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Safe and efficacious probiotics: what are they?

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Each year, >20 billion doses of probiotics are used by healthy people and by those diagnosed with a range of medical conditions. Compared to many pharmaceutical agents, probiotics are well tolerated and extremely safe, and serious adverse effects rarely occur. Nevertheless, as many new researchers enter the field and companies launch 'probiotic' products, it is essential that standards are set for naming a product 'probiotic' to show that it meets an acceptable level of safety and efficacy, and to understand the strengths and limitations of its activity. In this Opinion article, recommendations are made based upon the current understanding of scientific, clinical and regulatory issues, with a special focus on safety.

Key to the future of probiotics

The number of scientific publications on probiotics has doubled in the past three years and this recent interest [1] has been further stimulated by several factors: (i) exciting scientific and clinical findings using well documented probiotic organisms; (ii) concerns over limitations and side effects of pharmaceutical agents; and (iii) consumer demand for natural products. All this has led to predictions of a tripling in sales by 2010 (European and US Probiotics Market research, 6 August 2003; www.frost.com). The key to the future of probiotics will be the establishment of a consensus on product regulation, including enforcement of guidelines and standards, appropriate clinical studies that define strengths and limitations of products, and basic science studies that uncover the mechanisms of action of strains. This Opinion article presents a personal viewpoint on these issues.

Definition and guidelines for use of the term 'probiotic'

A number of definitions of the term 'probiotic' have been used over the years but the one derived by the Food and Agriculture Organization of the United Nations–World Health Organization (FAO–WHO) [2] and endorsed by the International Scientific Association for Probiotics and Prebiotics [3] best exemplifies the breadth and scope of probiotics as they are known today: "Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". This definition retains the historical elements of the use of living organisms for health purposes but does not restrict the application of the term only to oral probiotics with intestinal outcomes. This is important considering that vaginal applications of probiotics have existed for >20 years [4]. The guidelines that stipulate what is required for a product to be called a probiotic were published by FAO–WHO in 2002 [5]. They require that strains must be designated individually, speciated appropriately and retain a viable count at the end of their shelf life in the designated product formulation that confers a proven clinical end-point. Although member nations were encouraged to use these guidelines, the fact that some products continue to be of dubious quality and claim health benefits that are not supported by appropriate, peer-reviewed human studies [6–8] suggests that many regulatory authorities are not yet aligned. Although companies often genuinely try to inform consumers of the attributes of probiotics, some might consider new approaches so that evidence-based outcomes take precedence and product recommendations can be supported by well-designed human studies (Table 1).

The importance of safety within the guidelines

Safety is the state of being certain that adverse effects will not be caused by an agent under defined conditions. The reciprocal of safety is risk. The issue of safety for any product is arguably paramount during pregnancy and in newborn babies. The best example of the safe use of probiotics during pregnancy is that of *Lactobacillus rhamnosus* GG, which was used in 132 women who were at high risk of their newborn babies developing atopic dermatitis [9]. Two interesting outcomes relevant to adults and children emerged from this study. There were no reports of adverse effects in the mothers, which indicated that ingestion of the probiotic was safe. This is further supported by the long-term use of this probiotic in Finland (since the late 1980s) and the low rate of cases of bacteremia potentially associated with its use [10] (<0.05 cases per 100 000 in Finland [11]). Nevertheless, cases of bacteremia have been reported following intake of this probiotic and some deaths have occurred in patients with severe underlying disease [12].

The question of how to relay a perceived risk on labels of products that contain *L. rhamnosus* GG or other strains remains unresolved, in part because it is not clear which type of person should be advised not to take a probiotic. The case for advising immunocompromised or seriously ill surgical patients against taking *L. rhamnosus* GG is countered by studies that show it can be used safely (twice a day for two weeks) in HIV/AIDS patients [13]. In addition, studies have shown the benefits of *Lactobacillus plantarum* 299v in patients undergoing major abdominal surgery [14]. Benefits

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Table 1. Usage of the term 'probiotic' and how companies could raise standards in line with FAO–WHO guidelines[a]

