of synbiotic development.

Healthy Adult Microbiota Manipulation

Tanaka et al. (1983) investigated the effect of GOS administration in combination with *Bifidobacterium breve* 4006 in sixteen healthy adult men. The selective fermentation of GOS by bifidobacteria and the ability of *B. breve* 4006 to utilize it were established in vitro, prior to the in vivo study. Volunteers were divided into three treatment groups, each being administered 3 g d^{-1} or 10 g d^{-1} GOS alone, 3×10^9 CFU d^{-1} *B. breve* 4006 alone, or a combination of the two treatments for a two-week period. The prebiotic and synbiotic treatments both resulted in an increase in commensal bifidobacterial levels, whereas the probiotic alone did not mediate the same effect. Although this was a very early study and gut microbiota changes were evaluated using only culture techniques, the selection of probiotic and its complementary prebiotic, as well as the inclusion of the proper probiotic and prebiotic controls, allows us to draw conclusions on the mechanism of synbiotic efficacy. As such, the prebiotic appeared to enhance numbers of the probiotic and the comensal bifidobacterial populations.

In a later study, the synbiotic efficacy of yogurt supplemented with lactulose (0.5 g 100 ml^{-1}) and *Bifidobacterium longum* (10^8 CFU g^{-1}) in 10 healthy adults was investigated (Tomoda et al. 1991). The test interventions were plain yogurt, lactulose supplemented yogurt, *B. longum* yogurt, lactulose/*B. breve* yogurt. Over a three-month intervention period, volunteers ingested over three to six-week periods, with each treatment in a sequential manner. No significant differences were observed in fecal microbiota between the three active treatments. All treatments increased bifidobacterium levels compared with plain yogurt. No mention was made as to whether the probiotic was prescreened for its ability to ferment lactulose prior to the study. Although the proper controls were included in this trial, the sequential administration of the different treatments and the absence of washout periods prohibited realistic conclusions.

It was not until the late 1990s that interest in synbiotics was reignited. The majority of recent studies in healthy adults utilized commercially available prebiotic and probiotic combinations without prescreening for utilization of the prebiotic by probiotic microbes.

The effect of 5×10^9 CFU *Lactobacillus paracasei* B21060 and B21070 0.5×10^9 CFU *Lactobacillus gasseri* B21090 (Flortec, Bracco SpA) combined with inulin/oligosaccharides (dose and product not specified by the authors), ingested three times daily for 15 days, was investigated in 12 healthy adults (Morelli et al. 2003). A combination of PCR-ARDRA (amplified ribosomal DNA restriction analysis) and microbial culture techniques was applied to follow changes in *L. paracasei* and total *Lactobacillus* and *Bifidobacterium* spp. in feces. Increases in overall bifidobacteria and lactobacilli populations were observed, and the probiotic strains could be detected in volunteer feces throughout the study and three days following treatment cessation. A small number of volunteers, an absence of placebo, and lack of proper controls did not allow for solid conclusions on the mechanisms of synbiotic action. Furthermore, the authors focused solely on bifidobacteria and lactobacilli detection, and no attempt was made to evaluate the effects on other members of the fecal microbiota. In a later study, Morelli and coworkers (2006) investigated the presence of *L. paracasei* B21060 in the colonic mucosa and feces. Seven volunteers scheduled for colonoscopy were administered a combination of 5×10^9 CFU *L. paracasei* B21060, 0.5 g xylooligoasaccherides (XOS), and 3 g inulin three times daily for a period of 15 days. Two days after completing the synbiotic treatment they underwent a colonoscopy, during which biopsies from different sites were obtained. With the exception of one volunteer, *L. paracasei* could be detected in the cecum, transverse, descending, and sigmoid colon biopsies, and in 74.7% of the colonic samples. With regard to the association of *L. paracasei* to different colonic regions, the results are interesting, but the absence of a probiotic-only control fails to provide insight on any role for the prebiotic components of the treatment.

A long-term placebo-controlled crossover study investigated the effect of lactulose (10 g) and *Saccharomyces boulardii* (2×10^9 viable cells) in 30 young healthy adults (Vanhoutte et al. 2006). This was a well-designed study in which volunteers were divided into three groups, each receiving different probiotic and prebiotic doses. Each group ingested the prebiotic, probiotic, synbiotic, and placebo treatments, followed by a final washout over an 18-week study period. Unfortunately, the washout between treatments was only four days, which may not have been sufficient to ensure that there was no treatment carry over. DGGE (denaturing gradient gel electrophoresis) of 16 rRNA gene amplicons was used to detect changes in the overall composition of the fecal microbiota as well as group specific subpopulation levels. The addition of *S. boulardii* in the study diet did not appear to exert any effect, whereas lactulose alone gave a significant increase in bifidobacterial levels. One main shortcoming of this study was that *S. boulardii* was not evaluated for its ability to ferment lactulose, perhaps explaining why no change was observed.

