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New Insights into Microbiome Study for Environmental Health: Proceedings of a Workshop—in Brief

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New Insights into Microbiome Study for Environmental Health

Proceedings of a Workshop—in Brief

In the five years since the National Academies of Sciences, Engineering, and Medicine's Standing Committee on Emerging Science for Environmental Health Decisions (ESEH) held its first workshop on the microbes that inhabit the human body, known as the microbiome, the amount of research in the field has increased exponentially. On January 14 and 15, 2016, the committee held its second workshop on the topic, which highlighted what researchers have learned since 2011, and discussed elements that could help inform the research carried out in the next decade and aid in its integration into policy.
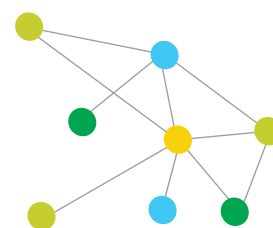
Microbiome research has progressed considerably beyond sequencing microbial communities and studying their composition. The federal government has invested nearly $1 billion in research related to the microbiome,[1] and now much more is understood about the microbiome's role in causing disease. In some cases, mechanisms or molecules that serve as mediators have been identified. New information regarding how early life exposures may affect risk later in life has also come to light.

Current knowledge shows that perturbation by almost any exposure, including drugs, age, genetics, sex, food, and the environment, leads to changes in the human microbiome, summarized Ian Wilson of Imperial College London. For example, there is evidence that metals, pesticides, and polychlorinated biphenyls (PCBs)[2] can interact with and modify the intestinal microbiota. Teasing out the impacts of such changes is challenging because research has also made it clear that many disparate groups of microbial communities can work together to perform the same general functions.

Workshop speakers highlighted several other areas of progress in understanding the microbiome. Scientific understanding of the potential for lifetime consequences from birth via Caesarean section (C-section) and early life exposure to antibiotics has progressed. Recent research also suggests that urban lifestyles may be placing humans at risk of losing important microbial diversity. Other new research details how the bacteria living in the human body can interfere with metabolism by reactivating compounds slated for excretion. An example of how an increased understanding of the microbiome is bearing fruit is work by Stephanie Shore of Harvard University's T.H. Chan School of Public Health. She presented data on her group's ongoing studies on the contribution of obesity to a form of asthma that does not respond to drugs and is highly sensitive to ozone. The fact that the symptoms can be alleviated by antibiotics, and that males and females respond differently, points to the microbiome's involvement. Using mice, the researchers were able to demonstrate the microbiome's potential for involvement by showing that fecal transplants could alter the recipients' responses to ozone. The group's work with metabolomic profiling suggests an unexpected role for altered conjugation of bile acids, an impact that gastrointestinal (GI) microbes have been shown to exhibit, Shore said. She and her colleagues believe that signaling through a G-protein receptor that responds to bile acids (TGR5), and is on smooth muscle, decreases airway responsiveness.

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[1] Stulberg, E, et al. 2015. An assessment of US microbiome research. *Nature Microbiology* 1:15015.
[2] Compounds consisting of two benzene rings in which chlorine replaces hydrogen.

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Research presented by some of the workshop speakers suggests that the microbiome's potential for impacting the dose of environmental contaminants to which people may be exposed and the resulting hazard may be a factor that is one day incorporated into decision making about the risks of chemicals.

INSIGHTS FROM NEW RESEARCH TOOLS

Technology is now available to shine a light on the GI microbiome, the subject of the most research to date, at a granularity that is revolutionary, said Andrew Patterson of Pennsylvania State University. 16S RNA amplification sequencing tools have provided many insights into the composition of microbial communities, but they do not provide much information about the microbes' function. Newer in vitro tools that provide the ability to better understand microbes' function include bioassays that measure the impact of exposures on the microbiome, as well as microbiome interaction with the body's receptors, in some cases. For example, the FXR assay for measuring activity linked to the farnesoid X bile acid receptor can show when changes to the microbiome may impact the body's system for regulating bile acid production, which can in turn affect weight regulation. Other insights have arisen from metagenomics, which involves analyzing microbial DNA extracted from entire communities of microbes. Similarly, metatranscriptomics allows researchers to monitor the gene expression of microbial communities. Mouse models with knockouts of specific GI genes or proteins allow an understanding of how those genes and proteins interact with various body tissues and how they may signal with the microbiota. Also playing an important role is metabolomics, the comprehensive, qualitative, and quantitative study of all the small molecules in an organism, which was also the subject of a recent ESEH workshop.[3] Metabolomics is helping researchers interpret the observations made with mouse models and other tools by "listening in on the chatter between the microbiome and small molecules," Patterson said.