How the use of 'probiotic' might confuse consumers and physicians	Suggestions for improvements
'Contains *Lactobacillus sporogenes*'. (e.g. http://www.modernherbalist.com/products.html; http://www.thorne.com/pdf/journal/7-4/lactobacillus_monograph.pdf; see Ref. [48])	If a named bacterium is associated with a brand, the label should at least omit the use of a species that does not exist, and rename it as *Bacillus* or whichever actual species is identified by DNA–DNA hybridization or alternative molecular typing methods.
'Probiotic face cream and aftershave'. (e.g. www.natren.com; http://www.naturella.com.pl/product_info.php/products_id/1535)	These products should be renamed because they misuse the term 'probiotic' and create confusion among consumers, many of whom do not appreciate the need for viable health-conferring organisms in probiotic formulations.
'Product contains L. Acidophilus, L. Rhamnosus, L. Plantarum, B. Longum and B. Bifidum'. This implies that combinations are better for health. (e.g. http://www.customprobiotics.com/)	Bacterial species should be italicized, written in full (e.g. *Lactobacillus acidophilus*) with strain designations to enable literature citation of studies performed with this exact formulation. Websites such as the following provide reliable information: http://www.usprobiotics.org; http://www.isapp.net.
'Improved liver, joint, muscle and sleep functions'. (e.g. http://www.buyprobiotics.com/)	Randomized, placebo-controlled studies published in peer-reviewed journals and preferably confirmed by independent studies are needed to verify these and other such claims of health outcomes.
Addition of 'probiotics' to other product formulations, such as those with complex natural herbs, fibers or oils. (e.g. http://www.mannapages.com)	To avoid implying that products contain probiotics that confer specific benefits on the host, companies should define the strains present, their viable count at end-of-shelf-life and how their inclusion provides a proven clinical outcome beyond that derived from the other contents.
'Soil-based probiotics that fight pathological molds, yeast, fungus, viruses and parasites and stimulate B-lymphocyte and related antibody production'. (e.g. http://www.upwardquest.com/; http://www.health24.com/natural/Probiotics/17-1940,16776.asp)	Many products and web sites genuinely attempt to help consumers and provide reference to scientific studies. However, efforts need to be made to avoid a layperson thinking that certain products kill fungi or viruses and can treat such infections, unless proven scientifically to do so. Also, the concept of stimulating immunity is complex and care should be given to what this actually means in practical terms for any and all consumers who might use a certain product.

[a]The author has no specific knowledge of the products cited in the table and does not endorse them nor imply that the companies or products named are in any way unreliable. Rather, the examples illustrate several important points about probiotics.

have also been seen in patients with inflammatory conditions of the intestine who received the high-dose, eight-strain probiotic VSL#3 [15], *L. rhamnosus* GG [16], *Saccharomyces boulardii* lyo [17], and even a Gram-negative probiotic, *Escherichia coli* Nissle 1917 [18]. Nevertheless, invasive fungemias associated with *S. boulardii* lyo [19], and endocarditis apparently caused by *Lactobacillus paracasei* subsp. *paracasei* [20] and *L. rhamnosus* GG [12], demonstrate that a proportion of recipients of probiotics, however small, does seem to be at risk of adverse effects. The reasons for susceptibility in some individuals remain unclear. Regulatory agencies might consider requiring probiotic products to include an insert, which could state that anyone who has a serious underlying medical condition of the intestine or bloodstream should inform their physician that they are consuming a particular probiotic, and immediately report any episodes of fever, chills or vomiting that arise.

Although the Finnish study resulted in a significant reduction in babies born with atopic dermatitis, a small number of newborns who were administered *L. rhamnosus* GG during the first six months of life later developed asthma [21]. In other studies of premature babies treated with probiotics to prevent necrotizing enterocolitis and death, no such asthma cases were reported; safety was assessed against risk of disease and by the lack of adverse effects on height or weight-for-heights [22,23]. Future use of probiotics in newborns should have long-term end points (of at least five years) in an attempt to determine that no significant increased risk of conditions like diabetes, allergies or inflammatory diseases arises. Such studies would probably be expensive and logistically difficult, however, in countries like Finland and Sweden they could be feasible because probiotics are readily available and patients are often particularly well monitored in studies. Only then can the true risk–benefit analysis be assessed. The link between probiotics and safety also requires that true probiotic products are evaluated. In one case, the issue of probiotic safety was raised by authors who neither cited clinically proven products and the extent of their use, nor took into account underlying medical conditions in subjects in which adverse effects occurred [24]. This only damages the reputation of the research field and does not help to identify cause and effect [25].

