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EXPERT OPINION

A Proposed New Path for Management of Health in Inherited Metabolic Disorders Using Engineered Probiotic Bacteria

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Abstract: Dietary supplements represent a growing segment of the healthcare industry as consumers seek alternative methods to manage health concerns. Probiotics are dietary supplements that are broadly used to aid digestion and immune and metabolic health. Patients with disorders restricting the intake of particular nutrients must balance adequate nutrition with limitations in the intake of natural food ingredients. Synthetic biology has emerged in the last decade, allowing scientists to specifically engineer probiotic bacteria to perform functions in the GI tract, such as the elimination or reduction of specific nutrients from ingested food sources. Engineered probiotic bacteria have the potential to provide health benefits and improve the quality of life of patients with genetic or acquired metabolic disorders. One way to make them available to consumers is to develop specialized products as dietary supplements. Developers of such innovative products must follow new FDA guidance and work closely with FDA in consideration of the design, development, manufacturing, and labeling of engineered probiotics as dietary supplements.

Keywords: probiotic dietary supplement, FDA, new dietary ingredient

Introduction

Consumers are becoming interested in personalized nutrition and are increasingly turning to dietary supplements to address their specific health concerns, including general health, energy and weight management, gastrointestinal health, bone and joint health, immunity, cardiac health, sports fitness, diabetes, insomnia, vaginal and urinary tract health, and nervous system functions.

The global dietary supplement market was estimated at $176.19 billion in 2023 and is expected to reach $346.36 billion by 2032.[1] Prospects for the growth of the US dietary supplements market are equally promising with a 2023 market size valued at $42.85 billion with expectations of reaching $71.16 billion by 2032.[2] Figure 1 illustrates the global growth of the dietary supplement market triggered by the Covid-19 pandemic and the general belief that an optimal nutrient status is protective against infection.[2] Relative to the specific types of dietary supplements classified according to their functional dietary ingredients, there is similar potential for growth. The purpose of this article is to introduce journal readership to the idea of developing a new genre of dietary supplements that would address personalized health concerns not currently addressed by dietary supplements in the marketplace. Herein, we focus on the regulatory challenges in meeting safety requirements and the paths required for label claim approval and marketing in the United States when a new dietary ingredient is involved.

In 1994, the US Congress established the Dietary Supplement Health and Education Act (DSHEA), setting the lawful definition of a dietary supplement as a product that is intended to supplement the diet with one or more dietary ingredients intended to be taken by mouth as a pill, capsule, tablet, or liquid, and is clearly labeled on the front panel as a dietary supplement.[3,4] The US Food and Drug Administration (FDA) defines a dietary ingredient as one that includes vitamins, minerals, herbs, other botanicals, amino acids, and other constituents (Table 1). These other constituents may be new dietary ingredients (NDI) that were not sold in the United States as constituents of a dietary supplement or

