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Review

Probiotics and prebiotics – Progress and challenges

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ABSTRACT

This is a particularly important time in the evolution of probiotic and prebiotic research. There has been strong growth in dairy food products containing probiotics and/or prebiotics, and a number of them are supported by clinical studies showing health benefits. By uncovering how probiotic and prebiotic interventions function in vivo, it will be possible to further expand dairy applications that improve general health, and in some cases provide adjunctive anti-disease benefits. However, it is important that probiotic products meet appropriate international standards, and contain appropriately speciated and characterized organisms, in shelf-stable formulations that have been shown in well-designed clinical studies to confer defined health benefits on the consumer. This review will focus on progress made over the past 3 years in understanding the important role of bacteria in health, beginning at conception through to older age. Studies showing that the body's microbiota can be modulated, to a certain extent, by use of probiotics and prebiotics, has led to the development and testing of products targeting immunity, regularity, allergy, gut and distant site infection, cardiovascular disease and other ailments. In the future, human and microbial genomic and metabolomic studies will provide a better understanding of the mechanisms conveyed by commensal and probiotic organisms in human and animal health. This will challenge the dairy industry and regulatory authorities as to how to communicate food benefits that go beyond general health and wellbeing claims. This will prove particularly challenging for recombinant strains carrying microbicides, immune-modulators and other disease-specific components.

Contents

1. Introduction

In recent times, there has been a growing appreciation for the important role of commensal microbes in human and animal health, be it through mediation of intestinal development and innate immunity, or digestion of food and protection of the host against disease (Sansonetti, 2006; Xu, Chiang, Bjursell, & Gordon 2004). This had led to attempts to manipulate or augment the microbiota through the use of probiotics ("live microorganisms that when administered in adequate amounts confer a health benefit on the host) or prebiotics ("nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria") (FAO/WHO, 2002; Reid, 2006; Reid, Sanders, et al., 2003). Critics have occasionally suggested that there are more reviews on probiotics than original papers, but a November 2007 PubMed search shows 237 articles under "probiotics AND dairy" of which only 66 are reviews or meta-analyses. With the recent report that fermented products are driving the growth of the dairy

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industry (Cogan et al., 2007), it seems timely to examine recent developments in this area. Indeed, medical evidence is key to expanded use of probiotics and prebiotics in dairy foods.

2. Probiotics and prebiotics for fetal and newborn health

The importance of lactic acid bacteria in life is perhaps best seen in relation to the health of women and babies. Women who are devoid of lactobacilli in the vagina have a reduced success rate of in vitro fertilization (Verstraelen & Senok, 2005). The cause may be an inflammatory process that inhibits sperm movement or egg–sperm binding, but there seems little doubt that bacteria influence this activity. Once pregnant, the loss of lactobacilli from the vagina and subsequent development of bacterial vaginosis (BV) is associated with increased risk of preterm labour (Leitich & Kiss, 2007). Clues regarding the effect of bacteria on fetal development come from large epidemiological studies showing that nutrition in mothers has long-term consequences for the baby (de Boo & Harding, 2006).

There has been one study of pregnant women and newborns that suggests that consumption of probiotic *Lactobacillus rhamnosus* GG can reduce the rate of newborns having atopic dermatitis (Kalliomaki et al., 2001). This finding has not been replicated at other sites, as yet. In an Australian study, 178 newborns of women with allergy who received either *Lactobacillus acidophilus* LAVRI-A1 or placebo daily for the first 6 months of life showed no difference in atopic dermatitis (probiotic, 23/89 versus placebo, 20/88; $P = 0.629$) (Taylor, Dunstan, & Prescott, 2007). However, at 12 months, the rate of sensitization was significantly higher in the probiotic group ($P = 0.030$), leading the authors to conclude that the treatment increased risk of subsequent cows' milk sensitization ($P = 0.012$).

A double-blind, randomized, placebo-controlled trial of 188 subjects with allergic disease, in which the mothers received *Lactobacillus reuteri* ATCC 55730 daily from gestational week 36 until delivery, and their babies continued with the probiotic until 12 months, showed less IgE-associated eczema during the second year (8% versus 20%; $P = 0.02$) (Abrahamsson et al., 2007).