Casiraghi et al. (2007) investigated the effect of a synbiotic milk product containing 10^7 CFU ml^{-1} *Lactobacillus acidophilus* 74-2, 10^7 CFU ml^{-1} *Bifidobacterium lactis* 420, and 2% Raftiline (inulin, DP10, Orafti) in 26 healthy adults in a randomized, placebo-controlled, two-arm parallel study. Significant increases in fecal bifidobacteria and lactobacilli were observed upon synbiotic ingestion, as evaluated using microbial culture techniques. The effect of a synbiotic on colonic nitrogen-protein metabolism was investigated in a recent study of 20 healthy humans over a 16-week period (De Preter et al. 2007). Volunteers were randomly assigned into two groups, each testing a different probiotic combination: either *Lactobacillus casei* Shirota ($2 \times 6.5 \times 10^9$ CFU d^{-1}) or *B. breve* (2×10^9 CFU d^{-1}) (Yakult). During each four-week treatment period, volunteers ingested the prebiotic (2×10 g d^{-1} FOS Synergy 1), the probiotic, or the synbiotic. Each treatment was followed by a two-week washout. Fecal and urinary excretion of ammonia and *p*-cresol were analyzed as indicators of proteolytic colonic fermentation. Synergy 1 alone mediated significant increases in fecal bifidobacteria concentrations and decreases in urinary *p*-cresol and ammonia. There was no additive effect observed with either synbiotic formulation with regard

to the proteolytic biomarkers. In a later study, the same group further investigated the effect on β-glucuronidase and β-glucosidase activities in a study of a similar design. Synergy 1 mediated a significant decrease in fecal β-glucuronidase activity, but when combined with probiotics this was not observed. Similarly, *B. breve* ingestion increased β-glucosidase activity, but this effect was not retained in the synbiotic. The results imply that there was neither synergy nor an additive effect between the probiotic and the prebiotic components of this synbiotic formulation.

Two recent studies investigated the effects on healthy elderly volunteers. Bartosch et al. (2005) investigated a synbiotic formula containing a total of approximately 3.5×10^{10} CFU *Bifidobacterium bifidum* BB-02 and 3.5×10^{10} CFU *B. lactis* BL-01(Rhodia) and 6 g Synergy 1 in a gelatin capsule in 18 healthy elderly volunteers in a double-blind, randomized, controlled study. The intervention mediated an increase in stool frequency and in fecal bifidobacteria and lactobacilli. At the end of the study, three weeks following treatment cessation, at least one of the probiotics was still detectable in feces. Ouwehand et al. (2009) investigated the effect of 2×10^9 CFU g^{-1} *L. acidophilus* NCFM (Danisco) in combination with 5 g Lactitol in a double-blind, placebo-controlled parallel study of 51 healthy elderly volunteers. They observed significant increases in fecal bifidobacteria and lactobacilli as evaluated by real time–polymerase chain reaction (RT-PCR) but no significant changes in immune markers IgA and PGE_2 compared to placebo.

Inflammatory Bowel Disease

IBD is a collective term describing three conditions: ulcerative colitis (UC), Crohn's disease (CD), and pouchitis. The disease is characterized by acute noninfectious inflammation of the intestinal mucosa and submucosa and is usually associated with diarrhea and rectal bleeding with an excess production of mucus. Geographically, there appears to be a higher incidence of ulcerative colitis in Westernized or industrialized countries (Maybery et al. 1991). The chronic nature of the disease and the need for constant medication pose a major financial burden on the health system. To date, there is no cure for IBD and treatment has been limited to maintenance of remission. Currently, drug therapy is mainly based on the administration of anti-inflammatory and immunomodulating drugs, nutritional support and in severe cases surgical resection. The disease etiology is as yet unknown. However, in germ-free rodents, intestinal inflammation cannot be induced, indicating that bacteria are necessary for the pathogenesis of chronic intestinal inflammation (Sartor 1997, Veltkamp et al. 2001). It has been suggested that IBD is at least partially due to a breakdown of tolerance to the normal commensal colonic flora (Macpherson et al. 1996) or disturbed colonic flora (Pathmakanthan et al. 1999). It is very likely that IBD is caused by a complex combination of genetics, environmental factors, and the immune system. Probiotic use against ulcerative colitis and pouchitis has been extensively investigated, and several studies report encouraging results. Thus far, single-strain bacterial products (Kuisma et al. 2003) have not been as successful as multi-strain bacterial products (Gionchetti et al. 2000, Gionchetti et al. 2003, Bibiloni et al. 2005, Mimura et al. 2004).

For synbiotics, Furrie et al. (2005) screened nineteen *Bifidobacterium* isolates, ten isolated from healthy colonic mucosae, five from healthy feces, and four obtained from culture collections, for their suitability as probiotics. The selected strains were tested for aerotolerance, acid tolerance, bile-salt resistance, adhesion to epithelial cells, and their ability to survive freeze drying and long-term storage. The ability to metabolize FOS as an energy source was also determined. Finally, the ability of the microbial strains to reduce production of proinflammatory cytokines (Interleukin 1α) was also investigated in the HT29 epithelial cell line. *B. longum* isolated from the healthy rectal mucosa was selected for further study. Eight volunteers ingested 2×10^{11} viable, freeze-dried *B. longum* in a gelatin capsule and 6 g Synergy 1(Orafti) twice daily for a four-week treatment

period. The eight volunteers in the placebo group were given identical capsules containing starch and 6 g of maltodextrose (Orafti). Sigmoidoscopy scores were determined at the start and end of treatment for both groups, and tumor necrosis factor α (TNF α) and IL 1α were monitored. TNFα, IL 1α, and antimicrobial human β defensin peptides were all significantly decreased, and mucosal bifidobacteria increased in the active group. Although this was a double-blind, placebo-controlled, randomized pilot study in a small patient group, it is one of the few studies that followed a rational procedure for probiotic selection that would complement the selected prebiotic as well as target the disease. Not only were the probiotic technological characteristics fully determined, including fermentation of the selected prebiotic, but the strain was specifically selected for its ability to downregulate in vitro production of cytokines that have been involved in the active state UC. It would have been interesting to see what effect the probiotic and prebiotic alone would have over a longer treatment period.