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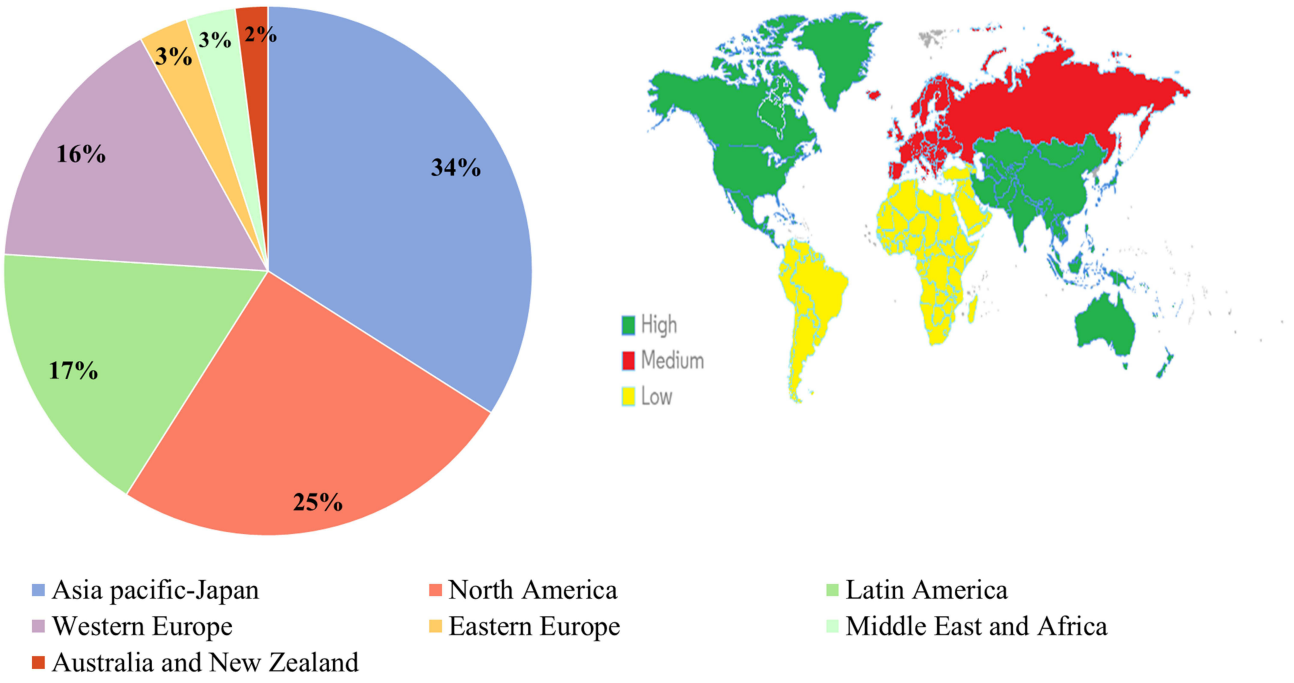

Figure 1 Geographic Distribution of Dietary Supplements. Geographical distribution of dietary supplements market and level of DS market growth worldwide (High, Medium, and Low). Reproduced from Djaoudene O, Romano A, Bradai YD et al. A global overview of dietary supplements: regulation, market trends, usage during the COVID-19 pandemic, and health effects. *Nutrients*. 2023;15(15):3320. Creative Commons.[2]

conventional food before October 15,1994. New dietary supplement ingredients are required to demonstrate evidence of safety to the FDA prior to marketing if they contain a new dietary ingredient.[5] Despite the fact that dietary supplements are regulated by the FDA as "foods", it is important to understand that their regulatory paths differ from foods in the conventional food supply or from drugs. According to the DSHEA classification of conventional food, dietary supplement or drug classification depends on the manufacturer's intended use and product information, which determines the claims that can be made on the label. Drug manufacturers' approved products may have the ability to diagnose, cure, mitigate, treat, or prevent a disease, whereas dietary supplements may not lawfully make these claims.[5] The FDA limits dietary supplements and/or conventional foods to three types of claims: health claims, nutrient content claims, or structure/function claims. FDA approval is required for the use of health claims on the product label. Specific to dietary supplements, health claims describe a relationship between the "active dietary supplement ingredient(s)" and the reduction in risk for a disease or health-related condition.[5]

Table 1 New Dietary Ingredients

(A) A vitamin
(B) A mineral
(C) An herb or other botanical
(D) An amino acid
(E) A dietary substance for use by man to supplement the diet by increasing the total dietary intake or
(F) A concentrate, metabolite, constituent, extract, or combination of any ingredient described in A-E above

Notes: A new dietary ingredient is defined by FDA as any one of the constituents, A–F, listed above that was not marketed in the US before October 15th, 1994. See FDA Draft Guidance Dietary supplements: New Dietary Ingredient Notifications and Related Issues: Guidance for Industry. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-new-dietary-ingredient-notifications-and-related-issues.[5]

