National Institutes of Health

PRECISION PROBIOTIC THERAPIES

Challenges and Opportunities

Executive Summary

April 26–27, 2022

Precision Probiotic Therapies: Challenges and Opportunities
Virtual Workshop, April 26 and 27, 2022

National Institutes of Health (NIH) Co-leads: National Center for Complementary and Integrative Health (NCCIH) and Office of Dietary Supplements (ODS)

Planning Committee:

NIH collaborators: *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD); National Cancer Institute (NCI); National Heart, Lung, and Blood Institute (NHLBI); National Institute on Aging (NIA); National Institute on Alcohol Abuse and Alcoholism (NIAAA); National Institute of Allergy and Infectious Diseases (NIAID); National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK); National Institute of Neurological Disorders and Stroke (NINDS); and Office of Nutrition Research (ONR)

U.S. Department of Agriculture (USDA): Agricultural Research Service (ARS)

Executive Summary

The goals of this NIH workshop were to identify 1) gaps in our current understanding of the biology of probiotics and the gut microbiota, and 2) research questions and challenges posed by the current knowledge gaps. The workshop identified many gaps in our knowledge and highlighted a variety of outstanding research questions and methodological challenges which would need to be surmounted for the successful clinical application of precision probiotic interventions. In the **Opening Remarks on Day 1,** NCCIH Director Dr. Helene M. Langevin noted that probiotic and prebiotic interventions are part of integrative health, and NCCIH's current strategic plan supports a robust portfolio of research around probiotics, in alignment with its focus on whole person health research.

In the **Day 1 Keynote Address,** Dr. Jeffrey Gordon of Washington University in St. Louis said research in Bangladesh has shown that undernutrition disrupts the development of the normal microbiota in children, and this disruption may be a causal factor in undernutrition and its sequelae as well as an effect of it. Complementary foods that can promote the development of microbial strains associated with growth, metabolism, and immune function are currently being tested in infants and young children. Dr. Gordon highlighted the need to better characterize normal human development and gut microbiota associated with healthy child development in different geographical regions and cultures to provide a baseline from which to distinguish deviations from normality. He also underscored the need for the development of consensus good practices for this research area (e.g., for research design, sharing information on the characterization of interventions, and sample collection). Dr. Gordon noted that stool is not an ideal specimen for analyzing gut microbiota, which presents a critical methodological challenge for gut microbiota research; better noninvasive methods are needed for its characterization. Dr. Gordon also highlighted the need to toggle between detailed mechanism of action studies and a systems focus, given the potential of the gut microbiota to act synergistically or antagonistically, and the importance of individual and environmental factors.

Speakers in **Session One: Current Challenges in Clinical Trials** discussed factors that complicate the optimal design (e.g., dose and duration) and interpretation of clinical trials of probiotics (e.g., substantial intra- and inter-individual variability in genetics, baseline gut microbiota, and environmental exposures including diet). Pleiotropy (the potential for a single gene or metabolite from a probiotic strain to

modulate multiple biological systems as well as interactions among organisms within the gut microbiota or between the microbiota and the host) is also a challenge. Given the dynamic complexity of the interacting systems, collecting and processing standardized data and biospecimens at multiple time points (before, during, and after probiotic supplementation) in clinical trials are critical. In addition, such data can support ancillary mechanistic studies, particularly if the data from all probiotics trials are stored in a data library for future assessment. The Microbial Metabolites DataBase (MiMeDB), which contains health, lifestyle, microbial, dietary, bioactive, metabolic, environmental, and medication data, may contribute to understanding how probiotics modify processes such as cholesterol metabolism and how manipulations of the microbiome may affect health.

Additional critical issues for clinical trials include product integrity and timing and duration of delivery. It was noted that in order to ensure product integrity, stability, and performance, researchers should work with probiotic product manufacturers during trial design to ensure cell viability (where required), delivery, and safety. Findings from early phase clinical trials of different investigational bacterial consortia suggest that dose duration is more important than dose level for achieving durable engraftment.

The **Session One Panel Discussion** focused on some of the outstanding challenges to conducting rigorous research on the health effects of probiotics, including the development of methods for assessing compliance with probiotic interventions and for dietary intake assessment, and the development of approaches to optimize the timing of probiotic dosing and sampling. Reverse translation studies (which begin with clinical trial findings and work backward to uncover their underlying mechanisms) could inform the development of such approaches. Understanding the mechanism(s) of action underlying the studied effect(s) decreases the risk of selecting a primary outcome that misses an actual effect of the intervention. The requirement to declare primary and secondary outcomes before conducting clinical trials is restrictive and absolutely necessary to avoid the proliferation of false positive results. Panelists also discussed having a common platform for communication, data exchange, and collaboration within and across organizations to facilitate research translation and reverse translation. Storing samples from trials in such a platform could contribute to big data and open science. The needs for systems biology research involving multidisciplinary teams and longitudinal studies were noted.