There is a conceptual rationale for using some form of probiotic to reduce allergies, but as the above cited papers show, it is not clear as to which probiotic would be best. In the Czech Republic, studies of newborns who were given a probiotic *Escherichia coli*, 10- and 20- year follow-up showed that allergies and some infections later in life could be prevented, compared to controls (Lodinova-Zadnikova, Cukrowska, & Tlaskalova-Hogenova, 2003). These clinical results imply that there are host or bacterial signals crossing the maternal–fetal membrane, and that fatty acids and cytokines present in breast milk influence whether or not the newborn will develop atopy (Laitenin, Sallinen, Linderborg, & Isolauri, 2006).

The use of prebiotics has been considered as a means of influencing the gut microbiota and risk of allergy. The gut is a complex environment influenced largely by the microbial content, secondary bacterial metabolites including antimicrobial substances, immune-modulators and quorum-sensing molecules, and by host factors including secretions (Louis, Scott, Duncan, & Flint, 2007). The ability to shift the composition of the microbiota by administration of prebiotics thereby has implications for many aspects of gut function. The modulation of bifidobacteria and lactobacilli have been the main focus of prebiotic research, to date, but other organisms within the gram-negative Bacteroidetes phyla and the low % G + C Gram-positive Firmicutes need to be investigated. The following is a good example of that point. A prospective, double-blind, randomized, placebo-controlled trial of 259 infants at risk for atopy, bottle feeding with 0.8 g 100 mL^{-1} prebiotics or maltodextrine placebo led to 9.8% incidence of atopy versus 23.1% in the control (Moro et al., 2006). The prebiotic use was associated with a significantly higher number of fecal bifidobacteria compared with controls but there was no significant difference in lactobacilli counts, and no examination of *Clostridium* or other species.

There is other evidence that probiotics and prebiotics can influence newborn health. Using a duplex 5′ nuclease assays, targeted on rRNA intergenic spacer regions to enumerate *L. acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *L. reuteri*, and *L. rhamnosus*, the fecal presence of these organisms was detected after feeding for 6 weeks with a standard formula, breast milk, or a standard formula supplemented with galacto- and fructo-oligosaccharides in a 9:1 ratio (Haarman & Knol, 2006). The *Lactobacillus* species distribution in the prebiotic supplemented formula was comparable to breast-fed infants, with relatively high levels of *L. acidophilus*, *L. paracasei*, and *L. casei*. However, as with many other studies, the long-term impact of the treatment was not reported.

Microbes associated with the vagina, feces, skin and mouth clearly contribute to the early infant microbiota, but which of the many microbes does the host allow to remain and why? *Bacteroides thetaiotamicron*, and likely other species, which presumably come from the environment soon after birth, have been shown in animal studies to induce angiogenesis and the development of the healthy intestine (Stappenback, Hooper, & Gordon, 2002). If bacteria do not presume this role, what are the implications for the host? The first few weeks of life are clearly important in terms of the organisms we inherit, yet we understand little about the influence of vaginal versus caesarean birth, and formula versus breast feeding.

Breast milk not only provides a range of substrates for bacterial growth (Ward, Ninonuevo, Mills, Lebrilla, & German, 2006), but it also appears to be a reservoir for some of the bacteria we inherit, including *Lactobacillus* sp. (Martin et al., 2005). Although this needs to be verified and an explanation given with mechanism uncovered as to how lactobacilli reach the mammary gland and if other bacteria do likewise, the end result is that infants are colonized predominantly by lactic acid bacteria. These organisms appear to be responsible for a less diverse microbiota and mainly acetic and lactic acid production, compared to formula-fed infants who have higher acetic and propionic acid in the feces (Edwards & Parrett, 2002). The role that lactic acid bacteria and their metabolic end-products play in developmental processes, including immunity, food processing and intestinal barrier function, remain to be elucidated.

Supplementation of milk with probiotics confers immunomodulatory effects, leading to a Th1 response and reduced allergic tendencies (Rautava, Kalliomaki, & Isolauri, 2002; Viljanen et al., 2005). Likewise, adding specific prebiotics (usually fructo-oligosaccharides and galacto-oligosaccharides) to formula milk implies that their effect on the microbiota is beneficial. A first step would be to try and determine if an optimal microbiota exists, when and how it forms, and what stages in life it changes significantly. These tedious, labour-intensive studies are necessary and now possible with molecular probing.