The fundamental difference in the way FDA regulates drugs compared with dietary supplements lies in the testing required to establish safety and efficacy prior to marketing. Federal law requires drug products to meet the standards of safety and effectiveness for their intended use prior to marketing, whereas the ingredients of dietary supplements only show reasonable evidence of safety. DSHEA does not require FDA review or approval of dietary supplements before entering the market. Once in the marketplace, the burden of proof of safety is the responsibility of the FDA to provide compelling evidence that a dietary supplement is not safe or lacks evidence for its safe use in humans.[3,4] FDA review is not required for dietary supplement ingredients sold in the United States prior to October 15, 1994; however, manufacturers of dietary supplements with new dietary ingredients must notify the FDA of their intent to market dietary supplements containing the novel ingredient within a specific time period prior to marketing and be prepared to provide evidence addressing the agency's concerns that the novel ingredient is safe for human use under specific conditions of use as a dietary supplement. Manufacturers of dietary supplements with novel functional ingredients should assume that the FDA does indeed have regulatory oversight concerning their products' entry into the marketplace and address these concerns well ahead of product marketing.

Probiotic Dietary Supplements

Probiotics represent an expanding share of the dietary supplements market.[6] Probiotics differ from other classes of dietary supplements by their functional dietary ingredients, which are live microorganisms (bacteria and yeasts) that, when ingested, aid digestion, destroy disease-causing cells or unwanted food components, produce vitamins, and other health benefits. Many probiotics sold as dietary supplements do not require FDA review or approval because the live microbial components of their functional ingredients have a long history of safe use in food fermentation, such as *Lactobacillus rhamnosus GG* and *Bifidobacterium longum* in dairy products or yeasts, such as *Saccharomyces boulardii* or are naturally occurring beneficial flora of the microbiome.[7,8]

Advances in food science technology have enabled the development of probiotic dietary supplements that could potentially lower the dietary disease burden of consumers with genetically inborn errors of metabolism that limit their ability to metabolize specific nutrients common to most foods. This can lead to toxic build-up of metabolites and ultimately, serious degenerative disease conditions. Such inherited disorders are rare (most qualify as rare diseases with less than 200,000 patients in the US, with most having <20,000 patients), but they are increasingly unbearable, given a critical lack of acceptable life-sustaining alternatives other than adherence to severely restricted diets or the use of costly medical foods. Some examples of rare inherited metabolic disorders that limit the intake of particular dietary components include phenylketonuria (phenylalanine), homocystinuria (methionine), tyrosinemia (tyrosine), urea cycle disorders (protein), and maple syrup urine disease (branched chain amino acids, such as leucine, isoleucine, and valine).[9,10] These disorders place a huge burden on patients to limit the intake of specific nutrients (proteins, amino acids, and, in some cases, carbohydrates), while supplementing with often unpalatable and nutritionally inferior medical foods. This can lead to nutritional deficits that complicate disease outcomes.[11–13] For example, deficits in vitamin D and decreased bone density are common in patients with inborn errors of metabolism and may be linked to inadequate intake of natural food sources such as dairy products that supply vitamin D.[14] Additionally, the high economic and social burden associated with the consumption of these medical foods leads to poor patient compliance and lack of adequate disease control.[15,16] Other more common disorders that require limited dietary ingredients include hyperoxaluria (oxalate)[17] and celiac disease (gluten).[18,19]

A proof-of-concept has already been established for engineered organisms capable of degrading dietary constituents important to several disease conditions within the human body, such as oxalate for the treatment of hyperoxaluria[20] and phenylalanine for the treatment of PKU.[21] The treatment for PKU entered Phase 3 clinical trials in 2023, however, development of this strain was suspended by the company in 2024 due to variability in the data at an interim analysis (https://www.synlogictx.com/). Supplements could be developed using naturally occurring probiotics selected for particular enzymatic properties or by genetically modifying a non-pathogenic microbe (including probiotic species) to enable the metabolism of a specific nutrient in foods to non-toxic metabolite(s) before it is absorbed. This would help to limit the dietary burden of harmful nutrients, reduce the risk of inadvertent excursions of exposure to the harmful components of the food, and significantly diversify the foods available for consumption, promoting an overall better nutritional status.