MICROBIOME IMPACT ON CHEMICAL DISPOSITION

One of the things learned is that gut microbiota have the ability to alter the body's absorption, distribution, and metabolism of environmental chemicals and other xenobiotics. The role that the microbiota play in modulating what compounds enter the body suggests that the concept of what constitutes a dose of an environmental compound may need to be reconsidered, Patterson said. For example, Kun Lu of the University of Georgia explained that gut bacteria can transform arsenic into more toxic forms, but they can also help metabolize other heavy metals, including cadmium and bismuth.

The microbiome's role in metabolism is complex, pointed out Matthew Redinbo of the University of North Carolina at Chapel Hill. Gut bacteria have systems that mirror the first phase of the metabolic process, which involves modifying xenobiotics in preparation for the later stage of metabolism.

Redinbo's research shows that although bacteria in our microbiome do not have the two phases of metabolism that humans have (phase 1, which converts xenobiotic compounds into more biologically active forms, and phase 2, which further transforms compounds, generally to a biologically inactive form); they are able to interfere with the second phase of human metabolism by reactivating compounds slated for removal. An example is glucuronidation, a phase 2 metabolic process that can inactivate drugs and carcinogens and target them for excretion. Researchers discovered that bacteria can use the glucuronide sugar molecule added during glucuronidation as an energy source. Removing it can return toxic molecules into circulation. Redinbo's team was able to find a way to nonlethally control the unwanted reactivation of glucuronidated molecules by selectively targeting and inhibiting the enzymes involved.[4]

RECEPTORS AND ENVIRONMENTAL FACTORS THAT AFFECT THE MICROBIOME COMMUNITY, COMPOSITION, AND STRUCTURE

Metabolomics studies reveal that a variety of cells' important receptors can interact with the microbiome. These include nuclear receptors such as peroxisome proliferator-activated receptors (PPAR-α, β, and γ) as well as the aryl hydrocarbon receptor (AHr), which is involved in the metabolism of environmental chemicals including PCBs and dioxin. Patterson's

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[3] See http://nas-sites.org/emergingscience/meetings/metabolomics-and-the-exposome.
[4] Wallace, BD, et al. 2010. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* 330(6005):831-835.

ABOUT THE STANDING COMMITTEE ON EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS: The Standing Committee on Emerging Science for Environmental Health Decisions is sponsored by the National Institute of Environmental Health Sciences to examine, explore, and consider issues on the use of emerging science for environmental health decisions. The Committee's workshops provide a public venue for communication among government, industry, environmental groups, and the academic community about scientific advances in methods and approaches that can be used in the identification, quantification, and control of environmental impacts on human health. Presentations and proceedings such as this one are made broadly available, including at http://nas-sites/emergingscience.

work shows that AHr activation significantly modulates gut microbiota community structure and that processes associated with the microbiome also help regulate the balance of AHr activity.

Research by Lu demonstrated how changes in microbial composition induced by environmental exposure can lead to changes in metabolomic signatures that indicate which metabolites are involved. His work exposing mice with altered gut microbiomes, driven by infections or genetic change, to arsenic highlights the microbiome-mediated metabolic changes linked to the perturbation of pathways associated with arsenic metabolism. More recently, Lu's team has shown that both arsenic and pesticide exposures alter the gut microbiome in a gender-specific manner. This suggests the possibility that the microbiome plays a role in diseases that affect the sexes disproportionately, such as autism and Parkinson's disease.

Recent research brought to light how significant a role the local environment can play in impacting the microbiome. For example, Wilson pointed out that research with differing mouse strains in differing environments has shown that housing conditions can profoundly impact mice microbiomes. If young mice are moved to new environments during a critical period when they are young, they will take on the microbiome of the new environment. This discovery may impact the interpretation of some animal research, although such confounding effects are possible to control. When Shore described her work on asthma, she impressed some workshop participants with her effort to ensure that animal test results were not impacted by various variables, including the presence of siblings and cage effects.

MODERN LIFESTYLE AND VULNERABILITY: EARLY EXPOSURE TO MICROBES

To fully appreciate the symbiotic relationship between humans and microbes, it is important to recognize that humans both evolved from microbes and co-evolved with them; our organs and organelles are ultimately fusions of bacteria, said Maria Gloria Dominguez-Bello of New York University. Microbes occupy all possible niches on our bodies and form a barrier to the environment; technically even the bacteria lining our guts are outside the body from a biological perspective. Immune systems evolved to keep microbes from entering cells and tissues, she pointed out.

Humans, like other species, have also evolved a strategy for passing along microbiomes from one generation to the next during the labor that precedes birth. This transmission occurs primarily in the vagina, Dominguez-Bello said. That birth by C-section disrupts this essential process is not new, nor is that babies born by C-section are at higher risk for obesity, type 1 diabetes, asthma, and celiac disease. What is new are insights gleaned from new research into the impacts of C-section birth on the microbiome. Dominguez-Bello's research shows that babies delivered via C-section exhibit microbiome signatures they receive via their skin—by being handled by their mothers and other caregivers—and the built environment, for example, the operating room.[5] Breast-feeding also impacts the microbiome, she added, but in a study she conducted, 70 percent of mothers who had C-sections did not breast-feed. Her research also shows that babies born by C-section who receive formula do not gain the bacterial diversity of children who are born vaginally and are breast-fed. The extended periods spent inside built environments also play a role in shaping microbiomes, she added.