Animal studies on a dextran sulfate sodium (DSS)-induced colitis rat model testing the effect of administration of *Bifidobacterium infantis* DSM 15158 or *B. infantis* DSM 15159, alone or in combination with Synergy 1 (Orafti), noted a significant reduction in disease activity indices [bacterial translocation, short-chain fatty acids (SCFAs), cytokine production, myeloperoxidase, and malondialdehyde] (Osman et al. 2006). Six groups of six Sprague-Dawley rats were pretreated for seven days with either one of the test probiotic strains alone or in combination with Synergy 1. Following the treatment, DSS colitis was induced. Rats continued the synbiotic or probiotic treatments for seven further days after colitis initiation. Although all treatments mediated a significant improvement in disease activity indices, there appeared to be an additive effect when Synergy 1 was used, as it mediated an increase in succinate production. There were clear differences between the efficacies of the two probiotic strains with *B. infantis* DSM 15,159 being superior in reducing malondialdehyde levels. This was a well-designed study comparing the effect of each of the synbiotic constituents alone and in combination and investigating the effect of strain specificity against the disease.

Studies on the effect of synbiotics against Crohn's disease are sparse. Fujimori et al. (2007) examined the effect of probiotic (3×10^{11} CFU *B. breve*, 3×10^{11} CFU *L. casei*, and 1.5×10^{10} CFU *B. longum* daily) and prebiotic (3.3 g psyllium three times daily) cotherapy in an open label study of 10 active CD patients. The study duration was 10 months, and seven patients reported improved symptom scores. In contrast to the above-reviewed study, psyllium is not an established prebiotic, and no attempt was made to determine if the ability of the test probiotic strains could utilize it. The absence of a control group and the small number of participants did not allow for the proper evaluation of the efficacy of this approach and of the selected synbiotic performance. A multicenter, randomized, placebo-controlled study investigating the efficacy of Synbiotic 2000 (10^{10} CFU of each of *Pediacoccus pentoseceus*, *Leuconostoc mesenteroides*, *L. casei* spp. *paracasei* F1977:1, *Lactobacillus plantarum* 2362, and 2.5 g each of β-glucans, resistant starch, inulin, and pectin; Medipharm) in 30 ileal resection CD patients over a period of 24 months failed to note an effect on remission or disease scores compared to placebo (Chermesh et al. 2007).

Research in inflammatory bowel disease using synbiotic treatments is still in its infancy. It is evident, however, that when an informed selection of the probiotic and complementary prebiotic is made, pilot studies have been successful. Evidence from animal studies indicates that efficacy may be strain dependent. The lack of studies on the efficacy of prebiotics against IBD does not allow us to speculate as to the mechanism of action against this group of diseases. This puts even more pressure toward the selection of complementary prebiotics, where their primary goal would be to enhance the survival and/or activity of the probiotic component of the synbiotic formulation. It is difficult to rationally design a synbiotic against a disease of unknown etiology. However, in

the past decade research in this area has come a long way in identifying immune biomarkers that could be potential targets and could be considered during synbiotic development.

Irritable Bowel Syndrome

IBS is one of the most common GI disorders in primary and secondary care. It affects 9%–22% of the population in the United States and 3.5%–25% worldwide. It is estimated that only 10% of the people exhibiting IBS symptoms seek medical advice. Within this population, the ratio of females to males is 3:1 (Alaradi & Barkin 2002). Typical IBS symptoms include abdominal pain and discomfort, bloating, and changes in bowel habit. Three disease phenotypes have been identified: constipated predominant, diarrhea predominant, and alternating between the two. The disease etiology is as yet unknown, and although an involvement of the gut microbiota has long been suspected, there is no conclusive evidence. Sensorimotor dysfunction and abnormal brain-gut interaction have also been suspected without the presence of conclusive evidence, and increased stress also appears to be a factor. Some IBS patients respond very well to exclusion diets, which may indicate a degree of abnormal colonic fermentation. Although not always successful, there are recent studies reporting encouraging results on the alleviation of IBS symptoms upon the ingestion of various probiotic strains (O'Mahony et al. 2005, Kajander et al. 2005, Whorwell et al. 2006).

The efficacy of synbiotic formulations against IBS is far less well documented. To date, there are two published studies. Dughera et al. (2007) investigated the effect of a commercially available synbiotic product delivering a total daily dose of 5×10^9 CFU *B. longum* W11 and 2.5 g short chain FOS (Actilight) in an open label, prospective, uncontrolled multicenter study of 129 constipated IBS subjects. Patients were evaluated at the start of the trial, after one month, and at the end of the three-month intervention. A significant increase in stool frequency was reported as well as alleviation of pain and bloating as compared with baseline samples. However, this was an open label study in the absence of a placebo, and although the results are encouraging, further RCTs (randomized controlled trials) are required to confirm efficacy.

The task of designing a synbiotic against IBS is particularly difficult, as the disease etiology is not yet known. Improvement of IBS symptoms may be possible; however, owing to the lack of information on the colonic microbiota composition associated with this disease, informed choices on selection of potentially efficient probiotic strains and complementary prebiotics remains a difficult task.