The functional goal of developing such probiotics is to lower the oral load of a ubiquitous nutrient in individuals, where it causes harm, while not completely eliminating its bioavailability. A claim to treat, cure, or prevent the disease, as would be needed for a drug, would not be part of the product label or its intended use; thus, it would not meet the criteria for a drug. However, such a novel probiotic may qualify for a health claim upon FDA approval because it reduces the risk of disease.[5] Safety concerns regarding the impact of the organism on the patient's microbiome could be mitigated by choosing an organism with a long history and safe use as a probiotic as a chassis (ie, host organism) for genetic modifications to introduce or remove genes to address dietary conditions. No genetically modified organism is currently approved to treat a metabolic disorder, however there is a genetically modified nutritional supplement approved in the US to prevent hangover symptoms that is designed to be ingested prior to alcohol consumption to breakdown acetaldehydes produced from alcohol (ZBiotics® Pre-Alcohol).[22]

Design Considerations

Before developing a probiotic dietary supplement, the disease target, probiotic chassis, engineering tools, and manufacturing must all be considered (Figure 2). The selection of a chassis organism for engineering is a key consideration in the design and safe use of engineered probiotics. Ideally, the organism would have no history of pathogenicity, and would already be a known and utilized probiotic for humans in its unengineered state. This provides an established record of safe human use, and will allow the focus to be only on newly introduced genes. If any genes are associated with pathogenicity or toxicity, they need to be removed without affecting overall strain fitness. For example, *Escherichia coli Nissle* 1917, a probiotic marketed under the brand name Mutaflor[23] contains genes for colibactin in its genome.[24] The products of these genes have been associated with genotoxicity,[25–27] and the entire gene cluster encoding these genes may be removed to improve the safety of the base strain.[28] The location of activity within the GI tract is also an important consideration. Some probiotic organisms are more naturally resistant to low pH and bile acids, such as *Lactobacillus* and *Bifidobacteria* species,[29] and may survive through the acid in the stomach, making them more suitable for effector