An interesting clinical trial showed that prebiotic galacto-oligosaccharides and fructo-oligosaccharides (6 g L^{-1}) caused a similar effect on metabolic activity of the gut (fecal acetate ratio, lactate concentration and lower pH) as found in breast-fed infants (Bakker-Zierikzee et al., 2005). In this case, the addition of *Bifidobacterium animalis* Bb-12 did not have a detectable effect on metabolic activity, but this organism or others (Mazmanian, Liu, Tzianabos, & Kasper, 2005) likely influence neonatal immune development, and so studies must be designed to examine multiple parameters over time, before their widespread use can be recommended. A case has been made for using probiotics in babies at risk of serious and often fatal infections, such as necrotizing enterocolitis (NEC), the more recent one was a prospective, masked, randomized control trial of 367 low weight (<1500 g) infants fed enterally with *L. acidophilus* and *Bifidobacterium infantis* and with breast milk twice

daily until discharged (Lin et al., 2005). The incidence of death or NEC was significantly lower in the probiotic group (9/180 versus 24/187). In summary, it appears that probiotics might reduce the risk of NEC in preterm neonates of less than 33 weeks gestation, but issues of short- and long-term safety, dosage, duration, and type of probiotic agents (species, strain, single or combined) need to be further investigated (Deshpande, Rao, & Patole, 2007).

3. Functionality at local and distant sites

For some time, it has been recognized that physiological benefits can accrue at sites distant from where probiotic and prebiotic products are administered. Evidence mainly comes from studies of the (i) head/neck, oral and respiratory tracts (Hatakka et al., 2001; Tubelius, Stan, & Zachrisson, 2005; Turchet, Laurenzano, Auboiron, & Antoine, 2003); (ii) pancreas and liver (Kanazawa et al., 2005; Olah, Belagyi, Issekutz, & Olgyai, 2005; Rayes et al., 2002); and (iii) kidney, bladder and vagina (Hoppe et al., 2006; Ohashi et al., 2002; Reid et al., 2001).

The reduction in workplace acquired and respiratory infections could arguably be due to mucosal stimulation of the immune response in the intestine which then affects other lymphoid system sites. In a study of 571 healthy children aged 1–6 years who daily consumed 260 mL milk containing *L. rhamnosus* GG, absenteeism due to illness was lower (4.9 vs 5.8 days, 16% difference, $P = 0.03$) and there was a relative reduction of 17% in the number of children suffering from respiratory infections with complications and lower respiratory tract infections and a 19% relative reduction in antibiotic treatments for respiratory infection (Hatakka et al., 2001). This finding of enhanced respiratory effects with daily consumption of probiotics is supported by a controlled pilot study of 360 elderly subjects who took milk supplemented with *L. casei* DN-114001 for 3 weeks. There was no difference in the incidence of winter infections, but duration of all pathologies was significantly lower in the treatment group (7.0 vs 8.7 days; $P = 0.024$) (Turchet et al., 2003). Although the study designs and subject groups differed, there appears to be an overall effect of different probiotic products on respiratory health. Influenza causes an estimated 500,000 deaths annually, while pneumonia is responsible for 85–90% of deaths in children under 5 years of age (approximately 150,000 annually). Thus, there is merit in further exploring the efficacy of probiotics to prevent and treat respiratory infections.

It has long been recognized that probiotic organisms can modulate immunity, and some products have promoted immune 'boosting' effects. However, this is by no means a general effect for all strains or species, nor is it necessarily a desired effect for some patients whose immunity is already overly expressed. Furthermore, just because an organism demonstrates a particular immune profile in vitro does not mean it will do likewise in vivo. A recent in vitro study using peripheral blood mononuclear cells (PBMCs), showed that *Bifidobacterium longum* strains greatly stimulated regulatory cytokine interleukin (IL)-10 and proinflammatory cytokine tumour necrosis factor (TNF)-alpha production, but *B. longum* W11 stimulated T helper 1 (Th1) cytokines while *B. longum* NCIMB 8809 and BIF53 induced low levels of Th1 cytokines and high levels of IL-10 (Medina, Izquierdo, Ennahar, & Sanz, 2007). Strains *L. rhamnosus* GR-1 and *L. reuteri* RC-14, known to have immunomodulatory properties (Kim, Sheikh, Ha, Martins, & Reid, 2006), have intriguingly been shown to increase the CD4 count and 'boost' immunity in HIV/AIDS subjects (Anukam, Osazuwa, Osadolor, Bruce, & Reid, 2008), yet increase T_{reg} cells and anti-inflammatory effects in patients with inflammatory bowel disease (Lorea Baroja, Kirjavainen, Hekmat, & Reid, 2007). Prebiotic compounds can also have immunomodulatory properties, with and without addition of probiotic bacteria (synbiotics). A study using *L. rhamnosus* GG and *Bifidobacterium lactis* Bb-12 plus 10 g of inulin enriched with oligofructose showed increased ability of PBMCs to produce IFN-gamma in recovering colon cancer patients (Roller, Clune, Collins, Rechkemmer, & Watzl, 2007).