Colon Cancer Risk

Colorectal cancer is one of the most common forms of malignancy in developed countries. Approximately 100 new cases of colorectal cancer are diagnosed daily in the United Kingdom. It is the third most common cancer, after breast and lung. The impact of colorectal cancer is even higher in terms of mortality, as it is the second most common cause of death from cancer in the United Kingdom, after lung cancer, with deaths exceeding 16,000 yearly (Cancer Research UK 2006). Although incidence is significantly lower in developing countries, worldwide it was the third most commonly diagnosed cancer and the fourth most common cause of death by cancer according to 2002 estimates (Cancer Research UK 2005). Large bowel cancer incidence is related to age with 83% of cases being diagnosed in people over 60-years-old. Lifestyle appears to have a central role in large bowel cancer risk, with diet being one of the most important factors identified to date. Bowel cancer incidence is higher in populations on Westernized diets, rich in red meat and fat. The EPIC study (European prospective investigation into cancer and nutrition)

suggested a significant increase (55%) in bowel cancer risk upon a 100 g d^{-1} increase in red and processed meat consumption, and a significant decrease with higher intake of fish (Norat et al. 2005). A lower cancer risk has been correlated with high dietary fiber intake (Park et al. 2005). Colon cancer risk increases by approximately 60% in men and 30% in women with a body mass index greater than 28.5. It is suggested that obese men have a 90% increased risk of dying from cancer, and the increased risk in obese women ranges between 23% and 37%. Central obesity has also been suggested as an indicator of colon cancer risk (Bianchini et al. 2002, Murphy et al. 2000, Moore et al. 2004). Studies indicate that fiber intake plays a role in colorectal cancer. Men and women in the highest quintile of fiber intake were at approximately 20% lower risk of bowel cancer manifestation compared to individuals in the lower quintile. More specifically, individuals with a high fiber intake were at 40% lower risk of developing left-sided colon cancer compared to those with low fiber intakes (Bingham et al. 2005). Dietary fiber increases fecal bulk and decreases transit time and as such reduces contact with fecal carcinogens.

The main body of evidence on the effect of synbiotics against colon cancer is either from studies on animal models of tumorigenesis, transgenic animals, and chemically induced models of mutagenesis, or from in vitro cell line models.

One of the first studies to investigate the potential application of synbiotics against colon cancer was by Rowland and coworkers (1998). They investigated the effect of 4×10^8 viable *B. longum* 25 cells g^{-1} diet combined with Raftiline HP at 5% (w/w) of diet in 60 three to four-weeks-of-age male Sprague-Dawley rats that were treated with azoxymethane so as to induce aberrant crypt foci (ACF) formation. The probiotic was prescreened for its in vitro ability to ferment Raftiline HP prior to the in vivo study. Rats ingested a control diet, probiotic only, prebiotic only, and the synbiotic diet. All animals ingested the control diet for a one-week period prior to azoxymethane dosing, following which, rats were divided in four groups of 15 each, ingesting one of the treatments for a two-week period. Cecal ammonia concentrations, β-glucuronidase, and β-glucosidase activities were measured along with ACF formation. *B. longum* 25 and Raftiline HP both reduced small ACF formation, cecal ammonia concentrations, and β-glucuronidase activity when tested individually compared to the control diet. When used in combination, they mediated a significantly higher inhibition of both small and large size ACF formation. Both Raftinline HP and the synbiotic caused significant increases in β-glucosidase activities; however, *B. longum* alone did not have an effect. The inclusion in this study of proper controls gives valuable insight into the behavior of this synbiotic. It is clear that the effect of the combination of the selected probiotic and prebiotic result in a superior formula that appears to be acting both synergistically (ACF inhibition) but also in an additive manner (reduced β-glucosidase production).

In a later study, using the same model of carcinogenesis, the effect of 10^{10} CFU g^{-1} *L. acidophilus* (LAFTI® L10), *B. lactis* (LAFTI B94, DSM Food Specialties) alone or in combination, and 10 g kg^{-1} diet resistant starch (Hi-maize958, National Starch) was investigated (Le Leu et al. 2005). The study end points included cecal bacteria enumeration, fecal and cecal SCFA levels, cell proliferation, and acute apoptotic response to a genotoxic carcinogen (AARGC). Probiotics and prebiotics were used either alone or in combination with resistant starch. When the synbiotic constituents were used in combination, no effect was observed on the response to the carcinogen. However, the combination of resistant starch and *B. lactis* increased the acute apoptotic response. The combination with *L. acidophilus* did not have the same effect, indicating a strain specific effect and synergy between *B. lactis* and resistant starch. Synbiotic ingestion resulted in lower fecal pH values and higher total and individual SCFA concentrations compared to the probiotics alone. The inclusion of resistant starch increased bifidobacteria and lactobacilli levels. The potential benefit of synbiotic ingestion against ACF in a male Wistar rat model employing 1,2 demethyhydrazine

to initiate ACF formation was investigated in a later study (Gallaher & Khil 1999). An undefined *Bifidobacterium* culture (10^8 CFU g^{-1}) was combined with either oligofructose (GRC), soybean oligosaccharides (Ajinomoto), or wheat bran oligosaccahrides (Megazyme) at 2% (w/v) of the diet. The probiotic was tested alone or in combination with one of the test probiotics, and results were compared to a control diet group. Neither the probiotic nor the oligofructose treatment had an effect on ACF formation. However, when used in combination, they mediated a significant decrease as compared to the control group, which signifies a synergy between the components of the synbiotic. The other test prebiotics were only used in combination to the probiotic and not alone, and the results were not as consistent as with oligofructose.