Speakers in **Session Two: Host-Microbiome Interactions in Precision Probiotic Research** discussed contributions of the host native gut microbiome and other environmental factors as key inter-individual variables to consider in precision probiotic interventions. Synthetic biology tools need to be developed for controlling engraftment of a single microbe to different microbiota. Tools are also needed for eliminating undesired microbes from the gut, including synthetic microbes. Speakers presented various examples of the specificity of metabolites produced by different gut microbes. Challenges in reproducing microbiome research results were discussed, such as individual variability in probiotic engraftment, with background diet accounting for only part of such differences. Documenting sampling times is critical for reproducibility. Speakers presented a noninvasive method to query the transcriptome of exfoliated gut epithelial cells of human infants; it can prospectively collect stool samples for later use as individual baseline data for N-of-1 studies and probiotic interventions. Using precision probiotics research to identify individual subgroups for microbiota-driven treatments, combined with data from multiple factors (i.e., genomics, microbiome, exposome) in artificial intelligence (AI) models, may eventually provide insights on microbiota-driven disease development mechanisms; but it was noted that the research community is still far from having the requisite evidence

base to support rational treatment individualization. Microbes respond to different diets as guilds, or ecosystems, rather than as individual species or strains. Two guilds differing in their associations with metabolic gene expression were described, based on high-quality microbial genomes. Finally, the role of the gut microbiota in drug metabolism was presented in the context of efforts to develop and validate methods for predicting interactions between microbial metabolites and drugs that may influence the activity or effectiveness of the drugs.

The **Session Two Panel Discussion** spanned topics including noninvasive techniques for probiotic research; variation in sample composition stemming from differences in sampling location and timing, diet, and transit time; methods for studying microbial metabolite-host interactions; difficulty in defining a "healthy gut microbiome"; and challenges in estimating effect size when studying health effects of small molecular metabolites. Panelists agreed that to facilitate data comparisons and machine learning, a consensus is needed on common data elements, minimal acceptable DNA recovery rates, and good practices for sampling and documentation. Parameters affecting experimental variability and available data on effect size must be carefully and conservatively assessed for each study, as effect sizes will vary between interventions and outcomes. Not enough is known about the many factors that affect metabolite production, and there is little clear and replicated evidence on the effects of metabolites on health. Also, very little is known about the role of the microbiome in drug metabolism and drug-probiotic compatibility. Models can improve understanding of the interplay between drugs, the gut microbiome, and probiotics. N-of-1 trials; small cohort, early-phase clinical trials; and other types of pilot studies may help guide effect size estimation as well as assessment of the likelihood of seeing a clinically (as well as statistically) significant effect. Continued cross-communication between clinical study and animal study research will advance this field.

In the **Opening Remarks on Day 2,** ODS Acting Director Dr. Joseph M. Betz said that currently, almost 6,000 probiotic products are marketed as dietary supplements. According to the Dietary Supplement Health and Education Act of 1994 (DSHEA), dietary supplements are generally recognized as safe (GRAS) unless they contain a dietary ingredient that has not previously been present in the food supply. Dietary supplements have limited premarket review, no premarket approval, no mandatory formulation standards, and no required product registration. In fiscal year 2020, ODS invested about $600,000 in probiotics and prebiotics research.

In the **Day 2 Keynote Address,** Dr. Gary Wu of the University of Pennsylvania discussed precision probiotics in the context of disease treatment, using inflammatory bowel disease (IBD) as an example. Interindividual variability in treatment response, the wide range of potentially active components in microbial therapeutic technologies, and the need for building spatial discrimination into treatments all underscored the challenges in this research. In the case of IBD, where the microbiota may be an important environmental disease trigger, identifying early predictive markers of individual response to biological therapies, including microbiological predictive markers, might aid in the development of companion diagnostics to show who is likely to respond to a particular treatment. Movement toward the development of live biotherapeutic products (LBPs) and diagnostics based on microorganisms will require human intervention studies.

Speakers in **Session Three: Next Generation Probiotics—New Strain Identification and Development** discussed why strains coexist for some species but not others, the niche range for individual strains, and the roles played by within-person evolution and other selective forces in determining colonization.

Restoring microbial diversity in diseased skin microbiomes and removing pathogens could be appropriate therapeutic targets, and microbial therapeutics must consider genetic diversity at both the species and strain levels. Immunoglobulin A (IgA) might be useful for identifying relevant fungi, bacteria, bacteriophages, and viruses for therapeutics such as vaccines to control microbial gene expression and disease-promoting capabilities. Engineered probiotics with phage-mediated lysis present an effective delivery method of therapeutic compounds and increase biocontainment. The human small intestine and gut mucosal interface are keys to understanding the potential of the gut microbiota and/or probiotics to modulate the immune system, given that these are anatomically the major sites of interaction between the two, but assessing these interactions in humans remains a major challenge. Three-dimensional (3D) imaging tools exist that can show the spatial structure of interacting host cells and mucosal microbial communities, allowing for a greater understanding of mechanisms and next generation probiotic development.

