Probiotics Retailer Education White Paper

Introduction

Probiotics are a unique class of ingredients that continue to gain popularity with consumers due to growing product innovation and the developing body of scientific research demonstrating a variety of health benefits. The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) define probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[1]

The human body plays host to trillions of microscopic live organisms, including bacteria, yeast, and fungi— many are beneficial, though some may be harmful. These organisms, which live on and in the body (such as on the skin and in the gut), comprise the human microbiome. Humans depend on these organisms, interacting with their organs and bodily systems, for a broad range of activities (e.g., digestion, immune health, supporting the gut-brain axis).

Probiotics are known to help the body in a number of ways, including by fighting off excessive "bad" bacteria and restoring a healthy microbial balance. Each region of the body has its own microbiome that can be disrupted by lifestyle (e.g., stress level), diet (e.g., alcohol intake), and use of antibiotics. Probiotics can modify niche microbiomes to help maintain healthy digestive and immune systems as well as healthy upper respiratory and urinary tracts and the oral cavity. Probiotics can limit the growth of "bad" microbes by outcompeting them and support existing microbiomes.

There are probiotics for many life stages and lifestyles: they can help infants to properly develop a healthy digestive and immune system, they can support the immune system of school-aged children, they can promote the growth of *bifidobacteria* in the colon of the elderly for proper digestion, and can assist with post-workout recovery and keeping the immune system ready to reduce body fatigue that comes from constant training.

Ingredients often associated with probiotics include prebiotics, postbiotics, and paraprobiotics. Table 1 defines each of these ingredient categories. The common theme is that all of these ingredients are expected to ultimately confer a health benefit and support a healthy microbiome.

Table 1. Definition of probiotic, prebiotic, postbiotic, and paraprobiotic

Term	**Definition**
Probiotic	Live microorganism which when administered in adequate amounts confer a health benefit to the host.[2]
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit.[3]
Postbiotic	Functional bioactive compound, generated in a matrix during fermentation, which may be used to promote health, including metabolites, short-chain fatty acids (SCFAs), microbial cell fractions, and functional proteins.[4]
Paraprobiotic	Inactivated microbial cells or cell fractions that confer a health benefit to the host.[5]

Benefits - beyond gut health

Different probiotic species and strains have unique bioactivities and provide varied health effects. Each probiotic (or combination of probiotics) may affect different systems in the body (digestive, immune function, brain health, heart health, women's health, etc.). See Table 2 below for examples.

Table 2. Select probiotics and their health benefits

Genus	Species	Benefit
Lactobacillus	*acidophilus*	Immune and vaginal health
	casei	Digestive health
	paracasei	Immune, digestive, and brain health
	plantarum	Brain health
	reuteri	Heart health
	rhamnosus	Immune and digestive health (infants and children); Immune and women's health
Bifidobacterium	*bifidum*	Immune health
	breve	Immune and digestive health
	infantis	Digestive health
	lactis	Immune and digestive health
	longum	Digestive and brain health
Bacillus	*coagulans*	Digestive health
	subtilis	Digestive health
Saccharomyces	*boulardii*	Digestive health
Streptococcus	*salivarius*	Oral health
	thermophilus	Digestive health
Pediococcus	*pentosaceus*	Digestive and infant health
	acidilactici	Digestive, oral, and immune health

Supported by a long history of safe use and rigorous clinical trials, probiotics are most widely known for their role in maintaining digestive health. However, emerging science show benefits of specific organisms for immune function, brain health, oral health and heart health, among others.

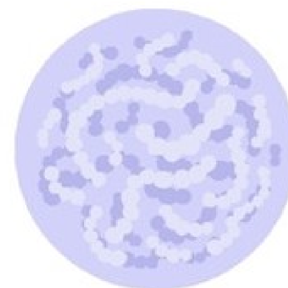
- ***Lactobacillus***: *Lactobacillus* is arguably the most well-known probiotic genus, although a recent taxonomic exercise comparing full genomic sequences resulted in the reclassification of many of the traditional *lactobacillus* species into 23 new genera.[6] As a byproduct of glucose metabolism, *Lactobacillus* species produce lactic acid, which has a variety of benefits to the human body including modifying the local pH around the colony, thus helping to stymie the growth of pathogenic microbes such as *H. pylori* and *E. coli*. *Lactobacillus* have been clinically studied for a variety of health benefits, including digestive health,[7] immune health,[8] lactose digestion,[9] iron absorption[10], vaginal health,[11] heart health,[12] mood and stress,[13] and skin health.[14]