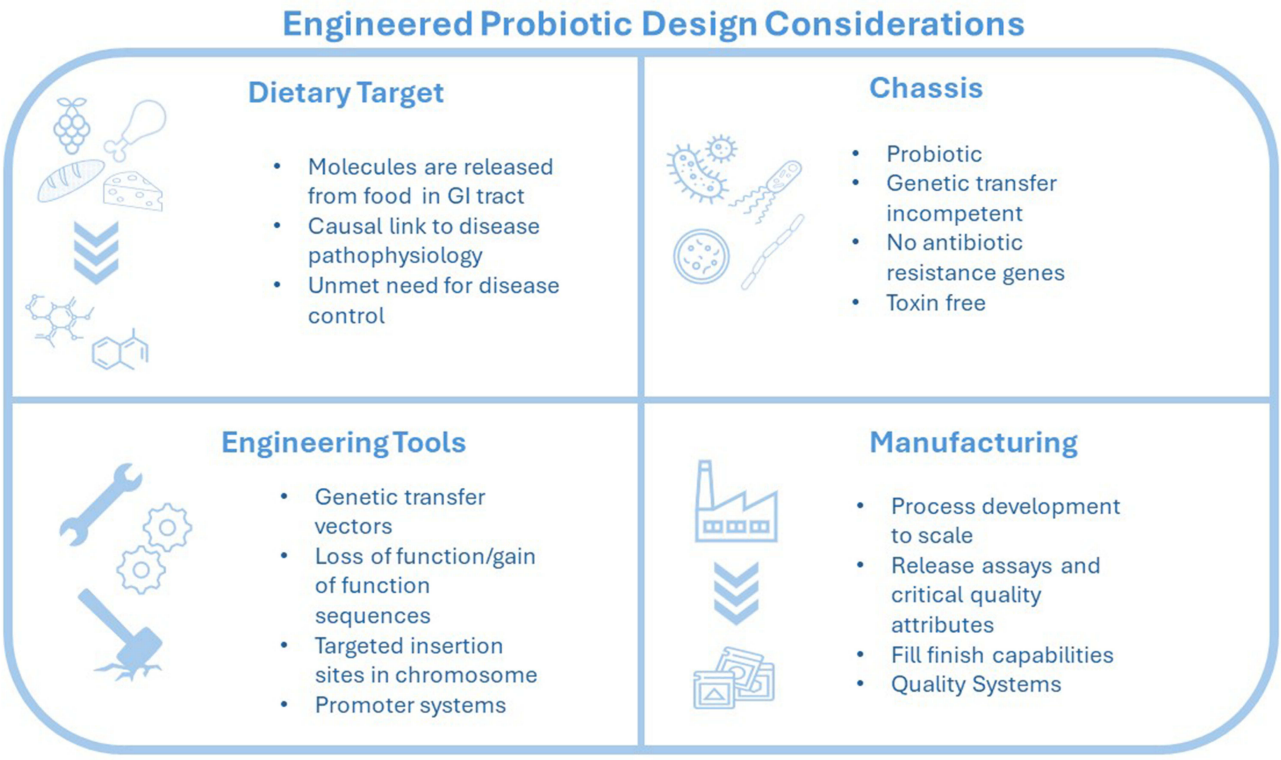

Figure 2 Design Considerations for an Engineered Probiotic. Designing an engineered probiotic includes a full characterization of the dietary target and underlying disease condition, characterization of the probiotic organism used as the chassis for introduction of new genes, availability of genetic engineering tools specific to the probiotic chassis, and capabilities to develop the manufacturing process and scale for production of the final product.

functions that operate in the upper GI tract. Alternatively, some species are well adapted to the anaerobic conditions of the distal small intestine and colon and may be more suited to effector functions operating in these parts of the GI tract.[30] Another consideration may be the propensity of the organism to share genes with other bacteria in the microbiome. For example, *Lactobacillus* species can transfer antibiotic genes to other cells through conjugation systems.[31] Species such as *E. coli* Nissle 1917, which lack endogenous phages or conjugation systems, may be more suited to retaining the engineered genes in the engineered organism.[32] Antibiotic resistance genes found in this strain must also be characterized or removed.

When engineering the selected chassis, genes should ultimately be stably integrated into the host chromosome rather than residing on plasmids that may be lost over replication cycles. Engineering tools for chassis organisms continue to expand with an extensive library of *E. coli* species because of their long history of use for recombinant DNA technology; however, there are growing tools for *Lactobacillus* and *Bacteroides* species as well.[33,34] In addition to introduction of desired genes, it may be beneficial to remove certain genes from the host chromosome to create auxotrophy, an inability to synthesize a particular nutrient for growth such that it must be supplied exogenously for the cell to replicate. This strategy has been employed to control growth during production by adding back the missing nutrients to the fermenter, but it does not allow replication in the human body or the environment, where nutrients are unavailable in sufficient quantities to support growth. For example, common auxotrophies for *E. coli* species include deletion of *thy*A, the gene for thymidylate synthase needed for replication,[35] or DAP, the gene for diaminopimelic acid used in the production of cell walls.[36] Without the addition of the products of these genes to the fermenter, growth is not possible, allowing for the control and containment of the engineered organism in the laboratory, host, and environment. These growth promoters are then removed during the purification and concentration of the organism as part of the manufacturing process.

Regulatory Paths to Developing Novel Probiotic Dietary Supplements

Consistent with the guidance provided in response to our specific questions, the FDA's Center for Food Safety and Applied Nutrition (CFSAN), Office of Dietary Supplement Programs (odsp@fda.hhs.gov), offered the following regulatory guidance for the approval of a novel genetically engineered probiotic dietary supplement. "The product must meet the definition of a dietary ingredient under section 201(ff)(1) of the FD&C Act (21 U.S.C. 312(ff)(1)"). The guidance for new dietary ingredients is summarized in Table 1.

FDA further stated that

> Probiotics, as live microbes, are often able to meet the 201(ff)(1) definition by being (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake as long as you have evidence it has been in use in the food supply as a dietary ingredient without chemical alteration.

A probiotic designed to address a particular disorder would likely be considered as a new dietary ingredient, as it would not have been present in the food supply. It is the responsibility of the manufacturer or distributor of a dietary supplement to ensure that its products are reasonably safe and to determine whether any of the dietary ingredients are new. If the supplement contains a new dietary ingredient, a New Dietary Ingredient Notification (NDIN) is required, which must be submitted to the FDA at least 75 days before marketing the dietary supplement.