Burns & Rowland (2004) used the Comet assay to evaluate the prophylactic potential of 10^8 *L. plantarum* (Rhodia), *Bifibobacterium* Bb12 (Chr. Hansen), *Enterococcus faecium* S13 (Danisco), *Bifidobacterium* sp. 420 (Danisco), *Streptococcus thermophilus*, and *Lactobacillus bulgaricus* against DNA damage of genotoxic fecal water on HT29 human adenocarcinoma cell lines (2004). The effect was strain specific with *L. plantarum* and *Bifidobacterium* Bb12 being the most effective. In a second experiment, the selected strains were preincubated with Raftiline HP, Raftilose, chicory inulin (Sigma), GOS (Borculo Domo), Actilight (Beghin Meiji), and Fibersol (maltodextrin; Matsutani), and the fermentation supernatants were incubated with the HT29 cells. The most pronounced effect was again observed with *L. plantarum* and *Bifidobacterium* Bb12 in combination with Inulin and Actilight. The degree of protection was even greater than when the bacteria were used alone in the absence of supernatants. Heat-killed bacteria did not have any effect. This suggested that cell viability was necessary to mediate the effect and that certain fermentation products enhanced the degree of protection. Two studies have investigated the tumor-preventative efficacy and fecal water genotixicity *Lactobacillus rhamnosus* and *B. lactis* each at 5×10^8 CFU g^{-1} diet either alone or in combination with 100 g kg^{-1} w/w Synergy 1 in 4 to 5-week-old male F344 rats (Femia et al. 2002, Klinder et al. 2004). Thirty-two rats were fed a control diet, 33 were fed Synergy 1, 32 were fed the probiotics, and 32 were fed the synbiotic. Ten days after being on the test diets, the rats were treated with AOM (azoxymethane) to induce cancer. Animals continued the dietary regime for a further 31 weeks, at which time their clinical condition was assessed. Rats fed the synbiotic and prebiotic diets presented significantly lower tumor numbers compared to rats not fed Synergy 1. Apoptosis was increased in the normal mucosa of the probiotic group; however, no variation was observed in the tumors. Colonic proliferation and SCFA levels were increased in the prebiotic and synbiotic groups. In the Synergy 1 ingesting groups, there was a reduced exposure to genotoxins in the feces, which correlated with tumor incidence in these animals. This implies that it is the fermentation of the prebiotic by the probiotic and the commensal flora that is central in the prophylactic effect. The results of this study indicate again the importance of bacterial fermentation products as protective agents against tumorigenesis.

One in vivo human study on the protective effect of synbiotics against colon cancer has been published (Rafter et al. 2007). The aim was to investigate the potential of a synbiotic preparation, 10^{10} CFU *L. rhamnosus* GG (Valio) and *B. lactis* Bb12 encapsulated in Eudragit L30-D55 (Chr. Hansen) combined with 12 g Synergy 1 in a 12-week randomized, double-blind, placebo-controlled study. Two groups were targeted: 43 polypectomized patients and 37 colon cancer patients. Following synbiotic ingestion, significant increases in fecal lactobacilli and bifidobacteria were observed, and a decrease in *Clostridium perfringens* evaluated using culture techniques. In polypectomized patients, the synbiotic improved the epithelial barrier function and reduced the ability of fecal water to induce colonic cell necrosis, and it prevented increases in Il2 by PBMCs. Analysis of biopsy samples at the end of the intervention indicated a reduced genotoxin exposure. In cancer patients, synbiotic ingestion increased the production of interferon γ. The results of this study are encouraging; however, they do not offer enough insight into the mechanism of synbiotic

action. As our knowledge in this area is not very advanced and is mainly based on animal models, it is imperative that proper probiotic and prebiotic controls are used.

Surgical Patients

Morbidity and mortality among severely ill patients, such as transplant and postoperative patients, has remained at high levels despite medical advances in both surgical procedures and pharmacological treatments. Sepsis in intensive care units (ICUs) is unacceptably high and is the tenth most common cause of death in the United States (Bengmark 2004). Patients undergoing extensive surgery are under severe risk of contracting nosocomial bacterial infections, but there is also evidence that disruption of the gut barrier integrity in postoperative patients may greatly contribute towards the increased incidence of sepsis in the ICU (Marshall et al. 1993, MacFie 1997, MacFie et al. 1999). Synbiotic supplementation of the diet and enteral formulas of pre- and postoperative patients may be a promising route of manipulating the composition of the gut microbiota toward a more beneficial community, and the potential trophic effect of the prebiotic on the gut mucosa could preserve gut barrier function and suppress gut pathogen translocation.

In a double-blind, placebo-controlled study of 137 patients scheduled to undergo elective laparotomy, the efficacy of a synbiotic in preventing postoperative complications was evaluated (Anderson et al. 2004). Patients were divided into a synbiotic ($n = 72$) and a placebo ($n = 65$) treatment group. Patients in the synbiotic group received three times daily capsules delivering 4×10^9 CFU *L. acidophilus* La5, *L. bulgaricus*, *B. lactis* Bb12, and *S. thermophilus* (Trevis®, Christian Hansen) and twice daily 16 g of oligofructose. Patients in the placebo group received placebo capsules and sucrose. Treatment was intiated one to two weeks preoperatively and continued until patients were discharged from the hospital. No difference was observed between the active and placebo groups with regard to bacterial translocation and colonization, systemic inflammation, and septic complications.

In a double-blind study investigating the effect of Synbiotic 2000 in 66 patients scheduled for liver transplantation, a significant decrease in postoperative infections was observed (Rayes et al. 2005). The treatment was added in the enteral feed formula and was administered for 14 days starting on the day of the surgery. Patients were divided in two treatment groups, one receiving Synbiotic 2000 and the other receiving only the fiber components of Synbiotic 2000. Interestingly, in the group receiving the full synbiotic formula, postoperative infections were significantly lower (3%) compared to the fiber only group (48%). In the synbiotic group, the required antibiotic treatment duration was also significantly lower. In a later study, the same group evaluated the efficacy of Synbiotic 2000 against bacterial infection rates following pylorus-preserving pancreatoduodenectomy (Rayes et al. 2007). This time, the synbiotic treatment was administered one day preoperatively and was continued for eight days postoperatively. Eighty-nine adult patients were divided into two treatment groups, and they received identical treatments to the previous study. The results were in agreement with the liver transplantation study with bacterial infections being significantly lower in the synbiotic group (12.5%) compared with the fiber group (40%), despite the shorter postoperative treatment duration. Although these findings are very promising, one shortcoming of these studies was that it is not possible to determine whether it was the synbiotic or just the probiotic components of Synbiotic 2000 that mediated the effect. Also, no conventional treatment group was included in the study, and as such, results cannot be evaluated properly.