The reported benefits of probiotic use for the healthy function of the liver and pancreas are clearly due to distant effects of the organisms. Intake of the probiotic *E. coli* Nissle 1917 for 42–84 days has been shown to improve liver function as determined by the Child–Pugh classification on days 42 and 84 in patients with liver cirrhosis, possibly due to decreased release of endotoxin (Lata et al., 2006). The Child–Pugh classification is a method of measuring bilirubin, prothrombin and other indicators to put patients into prognostic categories, although inclusion of ascites and encelopathy in the scoring has been criticized and led to a model for end-stage liver disease (MELD) criteria being proposed (Kamath et al., 2001). Another area of great interest in relation to the liver's role in processing food is the concern over cholesterol and coronary heart disease. Given that a 1% reduction in serum cholesterol might reduce the risk of coronary heart disease by 2–3%, it is of interest to note that rats fed with *L. plantarum* PH04 had 7% lower serum cholesterol and 10% lower triglycerides (Nguyen, Kang, & Lee, 2007). Although human data are still needed, this is an area of study which could impact the health of many people. This is also true for the use of prebiotics where the fermentation of mannitol, fructo-oligosaccharide and inulin favoured the production of formic, lactic and butyric acids, respectively, and correlated with cholesterol removal (Liong & Shah, 2005).

Using a different mechanism of action and reducing endotoxin release, the administration of *L. rhamnosus* LC705 and *Propionibacterium freudenreichii* subsp. *shermanii* was shown in human studies to block the intestinal absorption of aflatoxin B(1) and thereby lead to reduced urinary excretion of aflatoxin B(1)-N(7)-guanine (AFB-N(7)-guanine), and decrease the risk of liver cancer (El-Nezami et al., 2006). Yet another effect, namely, the attenuation of oxidative stress caused by flutamide metabolites and promotion of regeneration of new hepatocytes, has been reported in rats following the use of *Saccharomyces cerevisiae* (Mannaa, Ahmed, Estefan, Sharaf, & Eskander, 2005). This therapy was postulated to restore liver function beyond the normal status, but human trials are needed for verification.

The administration of certain prebiotics appears to be important in modulating the gut microbiota and abdominal organ health. In a study of 55 cirrhotic patients with minimal hepatic encephalopathy (MHE) randomized to receive a probiotic plus prebiotic (symbiotic) preparation ($n = 20$), fermentable fiber alone ($n = 20$), or placebo ($n = 15$) for 30 days, the controls had substantial derangements in the gut microecology, with significant fecal overgrowth of potentially pathogenic *E. coli* and *Staphylococcus* species, while the synbiotic treated subjects had significantly increased fecal content of non-urease-producing *Lactobacillus* strains and a significant reduction in blood ammonia levels and reversal of MHE in 50% of cases as determined by the Child–Pugh assessment tool (Liu et al., 2004). One prebiotic resistant starch (which escapes small intestinal digestion by microbes), in the form of high amylose cornstarch (HAS), has been shown in animal studies to decrease intestinal pH, increase short chain fatty acid formation, especially butyrate, inducing an apoptotic response to a genotoxic carcinogen in the colon (Le Leu, Brown, Hu, & Young, 2003).

