PROBIOTICS: strains matter

The tendency to generalise about 'probiotic' effects is widespread. Underlying such generalisations is the erroneous assumption that the body of research on specific probiotic strains can be applied to any product marketed as a probiotic. MARY ELLEN SANDERS, PhD, explains why not just any probiotic will do for your next product launch

Probiotics are live microbes that, when administered in adequate amounts, confer a health benefit on the host.[1] There is mounting awareness in the United States about probiotics, spurred in part by a growing number of high-profile probiotic-containing products, such as Dannon's successful Activa yoghurt. The potential of this product category seems evident, especially when considering the success of such products in Europe and Asia. One report estimated that more than 100 companies in the United States market probiotic supplements and approximately two million US adults use them.[2]

Furthermore, there are many people who stand to benefit from efficacious probiotic products. Studies documenting effects on a variety of gastrointestinal disorders including irritable bowel syndrome (IBS), vaginal infections and immune enhancement resulting in getting sick less often are compelling reasons for including probiotics as part of a healthy diet.[3-5]

PROBIOTIC COMPLEXITIES

When considering this product area, a few guidelines can help provide context for understanding the complexities of the science and use of probiotics.

Although a consensus scientific definition has been advanced, no legal definition of the term 'probiotic' exists. The term unfortunately can be (and is) used on products that do not meet the minimum criteria that the probiotic be alive, delivered in adequate dose (through the end of shelf life); and shown to be efficacious in controlled human studies.[6] The 'truthful and not misleading' FDA standard for content and support of structure/function label claims on products is not, in practice, enforced by the FDA. Therefore, it is incumbent on the industry to maintain integrity in formulation and labelling of these products so that consumers can be confident in this product category.

There is little scientific evidence that in order to qualify as probiotics, these beneficial bacteria must demonstrate specific physiological attributes such as be of human origin, adhere to intestinal cells or produce bacteriocins. Although many have used these criteria as a basis for selection of strains 'appropriate' for use as probiotics, no studies have compared isogenic strains (ie, strains that are identical genetically except with altered capacity for one specific attribute) with and without these traits in humans

FORMULATORS

WHAT LEVELS OF PROBIOTICS NEED TO BE DELIVERED IN PRODUCTS?

Probiotic levels used in product offerings must be based on levels found to be efficacious in human studies. Although it is tempting to offer a general recommendation for a minimum amount of probiotic that is needed to be effective, the reality is that such generalisations cannot be accurate.

This is because efficacious doses vary widely in documented human studies. For example, several studies of *L. reuteri* SD2112 and of *B. infantis* 35264 have documented that $1x10^8$ (100 million)/day is an adequate dose for several different health targets. However, the product VSL#3 is recommended at $1.8x10^{12}$ (1.8 trillion)/day for management of recurrence of certain inflammatory bowel conditions. This is over a four-log cycle difference in recommended dose.

It is likely that the required dose is dependent on a variety of factors, including physiological characteristics of strains being used, types of clinical endpoints being tracked, whether the endpoint is prophylactic or therapeutic, length of time of administration of probiotic, and if other bioactive ingredients are used in conjunction with the probiotic.

Unlike other food/nutrient issues, where a company can get away with putting in one-fourth the daily recommended amount with the understanding/hope that a consumer will get nutrient sources from places other than the specific functional food, there are not many other natural food sources to attain probiotics.

Food manufacturers should therefore include the required efficacious amount in each serving.

Finally, probiotic product labels should indicate the levels of each strain in the product – through the end of shelf life. This information should be tied directly to scientific publications that document that this formulation is efficacious. Labelling of levels 'at time of manufacture' is inadequate and has been shown to be linked to products less likely to meet label claims (www.consumerlab.com/results/probiotics.asp).

–*MES*

to determine their significance. Until such studies are conducted, it is more productive to focus on proof of their ability to improve human health, regardless of mechanism. Notably, one research group did conduct a study of colitis in mice comparing physiological effects of isogenic strains of *L. crispatus* which differed only in their ability to auto-aggregate (or clump).[7] They found that the aggregating strain, but not the non-aggregating mutant or heat-killed aggregating strain, reduced severity of colitis.

Another attribute that is sometimes questioned is the requirement that probiotics be alive. It is true that some research shows beneficial effects from cells killed by heat or radiation.[8] By definition, however, these substances do not qualify as bona fide probiotics. Studies have demonstrated superior activity of live compared to killed probiotics, in in vitro and in human studies.[9-11] Even though not all studies show an advantage to viability;[12-13] probiotics by definition must be administered alive.