Synbiotic 2000 was also effective in reducing endotoxemia and blood ammonia levels and increasing fecal *Lactobacillus* concentrations in patients with cirrhosis suffering from minimal hepatic encephalopathy (MHE) (Liu et al. 2004). The same experimental approach was followed as

described in the above Synbiotic 2000 studies, with 20 patients receiving the synbiotic and 20 receiving only the fiber components. However, a placebo group was also included that received a wheat-based, nonfermentable fiber (Medipharm). The gut microbiota of the MHE patients were characterized by an overgrowth of *Escherichia coli* and *Staphyloccocal* species. An increase in *Lactobacillus* upon ingestion of the synbiotic treatment normalized the flora. MHE reversal was observed in 50% of the synbiotic group patients with a significant reduction in endotoxemia. Treatment with the fiber components also had a beneficial effect in some of the volunteers.

As previously mentioned, timing of synbiotic intervention may be an important factor in preventing postoperative infection. This was investigated in 101 patients with biliary cancer undergoing scheduled high-risk hepatobiliary resection (Sugawara et al. 2006). Volunteers were divided into two groups, one receiving only postoperative treatment and one receiving both pre- and postoperative treatments two weeks prior and two weeks following surgery. The preoperative treatment of 4×10^{10} *L. casei* Shirota (80 ml Yakult 400), 10^{10} *B. breve* (100 ml Bifiel, Yakult), and 15 g GOS (Oligomate 55, Yakult) was administered orally and delivered daily. The postoperative treatment was delivered parenterally and delivered 15 g GOS, 10^8 CFU g^{-1} *L. casei*, and 10^8 CFU g^{-1} *B. breve*. Both probiotics were detected in patient feces in both patient groups; however, in the group receiving both pre- and postoperative synbiotic treatments, postoperative infections, white blood cell counts, and C-reactive protein were significantly lower compared to the group receiving only the postoperative treatment.

To date, all studies investigating the efficacy of synbiotic administration in surgical patients have used commercially available products. The results are indeed encouraging, and there seems to be scope for further studies and more widespread application of synbiotics in these patients. However, the lack of placebo and proper controls (e.g., prebiotic only, probiotic only) to elucidate the mechanism of the effects prevent the proper evaluation of the synbiotic effects. All studies to date have used multiprobiotic formulas, which limits insight into the actual functionally active ingredients mediating the observed effects.

PRODUCT FORMULATION

The concept of synbiotics offers a potential for increased efficacy to functional foods by exploiting the advantages that a combination of prebiotics with probiotics confers not only to health but also to product stability during storage. One of the main challenges that probiotic products have to meet is delivering an adequate concentration of live bacteria so as to mediate a desired health effect. Viability and stability during storage prior to ingestion have been two of the main issues that can greatly affect product efficacy and one of the challenges probiotic manufacturers must meet. The introduction of a prebiotic into the product should theoretically improve viability of the probiotic. However, fermentation of the prebiotic may have some undesirable effects on the food matrix, as lactate and SCFA production could mediate a decrease in the pH of the product and affect consistency. The synbiotic should be able to deliver a minimum dose of 10^8 CFU ml^{-1} product after storage at low temperature (Mattila-Sandholm et al. 2002).

The majority of probiotic or synbiotic foods available currently are either yogurts or dairy drinks, which, in addition to the traditional starter cultures, contain probiotic bacteria usually belonging to the genera *Bifidobacterium* and *Lactobacillus*. Any prebiotic introduced to the dairy product should be selective for growth of the probiotic. Prebiotic fermentation by the starter cultures should also be considered, as this may affect organoleptic properties of the product and reduce the amount of fermentable substrate available for the prebiotic. Several studies have investigated the effect on viability and palatability of dairy products when combined with a synbiotic. However,

there are few well-designed studies that have followed a rational selection of the probiotic and the prebiotic and therefore arbitrarily use the term synbiotic.

One study to investigate the formulation of a synbiotic food product was by Crittenden et al. (2001). They screened 40 *Bifidobacterium* strains for their ability to ferment resistant starch (Hi-maize™) to be used in a synbiotic yogurt. Of the strains screened, *B. lactis* Lafti™B94 was selected for its ability to ferment resistant starch as well as a range of other potential prebiotics, including FOSs, GOSs, soybean oligosaccharides, and xylooligosaccharides. The authors tested extensively the probiotic isolates for their in vitro ability to survive simulated gastric conditions and bile secretions.