- ***Bifidobacterium***: *Bifidobacterium* are the most prevalent food-based probiotics in the digestive system, where they primarily occupy the colon. *Bifidobacterium* levels are highest during infancy but gradually decrease over time due to factors such as diet, stress, and antibiotics, providing supplemented probiotics ample opportunity to assist in maintaining ideal levels. *Bifidobacterium* species have been primarily studied for digestive[15] and immune system health,[16] although research has shown benefits for glucose metabolism,[17] stress management,[18] weight management,[19] endurance,[20] and skin health.[21]

- ***Bacillus***: As a spore-forming bacteria, *Bacillus* spores act like a seed, protecting the bacteria's genetic makeup until they are provided with the proper conditions to germinate into a live cell. Because of this, *Bacillus* species are very stable and work well for gummies, chocolates, and other finished products that undergo harsh manufacturing or preparation conditions. While

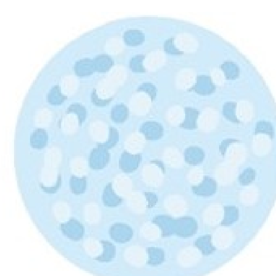
Bacillus are not known to colonize the intestine, they are a natural member of the gut microbiome, reproduce within the gut, and exert their effects as they travel through the digestive system. *Bacillus* benefits are generally seen in digestive health,[22] immune health,[23] mood,[24] protein absorption,[25] oral health,[26] and sports nutrition.[27]

- ***Streptococcus***: The most well-known of this genera is *Streptococcus thermophilus*, due to its use as a starter culture for many fermented dairy products and yogurt.[28] In addition to digestive benefits, *S. thermophilus* can also assist with blood sugar regulation[29] and liver health.[30] Another *Streptococcus* species, *S. salivarius*, is a prominent member of the oral cavity microbiome and has been shown to positively affect oral health by outcompeting other pathogenic *Streptococcus* species.[31]



- ***Saccharomyces***: Unlike most other probiotics, which are bacteria, *Saccharomyces* is a yeast. The most well-known is *Saccharomyces boulardii*, which can assist with digestive health.[32] *Saccharomyces* probiotics are typically more stable than traditional bacteria as well but can be difficult to quantitate via plate count enumeration (see Labeling section below for more information on measuring probiotic quantity).

- ***Pediococcus:*** *Pediococcus* have been historically used for fermenting sausage[33,34], sauerkraut[36], and other foods[35,36]. Benefits of *P. acidilactici* include addressing gut sensitivity[37,38], lactose sensitivity[39], gum health[40], and immune health[41,42]. Also, research shows that a specific strain of *P. acidilactici* helps with colic and microflora modulation in infants[43,44].



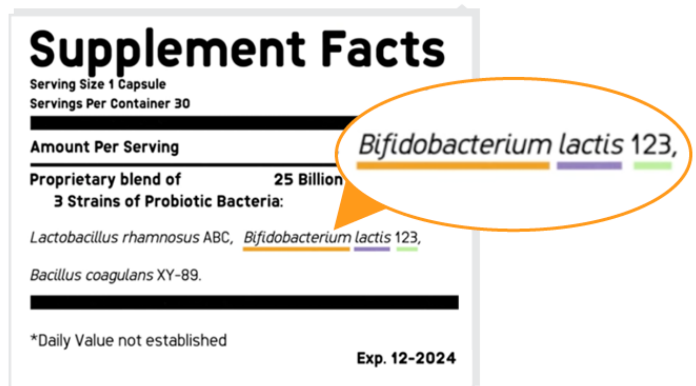
Some probiotic products contain one strain, while others may have many. A potential benefit of having multiple strains is to reach several health targets or facilitate additive effects in one health area. In addition, with every individual having a unique microbiome, multiple strains in a product increases the likelihood that one or more of the strains will resonate positively with a greater percentage of individual microbiomes.