Another distant site condition affected by the gut microbiota is oxaluria. It has been proposed that the presence of *Oxalobacter formigenes* in the gut, either as an indigenous constituent or as a transient probiotic, will degrade oxalates and prevent these substances from being deposited in the kidney through blood filtration. Common and widely used probiotic lactobacilli encode the capacity to degrade oxalate, and the genes involved in degradation of oxalate have been described in *L. acidophilus* NCFM (Azcarate-Peril, Bruno-Barcena, Hassan, & Klaenhammer, 2006). The only

human studies performed to date have been using *O. formigenes* administered orally for 4 weeks as frozen paste (IxOC-2) or as enteric-coated capsules (IxOC-3) (Hoppe et al., 2006). Nine patients (5 with normal renal function, 1 after liver–kidney transplantation, and 3 with renal failure) completed the IxOC-2 study; of these 3/5 with normal renal function showed a 22–48% reduction of urinary oxalate, while 2/3 renal failure patients experienced a significant reduction in plasma oxalate and amelioration of clinical symptoms. Seven patients (6 with normal renal function and 1 after liver–kidney transplantation) completed the IxOC-3 study; of these 4/6 with normal renal function responded with a reduction of urinary oxalate ranging from 38.5 to 92%. Fecal recovery of *O. formigenes* dropped as the ingestion was stopped, indicating an inability of the organism to colonize the gut.

The report that orally administered lactobacilli probiotics can ascend passively from the rectum to the vagina is a significant breakthrough in being able to deliver probiotics in foods and dietary supplements (Reid et al., 2001; Reid, Charbonneau, et al., 2003). This has now been confirmed by others (Antonio, Rabe, & Hillier, 2005; Morelli, Zonenenschain, Del Piano, & Cognein, 2004). What would appear to be distant site effects, are actually due to local effects induced by the passage of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 through the gut after daily intake in milk or dried form, then natural passage from the anal skin to the perineum and vagina, several centimetres away. The presence of lactobacilli in the vagina can further interfere with the ascension of uropathogens into the bladder, reducing the incidence of urinary tract infections (Reid & Bruce, 2006). A recent study of children with primary vesicoureteral reflux showed that prophylaxis with a strain of *L. acidophilus* was as effective as trimethoprim–sulfamethoxazole antimicrobials at preventing urinary tract infection (UTI), without increasing the risk of renal scarring (Lee, Shim, Cho, & Lee, 2007).

The final example of local and distant site effects comes from an animal study which showed that ingestion of *L. acidophilus* NCFM, an organism used extensively in the USA as a 'probiotic' in dairy products, induced the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells, and mediated analgesic functions in the gut – similar to the effects of morphine (Rousseaux et al., 2007). When it was administered at a clinically relevant concentration (10^9 colony-forming units per day for 15 consecutive days), to *Balb/c* mice and Sprague–Dawley rats, expression of opioid receptor μ1 (MOR1) and cannabinoid receptor 2 (CB2) was detected in approximately 25–60% of epithelial cells. As pain and discomfort are often symptoms of irritable bowel syndrome, it would be interesting to test *B. infantis* 35624, a probiotic shown to relieve abdominal pain in these patients (Whorwell et al., 2006). The problem that needs to be addressed in terms of translating the animal studies to humans is that there have been no reports to date on alleviation of pain in people who consume *L. acidophilus* NCFM or any other probiotic on a daily basis. If this were found to be the case, it would clearly provide the dairy industry with a major avenue for product sales.

4. Current challenges and future advances

Enormous potential for diverse metabolic and physiological capabilities exist in all strains, as illustrated by the 230 genes involved in cell envelope function in *L. plantarum* and the extensive differences between the chromosome organization and gene content of this organism and *Lactobacillus johnsonii* (Boekhurst et al., 2004; Kleerebezem et al., 2003). Key studies in the near future will be ones that uncover the diverse functionalities of lactic acid bacteria, in real time, in the host following specific dietary challenges (Barrangou et al., 2006; Wang, Beggs, Robertson, & Cerniglia, 2002). Combined with an examination of human genome-level gene expression changes that occur at the microbial–host interface, it will be possible to design strains that target specific conditions or signaling pathways and confer specific benefits using systems biology approaches. The opportunity has never been greater for microbiologists to uncover some fundamental roles played by microbes in human development and long-term well being, and to develop novel ways to administer strains (probiotics) and nutrients (prebiotics) to counter adverse conditions.