STRAIN-SPECIFIC EFFECTS

There is one particularly important consideration for probiotics: strain-dependent effects. Just as different breeds of dogs have attributes that are distinguishing, so too different strains of even the same species of bacteria may have different probiotic functions.

The scientific rationale that effects must be considered strain-specific is based mostly on in vitro and animal data where strain differences are evident. Attributes such as acid tolerance, sensitivity to therapeutic antibiotics, bile resistance, lactase activity, hydrogen-peroxide production, growth on prebiotics, genetic accessibility, production of antimicrobial compounds and stability in product have all been tested for a variety ▶

STRAIN	HUMAN DATA
Tested as single strains	
L. rhamnosus GG	Immune enhancement, infectious diarrhoea in children, primary prevention of atopic dermatitis
B. lactis BB-12	Immune enhancement, diarrhoea in children
L. reuteri SD2112	Reduced absences from work, diarrhoea, immune function
B. infantis 35624	Irritable bowel syndrome (IBS)
L. casei DN114-001	Immune enhancement
B. longum BB536	Allergy symptoms, intestinal micro-ecology
L. acidophilus NCFM	Symptoms of lactose intolerance, reduced small-bowel bacterial overgrowth
B. lactis HN019 (DR10)	Immune enhancement, especially in elderly
B. animalis DN173-010	Normalizes intestinal transit time
L. plantarum 299V	IBS, post-surgical gut nutrition
Lactobacillus casei Shirota YIT9029	Superficial bladder-cancer recurrence, intestinal microbiota, immune enhancement
L. salivarius UCC118	Inflammatory bowel disease
L. johnsonii La1 (Lj1)	immune function, *Helicobacter pylori* eradication
Escherichia coli Nissle 1917	Immune function, intestinal health
Saccharomyces cerevisiae (boulardii) lyo	Antibiotic-associated diarrhoea, *Clostridium difficile* infections
S. thermophilus (most strains)	Symptoms of lactose intolerance
Tested as blends	
L. rhamnosus GR-1 + L. reuteri RC-14	Oral consumption leads to colonization of vaginal tract and improved therapeutic outcome for women being treated for bacterial vaginosis
VSL#3 (8 strain blend of *S. thermophilus*, four strains of lactobacillus and three strains of bifidobacterium)	Inflammatory bowel conditions
L. acidophilus (CUL60) *B. bifidum* (CUL 20)	Reduction of C. *difficile* toxin in feces
L. helveticus R0052 *L. rhamnosus* R0011	*H. pylori* eradication, diarrhoea in children

SELECT SUPPLIERS

PROBIOTICS AND PREBIOTICS ARE GROWING QUICKLY

Biogaia: Reuteri culture comes in three different, producer-friendly forms: freeze-dried powder, freeze- dried DVS (Direct Vat Set) granules, and frozen pellets.
www.biogaia.com

Chr Hansen: The nu-trish brand probiotic culture range consists of Probio-Tec, Yo-Fast and other nu-trish culture blends with well-defined viscosity profile that ferment quickly.
www.chr-hansen.com

Danisco: Cultures division produces, develops and markets starter cultures, media, coagulants and enzymes for cheese, fresh dairy and other food products, and also supplies probiotic cultures for foods and supplements as well as natural food protectants.
www.danisco.com

DSM: Lafti line of probiotics are formulated for stability, survivability and concentration, and contains *L. acidophilus* (Lafti L10), *L. casei* (Lafti L26), and bifidobacterium (Lafti B94).
www.dsm.com

GTC Nutrition: NutraFlora short-chain fructo-oligosaccharides (scFOS) are a cane- or beet sugar-derived natural prebiotic fibre.
www.gtcnutrition.com

Jintan: Custom-makes triple-layered, enteric, seamless capsules specifically for probiotic supplements.
www.jintanworld.com

Lallemand: Canadian supplier delivers novel, high-quality probiotics and biosupplements to the nutraceuticals, functional-foods and pharmaceuticals industries.
www.lallemand.com

National Starch: Hi-Maize brand corn-based resistant starch has multiple benefits, among them it acts as a prebiotic for digestive health.
www.hi-maize.com

Nutraceutix: Bio-tract protects probiotic tablets from acids, LiveBac extends shelf life of probiotics, and 20 strains are offered by the company, which controls manufacturing processes from start to finish.
www.nutraceutix.com

Orafti: BeneoSynergy1 is the unique, patented oligofructose-enriched inulin prebiotic used in the landmark SynCan project on synbiotics and colon cancer.
www.orafti.com