Labeling

Proper labeling of probiotics comprises three components: **identity, quantity, and viability.**

Identity

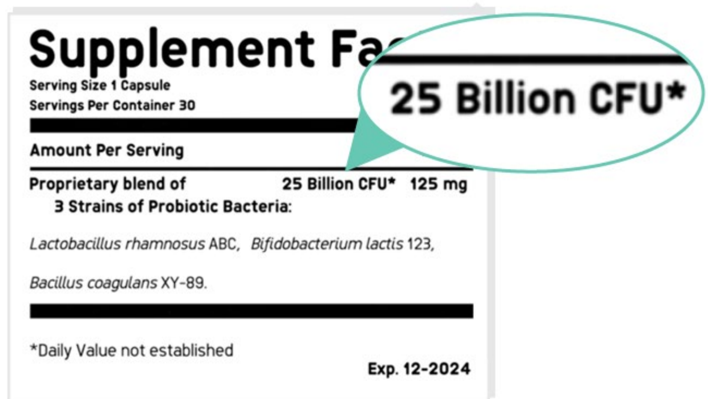
First, the label should identify the genus, species, and strain for each microorganism in the product. Not all probiotic strains are alike or serve the same function in the human body. Various probiotic strains may operate differently and affect different bodily systems (digestive, cognition, immune system function, etc.). While strain attributes and benefits may overlap, one cannot assume that research on one strain of probiotic applies to another strain, even of the same species. For example, *L. rhamnosus* GG and *L. rhamnosus* HN001 are strains within the same species that modulate immune health but their relevant doses differ.[45] Moreover, *B. lactis* HN019 has evidence of promoting digestive and immune health[46] while the *B. lactis* Bl-04 strain has been studied primarily for effects on immune function.[47] Identifying probiotics with strain specificity is essential for understanding the health benefit conferred.



Quantity

Second, the quantitative amount(s) of probiotics in a product should be expressed to reflect live microorganism count, such as Colony Forming Units (CFUs).[3] Often, a probiotic capsule contains millions or billions of live microorganisms – too many to count directly. However, a method exists to derive the number of live microorganisms by counting the colonies they produce. Each live microorganism is a CFU because it can self-replicate and give rise to a colony; colonies can be grown in a laboratory setting and can be feasibly counted. To enumerate CFUs in a given sample (e.g., capsule), the sample is diluted and plated, and microbial colonies are allowed to grow. The number of colonies is counted and plugged into a formula to derive the number of viable microorganisms, or CFUs, in the sample.

The CFU is currently the most prevalent scientifically accepted unit of measure for probiotics, used by scientific researchers, FDA, and other governmental organizations. Other commonly used units of measure include Active Fluorescent Units (AFUs) and live cells, which are measured by flow cytometry. Label descriptions that are used interchangeably with CFUs and live cells include live cultures, active cultures, and active cells.

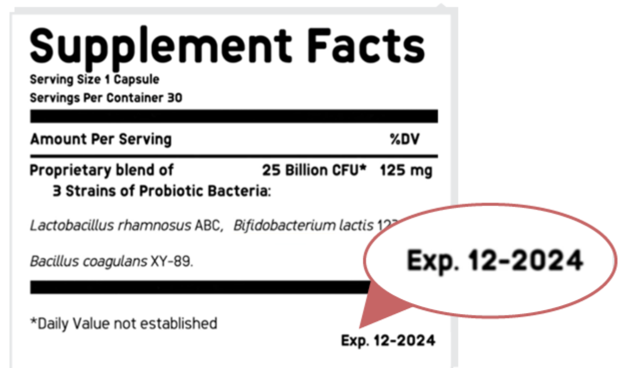


Measuring probiotic quantity by weight (such as in milligrams), as FDA regulations currently require, does not provide information on the number of live microorganisms in the product. In addition, some of these probiotics may die over their shelf life (see next section for more information on shelf life), and it is not possible to distinguish the weight of live microorganisms from that of dead microorganisms or from that of the fillers used to standardize the probiotic raw materials. If a label lists probiotic quantity by weight, it would be impossible for consumers to understand how much of the product is actually live microorganisms that confer a benefit. In addition, the number of live microorganisms expressed as CFUs cannot be derived from the weight amount as there is no correlation between CFUs and weight.