A newly updated (as of 2024) draft guidance on dietary supplements[5] provides information and considerations for preparing supplements for new dietary ingredients. The FDA recommends that this application contains information on the manufacture of the supplement, a description of the supplement, including its Latin name and level included in the new ingredient (or dose), evidence of safety for the dietary ingredient, and the recommended labeling. Other considerations to be mindful of when designing an engineered probiotic for use as a dietary supplement include that the ingredient must not already be authorized for investigation or marketed as a new drug or biologic. If substantial clinical investigations of the ingredients have already been conducted under IND application (Investigational New Drug) and made public, the ingredient may not be considered as a dietary supplement. Live microbial dietary ingredients are generally unacceptable for species known to be human pathogens, or that have limited use in human food. The level or dose of the engineered organism must be reconciled with the historical dose levels of the chassis organism in the diet. In general, the intake level of historically consumed articles should be the same as or higher than the proposed intake level

of the new dietary ingredient. If these levels are anticipated to exceed the historical intake, additional preclinical or clinical safety studies may be needed to support its use as a new dietary supplement.

In Europe, new nutritional supplements require approval through the European Commission, with the European Food Safety Authority (EFSA) conducting scientific evaluation and risk assessments on the safety of new ingredients. Within the EFSA there is a special working group to evaluate the safety of genetically modified plants and food ingredients, including genetically modified organisms. Since a new genetically modified organism for use as a nutritional supplement will be, by definition, not be in the permitted list of foods and supplements, it will need to be evaluated and approved by the European Commission under Directive 2002/46/EC. For food supplements that are claimed to have an effect on the nutritional or health status of consumers, EFSA carries out an assessment in line with Regulation (EC) No 1924/2006 on nutrition and health claims.

Manufacturing Considerations

If a dietary ingredient is chemically altered due to the manufacturing process, this necessitates a NDIN. An engineered probiotic is an altered form of a dietary ingredient. In the development of the manufacturing process, the dose of the organism and the expected market size are important considerations for developing processes that can be scaled to produce the desired batch sizes and number of batches per year. Careful consideration of release assays to ensure that the live biotherapeutic maintains viability and activity, and the maintenance of genes through many replication cycles is critical to the success of the product and acceptance by regulatory authorities. The Food and Drug Administration has issued guidelines for the development of live biotherapeutics.[37] In addition to considerations for stable production of engineered organisms, other considerations must include appropriate testing and cleaning of the facility consistent with FDA standards to ensure no cross-contamination from other organisms that may be produced in the same facility. Manufacturing under the FDA's good manufacturing practices (GMP) adds cost to the process but ensures quality and acceptance by regulatory authorities.

Summary and Future Perspectives

With growing awareness of how food impacts health and well-being, there is increasing consumer demand for food supplements that provide health and nutritional benefits. There are numerous rare and common disease conditions in which the limitation of a particular dietary component is essential for preventing disease symptoms. Often, these limitations incur a significant social and economic burden on patients and can lead to challenges in compliance and poor health outcomes. Engineered probiotics as food supplements may help reduce the disease burden by degrading dietary ingredients from whole foods prior to absorption by the body. This may allow patients to improve their compliance while gaining the benefits of increased consumption of whole foods with higher nutritional value. Engineered probiotic supplements could potentially be developed using the regulatory path for dietary supplements, allowing a more rapid and less costly path to make these innovations available to patients. Attention to the guidance on development of dietary supplements and direct interaction with CFSAN is important to assure safe marketability of future innovative products.

Disclosure

Dr Caroline B Kurtz reports that this work is an overview but includes discussion of work that was conducted at Synlogic. None of the manuscript preparation was funded by Synlogic and the opinions are those of the authors. Dr Kurtz reports consulting fees from Synlogic Inc, outside the submitted work. In addition, Dr Caroline Kurtz has patents PCT/US2022/076648, PCT/US23/77060, PCT/US23/68978, and US 17/792030 pending to Synlogic. Dr Calvo is retired from the FDA Center for Food Safety and Applied Nutrition. The authors report no other conflicts of interest in this work.

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