Probi: Biotech company develops and patents probiotic strains, among them *L. plantarum* 299v and *L. rhamnosus* 271. *L. plantarum 299* has not yet been commercialised, but it is in the out-licensing phase.
www.probi.com

Roquette: Nutriose is a range of soluble fibres that are resistant corn dextrin with 85 per cent fibre content (dry substance), which studies show matches up well with probiotics *Streptococcus thermophilus* and *Lactobacillus bulgaricus.*
www.nutriose.com

Sensus: Frutafit inulin and Frutalose fructo-oligosaccharides (FOS) are soluble dietary fibres with bifidogenic/prebiotic properties, suitable for a variety of food systems to enrich fibre, reduce calories, and replace sugars and fats.
www.sensus.us

Valio: *Lactobacillus rhamnosus* GG probiotic is the most researched in the world and was recently licensed to Dannon for the US yoghurt market. The Gefilus family containing LGG is marketed worldwide.
www.valio.fi

of strains in vitro.[14,19] Among tested strains, differences are clear. Frequently such testing compares strains of different species, but less commonly comparison of multiple strains of the same species has been conducted in in vitro tests.

In animal models, differences in responses evoked in tests of immune function are apparent. When one strain of each of *Lactobacillus salivarius* Ls-33 and *Lactobacillus rhamnosus* were tested via oral administration in a mouse model of colitis, researchers observed significant reduction in inflammation. However, one strain each of *Lactobacillus acidophilus, Lactococcus lactis* and *Streptococcus gordonii* showed no improvement.[20] The importance of testing specific strains for effects is further emphasised in a study that documented that a strain of *L. paracasei* isolated from an endocarditis patient actually worsened colitis in an animal model of severe inflammation.[21]

It is possible to visualize differences among strains of the same species at the DNA level as well. The chart on page 35 illustrates such differences among several strains of *L. crispatus.* Such results are typical among strains of lactobacillus species. Interestingly, findings with commercial bifidobacterium strains suggest more genetic similarity among strains of the same species.

In one recent assessment, researchers found that among 39 independent isolates of *B. animalis* subspecies *lactis* strains from commercial products, only four different types were identified, based on pulsed field gel electrophoresis (PFGE).[22] This finding may reflect a de facto greater similarity among strains of the same bifidobacterium species, that commercial products contain the same strains, or that differences are not evident from this type of chromosomal analysis. Interestingly, large strain-specific differences in immunopotential were observed among different strains of the same bifidobacterium species.[23]

STRAIN-SPECIFICS: HUMAN DATA

Head-to-head comparisons of different strains in human studies are rare. For example, *B. lactis* BB-12 was compared to *L. reuteri* SD2112 and to a placebo in a study determining the impact of supplementing ▶

infant formula with one of these strains at identical doses on incidence, symptoms and absences due to intestinal or respiratory infections in infants in day-care centers.[24] Both strains showed statistically significant improvements over the placebo control; however, the *L. reuteri* group outperformed both BB-12 and the placebo control, with significant decreases in number of days with fever, clinic visits, child-care absences and antibiotic prescriptions. The rate and duration of respiratory illnesses did not differ significantly among groups.

In another clinical study, *B. infantis* 35624 was compared to *L. salivarius* UCC4331 for its ability to reduce symptoms of irritable bowel syndrome.[25] *B. infantis* 35624 improved symptoms, whereas *L. salivarius* UCC4331 did not.

What these studies do not tell us is if two strains of the same species would have performed equivalently. However, it cannot be presumed that they will.

The implications of the strain-specificity of effects are:

1. Documentation of health effects must be conducted on the specific strain being sold.
2. Review articles that discuss the many studies done on specific strains are not sufficient evidence to support health effects of an untested strain.
3. Studies that document efficacy of specific strains at a specific dose are not sufficient evidence to support health effects at a lower dose *(see 'Formulators' sidebar, page 34).*
4. The role of carrier in delivering functional benefits is not well understood (*see 'R&D' sidebar, below*).

This issue is complicated by the fact that the mechanisms that lead to specific health ▶

R&D

PREBIOTICS AND PROBIOTICS: WHICH MATCH UP BEST WITH EACH OTHER?

Prebiotic fibres are a recent and novel food concept with a simple proposition: you are what you eat. In this case, the 'you' refers to probiotic bacteria living in the human gastrointestinal tract.