FDA's draft guidance[48] on labeling probiotic quantity recommends (does not require) listing both the weight as well as the number of CFUs per serving. This approach is impractical. Because there is no correlation between weight and CFUs, manufacturers would not be able to consistently produce probiotics that have uniform weight and CFUs, only one or the other. Between weight and CFUs, only CFUs is a scientifically accepted measure of live microbial cells.

Listing live microbial dietary ingredient quantity on a label gives consumers the most accurate information about the amount of viable microorganisms present in a product. FDA does not currently require labeling for live organisms but responsible marketers provide this information throughout the shelf life of the product.

Viability



Third, the labeled quantity of probiotics should reflect the quantity of live microorganisms at the end of their stated shelf life (i.e., the expiration or "use by" date), not at the date of manufacture. Labeling quantity at the time of manufacture may be misleading because, like many other ingredients (such as vitamins), probiotics can naturally lose activity over time. This fact is particularly relevant to probiotics that can die in the container over shelf life, so labeling quantity at the time of manufacture may not reflect the number of live microorganisms in the product by the time it reaches the consumer.

If the product label only includes the number of organisms at the date of manufacture, consumers will not know whether the probiotics will still be alive in the quantities needed to provide the desired health benefits at the time they take the product. Many of the benefits that probiotics are known for are attributed to the activities that these organisms participate in when they are alive. Research conducted on live cells to support the benefits of probiotics cannot usually be translated to their dead counterparts.

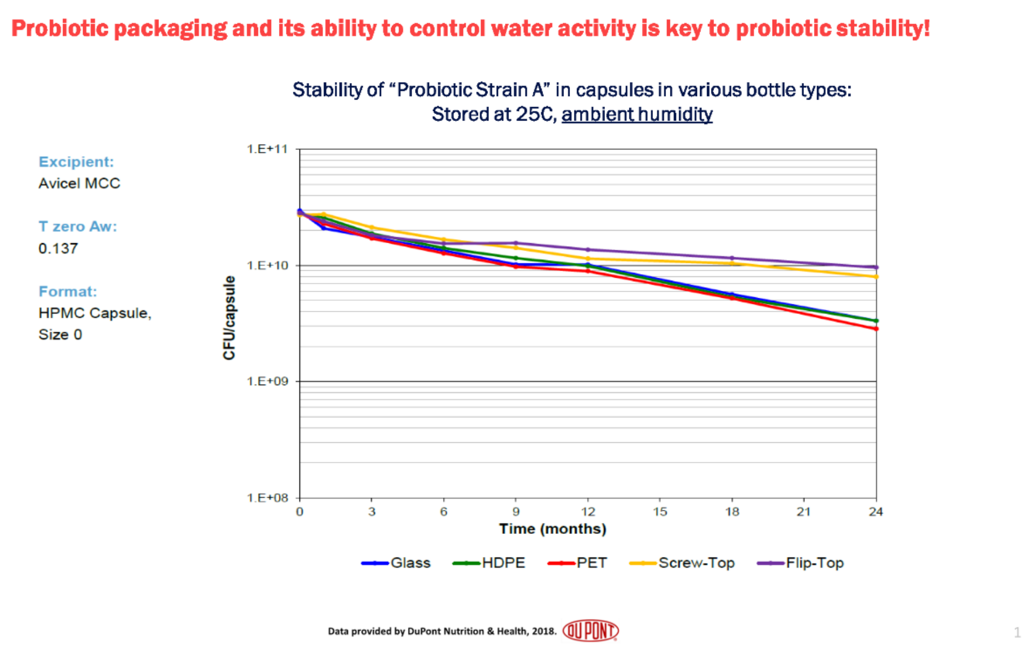
All dietary supplements that are labeled with an expiration date must contain at least the labeled amount of each dietary ingredient up until the expiration date. Although FDA regulations do not require supplements to be labeled with an expiration date/shelf date/best if used by date, responsible manufacturers should include an expiration date on the label and should have data to support it.[49]