Reducing the risk of adverse effects caused by probiotic organisms

A better understanding of the potential mechanisms whereby probiotic organisms might cause adverse effects will help to develop effective assays that predict which strains might not be suitable for use in probiotic products. Enhanced understanding will also improve guidelines for the use of specific products and will aid in pointing out clinical situations where probiotic use should be closely monitored.

Animal models

Animal models are commonly used to assess probiotic safety. These tend to use daily administration of large doses (10^9 colony-forming units) of probiotic organisms

to measure weight loss, intestinal inflammation, modification of cytokine levels in the ileum and colon, and bacterial dissemination through intestinal translocation in healthy and diseased animals [26]. These models have merit in identifying major effects on host tissues and induction of adverse behavioral effects but, as with all animal [27] and *in vitro* experiments, they do not necessarily predict the human situation.

Virulence factors

The extensive investigation of pathogenic microbes has identified factors that are clearly involved in pathogenesis. However, whereas adhesins on *E. coli* provide a means to infect the host [28], adhesins on lactobacilli might provide a mechanism to interfere with pathogenesis [29]. Thus, the presence or absence of adhesins per se is not useful to define a safe organism. Likewise, cell-wall components such as lipopolysaccharides can have toxic effects, yet probiotic *E. coli* Nissle 1917 and many *E. coli* in the healthy intestine clearly do not induce toxic effects. The requirement for commercialization of strains should be greater for species that are known to cause diseases in humans and harbor virulence factors (such as *Enterococcus, E. coli* and *Bacillus*) than for lactobacilli and bifidobacteria, which have long track records of safety in foods [2,5]. Still, consideration must be given to the end goal of the product. For general use in otherwise healthy subjects, ideally, probiotic strains should not express toxins and transmissible drug resistance genes. But if a probiotic was developed as a drug with the intention of inducing an immune response designed to fight disease and was administered with an antibiotic, the properties of the organism might well require that it has drug resistance genes and cell-wall adjuvants.

Immune effects

Bifidobacterium lactis BB12 can transiently trigger innate signal transduction together with interleukin (IL)-6, NF-κB RelA and p38 MAPK gene expression in the intestinal epithelium in the early stages of bacterial colonization [30]. This could well promote innate immunity, but studies are needed to determine if strains such as BB12 induce inflammation in immunosuppressed animals or in cases where antibiotics have destroyed a large population of the indigenous or autochthonous microbiota.

Cell-wall components of Gram-positive probiotic strains have been examined for their immunopotency and as anticancer agents. Peptidoglycan from *Lactobacillus* activates Toll-like receptor (TLR)-NF-κB and Jak-STAT signaling pathways, promoting a Th-1 anti-tumor response [31]. Furthermore, *L. rhamnosus* GR-1 can induce anti-inflammatory effects in macrophages, which cause suppression of TNF-α (H.I. Sheikh *et al.*, unpublished). It has been proposed that peptidoglycan recognition proteins can distinguish between peptidoglycans from different bacterial species, based upon the composition of the peptide stem and sensing of different peptide bridge components that crosslink the stems [32]. Together, these studies indicate a strong host-receptor activity, which not only detects certain organisms (such as probiotic lactobacilli) but also provides a means for these organisms to protect the host through innate immunity and fight off disease through the induction of inflammatory or anti-inflammatory responses. Thus, studies that use induction of inflammation as a parameter for safety assessment must carefully determine the inflammatory or anti-inflammatory factors that are induced and the end result of the process, with respect to what the probiotic is designed to do for the host. In some cases, the aim will be to enhance immunity to counter cancer or infection, whereas in other cases the aim could be to downregulate inflammatory processes. To some extent, animal models can predict the types of responses humans might have to probiotics but, ultimately, the only true test is to study the human response itself.