The **Session Three Panel Discussion** began with a debate on the definition of next generation probiotics. Panelists agreed that in many cases, colonization and engraftment are desirable but not essential. With very few exceptions, no "low-hanging fruit" are currently ripe for development in the probiotic field. Panelists discussed general features that determine whether an organism can colonize, techniques for improving host response to microbial therapeutics, implications for disease of changes in oral microbiomes and their metabolites, and the impact of lifestyle interventions and stress management on inflammatory diseases. Some data suggest that stress can alter the mucosal-associated microbiome composition and gut immune function; multimodal treatments and lifestyle alterations may improve the quality of life. Regarding critical gaps in knowledge and methodologies and needs for new resources or methods, panelists mentioned in situ transcriptomics profiling; gaps in understanding strain variation, absolute quantities, and spatial variation; the need for human studies and models based on the human reality as well as sharing data and biospecimens; inviting larger and diverse communities to participate in clinical trials; and collecting and preserving samples for later culturing of organisms.

Speakers in **Session Four: Emerging Technologies for Precision Probiotics** presented new technologies that may help to address some of the research gaps. Tissue chips containing 3D models of the human intestinal microenvironment can model the human-host microbiome crosstalk for specific disease models; they also have potential uses in studying bacterial toxins, probiotics, microbiota, epithelium morphology, tissue-specific immune cells, and environmental influences. Ingestible enteric-coated osmotic capsules allow for spatially targeted sampling of the microbiome from different areas of the gastrointestinal tract. In vitro human gut models can incorporate interindividual variability in bowel transit time, microbial quantity, and other factors that influence microbial engraftment. A major remaining challenge is that between two-thirds and three-quarters of the enzymes produced by the gut microbiota still lack functional annotation. Preclinical studies that use animal models with more natural microbiota (as opposed to standard pathogen-free mice) have been found to better predict human immune responses. An online platform of human microbiome bioactive molecules would be helpful to provide methodologies for sampling and profiling multiomics samples at scale and for screening and prioritizing these molecules. This platform could also provide computational methods for processing combined datasets and data resources for IBD.

The **Session Four Panel Discussion** focused on potential opportunities and impediments for harnessing technologies for precision probiotics research at every scale, from large population and multiethnic studies to microbiological and immunological mechanistic studies on microbiome-host interactions.

Panelists said that among the resources or methodologies that can help fill current knowledge gaps are single cell-based sorting techniques with flow cytometry-assisted sorters; in situ transcriptomic technologies; use of bacterial spores as probes of gut microbiota and metabolites; bidirectional use of ingestible capsules (i.e., for delivering a microbiome and sampling); and resources for mid-sized multidisciplinary research collaboration mechanisms similar to the NIH Human Microbiome Project (https://commonfund.nih.gov/hmp) and studies in larger, more diverse populations, with cloud-based open access to data and tools. There is a current Funding Opportunity Announcement on Identification and Characterization of Bioactive Microbial Metabolites for Advancing Research on Microbe-Diet-Host Interactions (R01 Clinical Trial Not Allowed), number PAR-21-253. Panelists agreed that the nature of the field requires larger cohort studies by multidisciplinary groups, and more opportunities for young investigators (i.e., via the new innovator award and smaller award mechanisms) are needed. Opportunities to collaborate with the Nutrition for Precision Health initiative (https://commonfund.nih.gov/nutritionforprecisionhealth) are needed. Regulatory hurdles in LBP need to be resolved and companion diagnostic development for precision probiotic interventions also is needed.

In the **Closing Remarks,** Dr. Hye-Sook Kim of NCCIH said that in addition to collaboration and open science, innovative clinical study design, longitudinal studies, companion diagnostics and prognostics, mechanism of action studies, and accurate outcome measurements are important considerations for advancing precision probiotic interventions. Standardization of sampling, data collection methods, and data formats are needed. Microbiome researchers can participate in the NIH Bridge to Artificial Intelligence (Bridge2AI) program, which is creating a machine learning and AI-ready dataset that can influence the generation of standardized human data. Dr. Mukesh Verma of NCI said that the NIH Environmental Influences on Child Health Outcomes (ECHO) (https://www.nih.gov/research-training/environmental-influences-child-health-outcomes-echo-program) study is following mothers before and after pregnancy and collecting microbiome samples and other samples that could be used for multi-omics analysis. Dr. Barbara C. Sorkin of ODS noted the need for community consensus on metadata for characterizing interventions, probiotics, study methodologies, sampling, and study documentation. There is a need to do pilot clinical trials to estimate effect sizes and answer additional questions, and probiotics need to be studied in the context of microbial and human evolution. The definition of “healthy gut microbiota” needs additional investigation, with consideration of the entire microbiota (bacterial, fungal, and viral). Drs. Kim and Sorkin agreed that microbiome studies are very complex, given multiple, multidirectional interactions between interventions, host genetics, gut, and other microbiota, and host environment and diet; however, microbiome therapeutics have the potential for both disease treatment and prevention. The Precision Probiotics Therapies—Challenges and Opportunities workshop was recorded and is available for viewing on the NIH VideoCast website.