Desai et al. (2004) investigated the effect of commercially available oligosaccharides (Hi-maize™, Raftilose, lactulose, and chicory inulin; Sigma) on seven *Lactobacillus* strains (*L. casei* ASCC 1520, *L. rhamnosus* ASCC 1521, *L. casei* CSCC 2607, *L. zeae* ATCC 15820, *L. casei* ASCC 290, *L. paracasei* ASCC 292, and *L. casei* ASCC 279) in skimmed milk during refrigerated storage. Although the authors did not preselect the probiotic strains so as to ensure test prebiotic fermentation, their aim was to develop a synbiotic food whereby the prebiotics would enhance viability of the probiotics. It was observed that despite a decrease in viability in all test products, viability was in general improved as compared to the prebiotic negative control, and the most improved effect was observed with inulin. The growth, activity, and viability of the *Lactobacilllus* strains was both strain and prebiotic dependent, and prebiotic addition improved growth rates while decreasing fermentation time. A more novel approach of encapsulating the synbiotic to improve its stability both during storage in yogurt as well as during ingestion was investigated by Iyer & Kailasapathy (2005). Prebiotics were screened in vitro for their ability to support the growth of *L. acidophilus* CSCC 2400 or CSCC 2409. The efficacy of selected complementary prebiotics (Hi-maize™ starch, Raftiline and Raftilose) in improving viability when coencapsulated with a probiotic was assessed under acidic conditions and during storage in yogurt. Hi-maize™ at concentrations of up to 1% (w/v) significantly increased probiotic viability during incubation at pH 2 as compared with the fructans; however, further increases in prebiotic concentration did not further increase viability. The effect of coating was also investigated and chitosan encapsulation was proven to offer superior protection as compared with alginate and poly-L-lysine. In a similar study, Crittenden et al. (2006) investigated the effect of encapsulation within a film-forming-carbohydrate-oil emulsion on viability of *B. infantis* Bb-02 during nonrefrigerated storage and GI transit. Briefly, spray-dried *B. infantis* in FOS (Raftilose P95) capsules was stored in foil sachets at room temperature for a two-month period and their viability compared with that of nonencapsulated bacteria. Encapsulation significantly improved viability of *B. infantis* during storage but also during passage through an in vitro model of the human stomach and small intestine. FOS microencapsulation also improved probiotic viability under storage in an open container at 25°C and 50% humidity, which further indicates that this approach could improve product stability and shelf life. Although there is a synergy between the prebiotic and the probiotic and a superior formulation is achieved by combining the two, enhancement of viability during storage and passage through the GI tract is through the encapsulation process rather than fermentation of the prebiotic. Here the authors tested two different capsules; however, they both contained FOS, and they both offered the same levels of protection. It would have been interesting to see the effect of other potential prebiotics and prebiotic-free capsules to investigate if it was the synergy between the prebiotic and *B. infantis* that resulted in increased viability. Homayouni et al. (2008) investigated the survival of *L. casei* Lc-01 and *B. lactis* Bb-12 when encapsulated in alginate in the presence of 1% Hi-maize™ in ice cream containing resistant starch. Viability was only compared with the nonencapsulated forms and not with capsules or ice cream in the absence of resistant starch. Although the product was characterized as a synbiotic, it is apparent that there was no

added effect on viability due to the presence of the prebiotic. The superiority of the encapsulated formula was due to protection lent by the encapsulation.

Buriti et al. (2007) investigated the stability of fresh cream cheese supplemented with inulin (Raftiline) and *L. paracasei*. The probiotic was not prescreened so as to determine its ability to ferment inulin. The authors did not observe an improvement in the viability of *L. paracasei* during

Table 1 Emerging studies on synbiotic application in disease

Intervention (treatments per day)	Study design	Duration	Evidence of synbiotic efficacy	Reference
Refractory enterocolitis				
3 × daily: 10^9 *Bifidobacteriumbreve Yakult: B. breve and Lactobacillus casei Shirota: L. casei* 1 g galactooligosaccharide (GOS)-oligomate (Yakult)	Seven short bowel patients with refractory enterocolitis, 2–24 years	15–55 months	Improvement in intestinal bacterial flora composition (culture), increased short chain fatty acid (SCFA) production, accelerated body weight gain	Kanamori et al. 2004
***Helicobacter pylori*–colonization**				
Antibiotic triple therapy, 10^9 *Lactobacillus acidophilus* LB (lacteol forte), 250 g lyophilized *Saccharomyces boulardii* (Perenteryl, Merck) & 5 g inulin (Orafti)	254 *H. pylori*–positive children distributed in three treatment groups Randomized open study. Spontaneous clearance assessed in 81 positive untreated children	Group 1: eight days Groups 2 and 3: eight weeks	Synbiotic more efficient than *L. acidophilus* LB (12% eradication) but not as effective as antibiotic (66% eradication) based on C-Urea Breath Test	Gotteland et al. 2005
MRSA (methicillin resistant *Staphylococcus aureus*) enteritis				
3 g GOS (oligomate) 3 g *L. casei Shirota* & 120 mg vancomycin supplemented by 3 g *B. breve* (BBG-1)	Case study; three-month-old Down syndrome boy	*B. breve* introduced following 42 days on initial treatment	MRSA dominant flora eradicated by vancomycin treatment, anaerobic flora, SCFA levels, and stool appearance normalized by successive synbiotic treatment	Kanamori et al. 2003
Mineral bioavailability				
Folow-up infant formula plus: 5.5×10^7 CFU *Bifidobacterium bifidum and Bifidobacterium longum* 12, 50, 100 g kg^{-1} GOS (oligomate) -synbiotic combinations of the above	54 three-week-old weanling male Srague-Dawley rats Samples on days 8–10, 18–20, and 28–30	30 days	Increased Ca, Mg, P bioavailability with probiotic and prebiotic formulas, most efficient formulas tended to be the 50 g kg^{-1} and100 g kg^{-1} synbiotics; synbiotics significantly reduced lumen pH and increased crypt depth cell density in colon. Increased Ca and P in femur and tibia, Mg in tibia. Site of Ca absorption distal colon, Mg proximal and distal.	Pérez-Conesa et al. 2006; Pérez-Conesa et al. 2007

storage at low temperature, and they noted stability of inulin in the product, which indicates that the probiotic could not ferment it. The results in the product prior to ingestion strongly suggest that there will be no synergy in vivo. In a later study, the same group investigated the sensory quality, and prebiotic and probiotic stability in petit-suisse cheese (Cardarelli et al. 2008). Again, the same approach was followed as the probiotic strains (*B. animalis* subsp. *lactis* and *L. acidophilus*) were not preselected to ensure fermentation of the test prebiotics (Beneo ST and Beneo P95, Orafti). Their results suggested again that the test prebiotics were not fermented and as such were stable in the product. Similar results were reported for the combination of *L. paracasei* subsp. *paracasei* LCB82 and inulin (Raftiline GR, Orafti) in chocolate mousse. Probiotic viability and prebiotic levels were not affected during storage (Casale Aragon-Alegro et al. 2007).