The uncovering of new strains with probiotic potential will present interesting challenges for the dairy industry, as not all organisms survive in milk products, or produce a suitable, shelf-stable and flavourful product. The next generation of dairy products might contain *Lactobacillus helveticus* for enhanced anti-infective immunity (Vinderola, Matar, & Perdigon, 2007), or *Bifidobacterium adoloscentis* for anti-allergy effects, or *B. thetaiotamicron* for early childhood gut maturation and immunity (Wilks, 2007), or *Weissella cibaria*, isolated from humans and animals worldwide, as well as from fermented foods, and of potential use for oral health (Meurman & Stamatova, 2007). Such strains may presently be outside the realm of mainstream dairy products, but their development could further expand the market opportunities for the industry.

With the targeting of foods for health benefits outside general wellbeing, will come closer scrutiny from regulatory agencies of end points of such treatment. A growing area of interest is the impact that strains have on gene transfer, including antibiotic resistance traits (Mayrhofer et al., 2007), even though lateral gene transfer has played an important role in allowing gut-dwelling *Bacteroidetes* to vary their cell surface, sense their environment, and harvest nutrient resources (Xu et al., 2007). *L. casei* is perhaps the most commonly ingested probiotic, yet one study has raised a concern with regard to early administration of *L. casei* increasing lymphocytes in the lungs of female mice and eosinophils in the lungs of male mice, thereby increasing allergy symptoms (Ezendam & van Loveren, 2007).

A potential major problem for probiotics is the misuse of the term. This can arise from products being poorly manufactured, or being referred to as probiotic without any relevant documentation. The net effect, deleterious to the overall field of probiotics, might be that such products are found to be ineffective, when in fact they were not even probiotic in the first place. It is the responsibility of manufacturers and scientists to only use the term 'probiotic' as outlined in the United Nations and World Health Organization Guidelines (FAO/WHO, 2002). This outlines the following key points:

(i) Probiotics are well-defined bacterial types administered to the host in sufficient numbers at the end of product shelf life, to confer defined and proven physiological benefits. They are not commensal organisms found in the human gut.

(ii) Probiotics are not genera or species, such as *L. acidophilus* or *L. rhamnosus*. These are simply bacterial types, not probiotics until proven to confer a specific benefit. Products are often called 'acidophilus', but *L. acidophilus* is not only re-classified into *Lactobacillus gasseri*, *Lactobacillus crispatus*, *L. acidophilus*, *Lactobacillus gallinarum*, *Lactobacillus amylovorus*, and *L. johnsonii*, it is neither the dominant constituent of the intestinal microbiota, nor the main *Lactobacillus* species at that site. Furthermore, a review of PubMed and dairy literature fails to show convincing evidence that any strain of *L. acidophilus* meets the FAO/WHO standards of being a probiotic by conferring proven physiological benefits on humans. *L. acidophilus* NCFM, an organism used extensively in the USA as a 'probiotic' in dairy products, has been purported to benefit patients with lactose intolerance, but this is not supported conclusively by human studies (Sanders & Klaenhammer, 2001). One trial shows alleviation of small bowel bacterial overgrowth (Dunne et al., 1998), but this needs to be repeated. A *L. acidophilus* strain R0052 used with some success to prevent NEC in

newborns is actually a *L. helveticus* (Naser et al., 2006). The strain *L. acidophilus* La5 has been shown to confer health benefits, but only in combination with other strains. Meanwhile, *L. acidophilus* L1 lowered serum cholesterol in one treatment period but not the next (Anderson & Gilliland, 1999).

(iii) Viable numbers of probiotic organisms used in a product must be consistent with those tested successfully in a clinical trial. In other words, one cannot add 1000 colonies of *L. reuteri* SD2112 or another known probiotic which has been shown at a dose of 1 billion colonies to confer benefits, then call the new product a probiotic. If strains are combined, such as *L. rhamnosus* GR-1 and *L. reuteri* RC-14 (Reid & Bruce, 2006), addition of the second strain must be justified clinically.

(iv) The literature is strewn with experiments on 'probiotic' strains, many using in vitro adhesion or inhibition assays that do not prove functionality in vivo. Until these strains have been shown to fulfill the guidelines and confer health benefits on a host, they should be termed potential probiotic strains or simply bacterial strains.