Fledgling research over the past five years indicates that certain energy substrates work better than others in making probiotics more numerous and healthier – with ultimate benefits seen in their human hosts. Knowledge of the effect that prebiotics have on the viability of the probiotics will allow the rational design of synbiotic combinations.[1]

National Starch's Hi-Maize brand resistant starch was studied by Australian researchers, who examined 40 different bifidobacterium isolates to see which matched up best with the functional fibre. DSM's Lafti brand *B. lactis* B94 proved best.[2]

"It basically comes down to what the bacteria are going to use as food sources," says Rhonda Witwer, National Starch's business development manager, nutrition. "If they have good food sources they'll produce a fermentation by-product that promotes health, and if they don't have a good starch-based or carb-based food source, they'll use protein and produce ammonia and phenols and things that are not as healthy."

Witwer noted that while prebiotic stalwarts inulin and fructo-oligosaccharides (FOS) provide unique benefits such as calcium absorption, Hi-Maize has demonstrated effects on insulin sensitivity and genetic expression of satiety hormones, which address diabetes and weight management.

"In terms of prebiotic fibres, there's nothing to stop a formulator from using Hi-Maize and inulin/FOS to get the best of both ingredients," Witwer says. "Different fibres produce different results because they are producing different ratios of short-chain fatty acids and fermentation by-products."

Another study assessed which cereal substrate (malt, barley, wheat) worked best for the growth and metabolic activity of four probiotics – *L. fermentum, L. reuteri, L. acidophilus and L. plantarum*. Sugar content, amino nitrogen concentrations and, most importantly, pH levels, were the main determinants of strain health. The malt medium supported the growth of all strains more than barley and wheat, while *L. plantarum* and *L. fermentum* were more resistant to acidity than the other two strains.[3]

The SynCan project, a major European human study published in February 2007, validated the synbiotic thesis that adding prebiotics to probiotics gives not just an additive but a bona fide synbiotic effect – meaning better results using less of each functional ingredient than larger quantities of either alone.

The study used Orafti's BeneoSynergy1, which is a patented oligofructose-enriched inulin fibre, as prebiotic food source. Researchers combined it with *Lactobacillus* GG and *Bifidobacterium* Bb-12. Results of this 12-week human clinical showed a favourable effect on a number of early markers of colon cancer.[4]

"Synergy1 is a next-generation product – it is designed to have a range of molecular chain lengths so you get a staged digestion, like a timed-released reaction," explains Joseph O'Neill, executive vice president of sales and marketing at Orafti.

"Oligofructose on its own is fermented quickly, whereas the longer chain length inulin takes longer to break down and so it makes its way further down the colon. It creates a lower pH throughout the colon."

It is still down the road a ways before research is able to definitively pair up specific prebiotic fibres with a preferred probiotic. But research is continuing apace, and product developers are looking more keenly at the accumulating research to develop foods with a maximum health profile using the fewest value-added functional ingredients. The synbiotic paradigm promises as much.

– *Todd Runestad*

effects are often not known. When these are better understood, it may be possible to predict functionality in vivo. Certainly there are some physiological characteristics that are present in essentially all strains of given species. Such physiological similarities contribute to their grouping into the same species. For example: reuterin production by *L. reuteri*, although levels produced vary by strain;[26] high in vivo lactase activity in strains of *S. thermophilus*;[27] lactate production by all lactobacilli and acetate production by bifidobacteria. However, how such physiological or metabolic characteristics are expressed in vivo among different strains drives the need to confirm functionality in the target host.

Taken from a different perspective, the current body of published literature suggests that similar effects are observed for a variety of different strains. Effects on diarrhoeal illnesses, enhanced immune responses and improvement of symptoms of lactose intolerance are associated with more than one strain. Therefore, multiple strains of the same species may in fact have functional traits in common. But they may not. So again, studies on the specific strains are still needed.

BE PRO-ACTIVE

The tendency to generalise about 'probiotic' effects is widespread. Underlying such generalisations is the erroneous assumption that the body of research on specific probiotic strains can be applied to any product marketed as a probiotic. Although it is cumbersome to always need to define what strains at what doses are known to lead to health effects, it is essential to do so to prevent misrepresentation of a product.

In addition, commercial products that are marketed with no specific human studies documenting effects should not be marketed as probiotics, but perhaps as 'potentially beneficial cultures.' The term 'probiotic' should be used only for products composed of microbes that are alive, delivered in adequate dose (through the end of shelf life), and shown to be efficacious in controlled human studies (*see box, page 35*). As mechanisms leading to probiotic effects are better understood, perhaps extrapolation of results from certain strains to strains possessing the pertinent biological and physiological traits will be possible. Certainly, genomic sequencing efforts will grease the wheels for such advances.[28]

For more information, see www.isapp.net and www.usprobiotics.org.

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