CONCLUSIONS AND FUTURE PERSPECTIVES

Further evidence is emerging on the use of synbiotics on refractory entorocolitis, atopic dermatitis/immunomodulation, cholesterol profile improvement, *Helicobacter pylori* colonization, mineral bioavailability, and antipathogen activity. Study results are summarized in **Table 1**.

It is clear that the scope is broadening for the application of synbiotics in both health and disease. However, with few exceptions, the term synbiotic has been loosely used by the majority of published studies currently available. In most cases, there was no rational selection of the prebiotic-probiotic combinations, and some of the research lacked proper controls to confirm or deny the presence of a synergistic or additive effect. Furthermore, several studies used dietary fibers that are not recognized prebiotics. This combined with the fact that in most cases no attempt was made to confirm growth of the probiotic on the prebiotic implies that the nature of the effect is questionable.

The formulation of successful synbiotics is a particularly complex issue. Studies to date have not investigated the issue of minimum effective dose to mediate the desirable effect in the absence of side effects. In the future, it is imperative that both the probiotic and prebiotic components are rationally selected and the appropriate biomarkers are targeted during in vivo trials.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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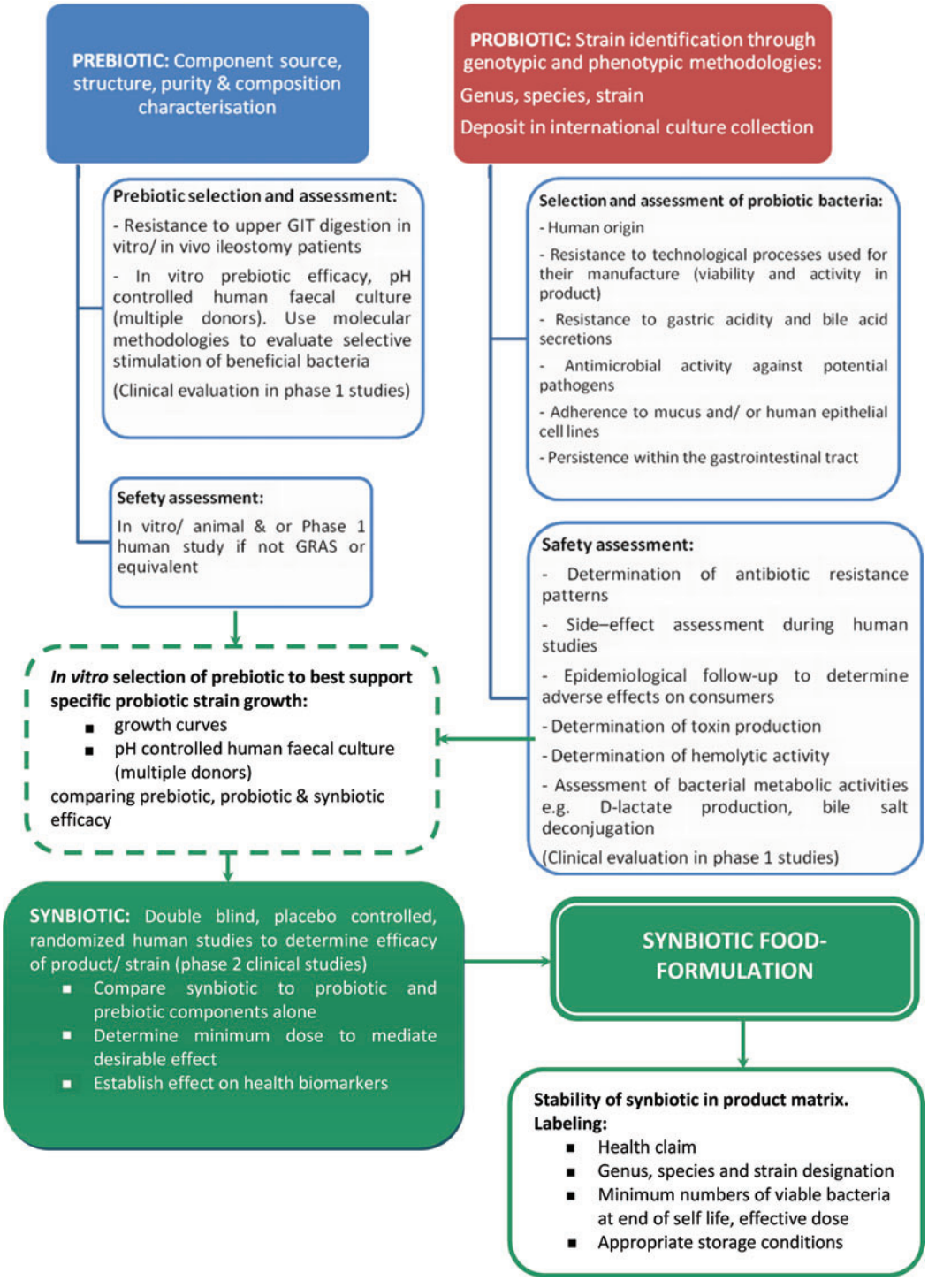

Figure 1

Suggested steps for establishing a synbiotic formulation.

Annual Review
of Food Science
and Technology

Volume 2, 2011

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Errata

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