(v) Genetically engineered bacteria can be probiotic, if properly documented. Studies have shown that a vaccine produced using constructs combining epitopes from mutans streptococcal glucosyltransferases (GTF) and glucan binding protein B (GbpB) has great potential to interfere with the development of caries (Smith, King, Rivero, & Taubman, 2005; Taubman & Nash, 2006). The creation of a *Lactococcus lactis* LL-Thy12 strain expressing human interleukin-10 (IL-10) is a development of potential clinical significance (Braat et al., 2006). The replacement of the thymidylate synthase gene with a synthetic sequence encoding mature human IL-10 provides a means to treat inflammatory bowel disease as well as contain the organism due to its inability to survive without exogenous thymidine added.

L. jensenii recombinants secrete 2-domain CD4 proteins to competitively bind HIV precluding it from attaching to host cells, and secrete Cyanovirin-N, a microbicide designed to inhibit HIV binding (Chang et al., 2003; Liu et al., 2006). The use of *L. jensenii* 1153 has been claimed to be preferable to *L. lactis*, *L. plantarum* or *L. gasseri*, which have been used by others to secrete cyanovirin-N (Pusch et al., 2005, 2006). However, neither *L. lactis* or *L. plantarum* are common inhabitants of the vagina, whereas *L. gasseri* is. In contrast, *L. reuteri* RC-14 has been shown to persist in the vagina for several weeks (Morelli et al., 2004; Reid et al., 2001) and 12 recombinant strains have been created, the first using chromosomal integration rather than plasmid expression (Liu, Reid, Jiang, Turner, & Tsai, 2007). These strains secrete 3 microbicides, PRO 542, a recombinant CD4-immunoglobulin G2, macrophage inflammatory protein 1b (MIP-1b), the normal ligand for CCR5, and T-1249, the 'next generation'

T-20-like peptide fusion inhibitor that retains activity against T-20-resistant HIV-1. In vitro studies showed inhibition of viral entry and killing of the virus. None of the 3 approaches to anti-HIV recombinants included a suicide gene system, and thus containment is not assured. This raises the question of whether or not all genetically modified bacteria created for human use should have a containment system.

(vi) In order to attain more widespread credibility amongst the scientific and clinical communities, products must contain speciated strains, sufficiently viable at end of shelf life, and with appropriate label claims (Reid, Kim, & Kohler, 2006). Differences in growth parameters and stress responses are observed among probiotic strains of the same species. Heat and oxygen tolerance, stress resistance, and other factors affect viability (Simpson, Stanton, Fitzgerald, & Ross, 2005). Studies are needed to assess the contributions that different delivery vehicles make to the efficacy of products. For example, dairy foods in which probiotic strains grow, will contain metabolic end-products, and it could be these substances, or prebiotic compounds in the milk, that induce biological effects.

Likewise for prebiotics, the necessary quantity and type of substance needed to confer health benefits must be defined in each case. At present, many products contain small amounts of inulin or fructo-oligosaccharides, without the clinical data to show if such amounts are sufficient for health benefits.

5. Conclusions

There is increasing optimism that the manipulation of the human microbiota through prebiotic use or probiotic administration, can provide significant health benefits including disease remediation or prevention. Dairy products provide a universal delivery system for prebiotics and probiotics, which augers well for market growth in the industry. However, these are not magic bullets, and expectations must not be raised for disease cure or even clinically observable changes, when in some instances down regulation of inflammation or reduction in cholesterol or cancer risk, may not be sufficient to interrupt a disease outcome. Advances in genomics and metabolomics provide a means to understand, and manipulate, the mechanisms responsible for microbes maintaining health or interfering with disease processes. Bioinformatic tools are necessary to help capture and interpret the mounting data from these studies, and identify key components that may improve the clinical outcomes. When probiotic organisms, particularly recombinant strains, are delivered in dairy formulations, the influence of the carrier (milk, cheese, yoghurt) must be examined. In child health, probiotic and prebiotic products have the potential to make the biggest impact as the gut, immune system and other organs are developing. Likewise, studies on this age group need to be closely monitored for short- and long-term safety. The distant site effects of probiotics are growing in number, and while cause and effect studies will prove difficult, they are essential to understand the scope of influence of these microbes. In the foreseeable future, probiotic and prebiotic products will contribute to the management and prevention of increasingly prevalent conditions such as allergies, hypercholesterolemia, obesity, HIV/AIDS and antibiotic resistant infections.

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