It's not uncommon for manufacturers to provide more than the labeled amount of an ingredient that allows for some deterioration over its shelf life to ensure that the product contains at least the labeled amount by the end of the stated shelf life (overages). That is true for probiotics too. Based on the strains used, the length of the shelf life, the finished product matrix, and the product packaging, transportation, and storage, probiotic quantity at time of manufacture could be significantly higher than the label claim. As an example, probiotics degrade at different rates when packaged in a glass bottle vs. HDPE (plastic) vs. PET (plastic), and whether the bottles are screw-top vs. flip top because each packaging type offers different levels of protection against moisture, which is a key factor in probiotic stability and viability.

Proper storage and handling

Ensuring that probiotics remain viable throughout their lifecycle can be a challenge, but it is critical to do so. As live organisms, probiotics are generally sensitive to changes in temperature and humidity. The extent to which an individual product is impacted by temperature and humidity depends on the probiotic strains in the product, formulation matrix and dosage form, manufacturing and packaging conditions, and product packaging, among other factors. As an example, an unpublished packaging study showed that probiotics are more stable in screw-top and flip-top desiccant-lined vials as well as glass bottles compared to either HDPE or PET bottles.[50] Blister cards, in particular foil/foil blister cards, are also a preferred packaging option for probiotics as each blister acts as its own sealed container, such that the other capsules are not subjected to increased water activity caused by repeated opening and closing of a bottle over time.

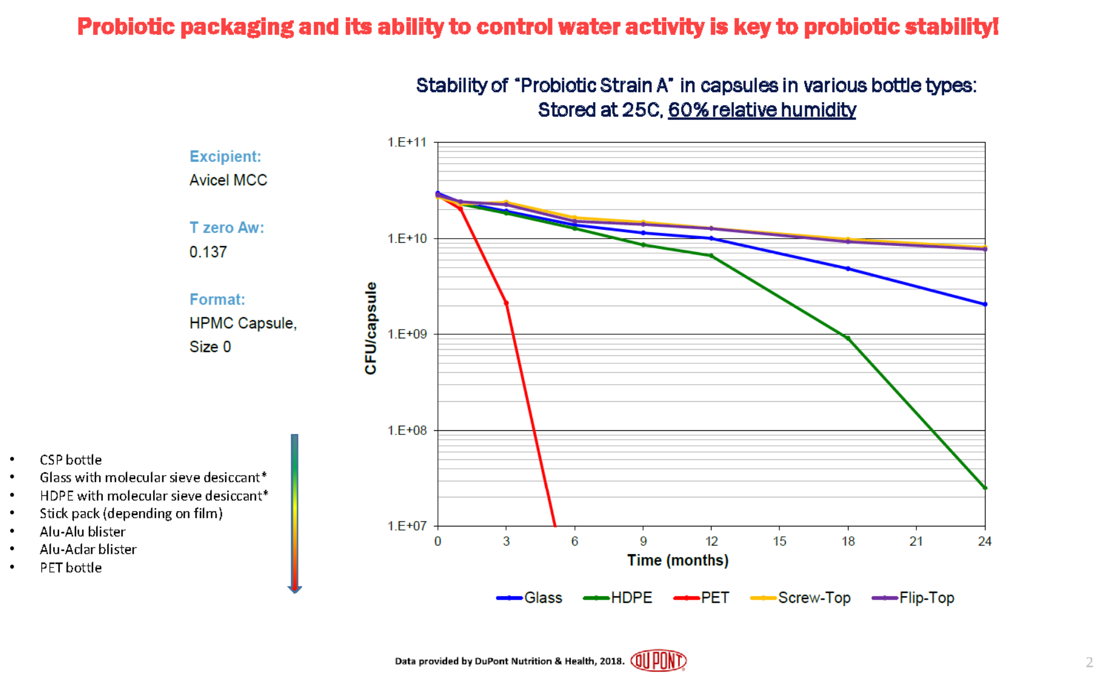
Chart 1. Stability of “Probiotic Strain A” in capsules in various bottle types – Stored at 25C, ambient humidity



Probiotics survive better if kept refrigerated throughout their journey to store shelves; however, refrigerated shelf space may not be possible. Often, probiotics are manufactured with sufficient overages to ensure claimed quantity at the end of shelf life when stored as directed. However, refrigeration at the retail store should be considered if probiotic products contain highly unstable strains, including most of the *Bifidobacterium* genus.