Drug resistance and biofilms

Enterococcus faecium [33] is commonly used in animal probiotics to modulate immunity by decreasing the adhesion molecule sICAM-1 in addition to CD54 (on monocytes) and CD11b (on lymphocytes). A major concern with any probiotic strain (but especially enterococci) is that they often carry transmissible antibiotic-resistance determinants. In particular, enterococci harbor vancomycin resistance, which can be passed on to other organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) [34]. This has led to recommendations that safety checks are carried out on human probiotic strains to ensure that no such transmissible genes are present [35]. In the case of some *Bacillus* 'probiotics' promoted for their ability to survive and proliferate, these should not contain virulence-associated genes that can aid in their spread to other individuals or to distant sites in the body [36]. One such factor found in *Bacillus subtilis* is guanidino kinase, which is involved in biofilm formation [37]; other factors are Fur box genes, which are involved in bacterial iron uptake and metabolism [38]. However, because virulence is the result of a complex process and usually involves tightly regulated gene expression that affects multiple factors, the presence of a virulence-associated gene in a bacterium does not necessarily imply virulence or pathogenesis. Nevertheless, testing for known virulence genes, particularly those present in transmissible forms, should be a requirement for strains that have the potential to cause infections [2].

Use of species known to cause disease

It is not uncommon for strains of *E. coli* to be found in the newborn gut following transmission from the mother during childbirth [39]. Recently, in the Czech Republic, a 'probiotic' *E. coli* strain was deliberately administered to newborns and was found to stimulate local and serum antibody responses and reduce pathogen colonization of the intestine. At follow-up after ten years, treated patients had a lower frequency of repeated infections [40]. Another study using an avirulent *E. coli* strain instilled intravesically (directly into the bladder) has shown success in reducing symptomatic urinary infections in patients with spinal cord injuries [41]. Given the potential probiotic attributes ascribed to other Gram-negative organisms (e.g. strains of *Bacteroides* that induce angiogenesis and immune development in newborn animal studies [42,43]), it seems likely that other Gram-negative probiotics will also eventually become available for human use. If so, as

with *Enterococcus, Bacillus, Saccharomyces* and *E. coli* probiotics, it will be important to introduce long-term monitoring protocols both at the manufacturing site and where patients are assessed, to ensure that no virulence determinants invade the strains or induce serious adverse reactions. In the case of *Bacteroides*, further monitoring will be required, especially in diabetic and surgical patients, because these strains have lipopolysaccharides that signal through the TLR-2 and TLR-4 pathways [44,45] and can induce endotoxicity in the host.

In summary, the onus is on both the producer and regulatory agencies to determine the minimum requirements for safety testing of probiotic strains. The absence of known virulence genes (especially those producing toxins, proteases and hemolysins that can adversely affect human cells) and transmissible drug-resistance genes should be a minimal requirement for pre-human testing. In my view, testing should also take the end-product formulation into consideration because this can induce adverse effects in some subjects (e.g. milk and lactose intolerance) or negate the positive effects altogether.

Concluding remarks and future perspectives

For the most part, probiotic strains are safe and well tolerated by humans of various ages. Rare cases of bacteremia have arisen and have been successfully treated with antibiotics, except in some patients with serious underlying diseases. No generalized warnings come with probiotic products and, at present, it would be difficult to deduce which warnings, if any, would be appropriate. Nevertheless, product labeling should contain more specific guidelines for consumers. These could include recommendations to inform physicians about the preferred use of a product, information about side effects such as gas production or loose stools (which might temporarily affect some people), and a warning that adverse side effects such as fever, diarrhea and vomiting require prompt referral to a physician. For probiotic use in critically ill and immunocompromised patients, labels should specify upper and lower dosage limits and any adverse effects should be noted in pre-launch clinical trials. Antibiotic sensitivity patterns should also be noted in case eradication of the probiotic organism is required.

It is unacceptable that regulatory agencies allow products to be called probiotics without appropriate clinical documentation. Likewise, it is unacceptable for products not to have end-of-shelf-life viability counts appropriate for the claims that are based upon clinical trials. As new probiotics emerge alongside genetically modified organisms (GMOs) that are designed specifically to treat disease [46], long-term monitoring will be important to insure that safety issues and (in the case of GMOs) proper environmental containment issues are addressed. More clinical studies must be performed, preferably comparing one probiotic product against another or against standard medical practice [47]. In this way, the strengths and limitations of probiotics will be determined. Documentation of proven clinical efficacy and known mechanisms of action in addition to clearly outlined dosage, duration of use and safety parameters will enable caregivers to recommend products and enable consumers to purchase probiotic foods and over-the-counter products with a high level of confidence.

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