Humidity is an important consideration for probiotic storage as stability is affected by moisture levels, which, in turn, is controlled to some degree by packaging type. According to the same unpublished packaging study, a given probiotic product stored at ambient humidity, degradation occurred much more slowly over a span of 24 months as compared to storage at 60 percent relative humidity.[51] In the latter case, probiotics packaged in less water restrictive packaging, such as PET, died off in less than 6 months.

Chart 2. Stability of "Probiotic Strain A" in capsules in various bottle types – Stored at 25C, <u>60% relative humidity</u>



Manufacturers should provide storage and handling instructions to their retailer customers, taking into account individual formulations and packaging. Retailers should ensure that the products are stored under the appropriate conditions throughout their lifecycle, including at the warehouse, during shipping, and on retail shelves. Retailers should also advise their customers about the proper storage conditions for the products they purchase, as indicated on the product label.

Proper storage and handling are critical for the products to maintain the labeled quantity of live organisms through the end of the stated shelf life. When defining storage temperatures, firms may consider the following storage conditions from the United States Pharmacopoeia (USP) General Chapter <659> Packaging and Storage Requirements[52] (see Table 3).

Table 3. USP General Chapter <659> storage condition definitions

USP General Chapter <659> storage condition definitions	
Storage	**Storage condition**
Refrigerated	2°C to 8°C
Cold	Not exceeding 8°C
Cool	8°C to 15°C
Controlled room temperature	20°C to 25°C

In addition, room temperature is generally accepted to be 25°C for the Continental US per International Council for Harmonisation stability testing guidelines.[53]

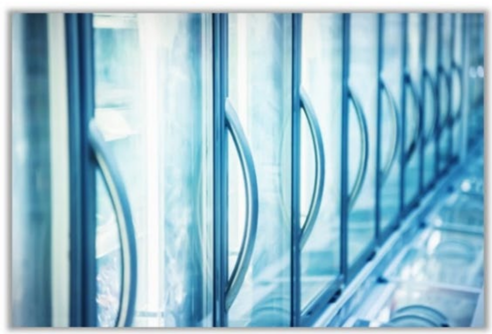
Conclusion

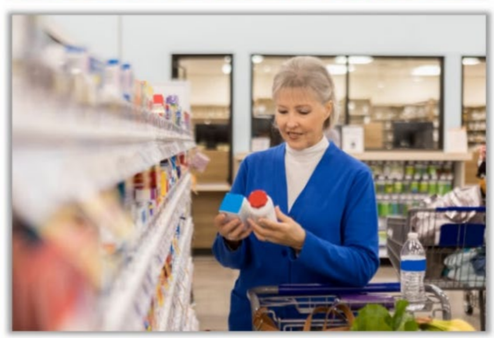
Probiotics have become increasingly popular class of dietary supplements with diverse ingredients conferring a variety of health benefits backed by science. Although well known for promotion of gut health, probiotics are studied for their effects on cognitive and immune function, as well as heart and women's health, and product claims are made according to the available scientific evidence.

Research shows that specific microbial strains favor different body systems and interact with the existing microbiome in different ways, leading to diverse benefits. The product label is key to understanding the product offerings. It is highly recommended that probiotic labels contain complete identity information, i.e., genus, species, and strain, for each microorganism; accurate quantity of live microorganisms in each serving in terms of CFUs or AFUs/live cells; and an expiration date indicating the last date by which a probiotic can be expected to contain at least the labeled quantity of viable cells.

As the prevailing definition of probiotic requires that they be live microorganisms, it is critical for manufacturers, retailers, and even consumers, to properly handle and store probiotics to maintain their viability over the course of their shelf life.

Storage and handling conditions should be clearly communicated and consistently implemented to ensure consumers get the most out of the probiotic products they purchase.





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