Dominguez-Bello told workshop attendees about her recent research showing that swabbing babies with fluid taken from their mother's vagina shortly before birth by C-section increased the diversity of their microbiomes.[6] She also spoke about her work with people in primitive cultures in small "uncontacted" villages in her native Venezuela; their microbial diversity is much higher than that of people in the developed world. This suggests that both urbanization and the built environment are fragmenting diversity so that key portions of the microbiome exist only in isolated subpopulations, and they reduce overall diversity across broader swaths of human populations.

Humans may need to worry about permanently losing important microbial diversity, Dominguez-Bello cautioned. Martin Blaser of New York University estimated that humans may have already lost 50 percent of their diversity. Both researchers stressed the importance of studying the microbial diversity found in people who have limited contact with the developed world while the opportunity exists.[7,8]

EFFECTS OF XENOBIOTIC EXPOSURES IN EARLY LIFE

Not only are birth method, breast-feeding, and other early exposures to microbes important in establishing microbiota in babies, early life exposures to xenobiotics can also have lasting impacts. Individual workshop speakers highlighted some of the newest data showing the effects of exposures to both environmental contaminants and antibiotics *in utero* and during known windows of susceptibility in early life.

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[5] Shin, H, et al. 2016. The first microbial environment of infants born by C-section: The operating room microbes. *Microbiome* 4:4.
[6] Dominguez-Bello, MG, et al. 2016. Partial restoration of the microbiota of Cesarean-born infants via vaginal microbial transfer. *Nature Medicine* 22:250-253. doi: 10.1038/nm.4039.
[7] Clemente, JC, et al. 2015. The microbiome of uncontacted Amerindians. *Science Advances* 1(3):e1500183.
[8] Blaser, MJ, and S. Falkow. 2009. What are the consequences of the disappearing human microbiota? *Nature Reviews Microbiology* 7:887-894.

Arsenic is a known human carcinogen that has been shown to pass through the placenta; it was historically used as an antibiotic. Epidemiological studies in Bangladesh, Chile, Mexico, and Thailand linked *in utero* and early life exposures to the metal with impacts on the respiratory and immune systems and an increased risk of infection, explained Margaret Karagas of Dartmouth College. The U.S. Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) documented that children whose parents report feeding them rice (a grain that absorbs arsenic present in water and soil) have higher levels of arsenic in their urine. Karagas presented results from epidemiological studies conducted with a birth cohort in New Hampshire, a state where arsenic was mined and can exist in well water. Her group's investigations into the effects of exposures to arsenic via rice-based food *in utero* and in early childhood suggest that arsenic may be associated with high levels of respiratory and gastrointestinal infections seen in children due to altered T cell functions. They also found significant associations between the composition of the 6-week-old infants' microbiomes and their mode of delivery (as seen by Dominguez-Bello and other researchers) as well as an association between the concentration of arsenic in the newborns' urine and their microbiome composition.[9] The researchers hypothesize that arsenic's antibiotic properties may explain its impact on the microbiome. They also suspect that the microbiome may be transforming the arsenic to more toxic forms in the infants' guts.

Blaser presented a comprehensive series of studies by his group and others linking early life exposure to antibiotics with changes in microbiome structure and function. These studies, according to Blaser, support the hypothesis that antibiotic exposures combined with a high-fat diet are linked to obesity in the United States. This is one of the topics discussed in his book, *Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues.* Specifically, he pointed to data showing that antibiotic use is higher for infants less than 1 year old than for any other age group. He described his studies with mice showing that those exposed to a high-fat diet from food animals receiving subtherapeutic antibiotic treatment—treatments used in many U.S. food animals—gained much more fat than controls on the same diet. His subsequent mouse studies using pulsed dosing show that the effects of early life exposure on the microbiome are reversible, but effects on the animal's phenotype that made them gain more fat persist. Blaser says his work suggests that the gut microbiome can also affect how the immune system develops.

THE ROLE OF THE MICROBIOME IN VARIABILITY

The microbiome is the largest contributor to differences in how food is metabolized, according to an extensive dataset collected by Eran Elinav of the Weizmann Institute of Science in Israel. Diets are a major contributor to human interindividual variability, a topic of a recent ESEH workshop.[10] Elinav explained that he views the microbiome as a signaling hub impacted by our genetics, immune system, lifestyle, and hygiene, as well as our diets. "When these interactions go wrong, the propensity for a number of multifactorial diseases develops," he said.

Elinav described the institute's cohort study of 1,000 healthy Israelis. The extensive data collected by the study include detailed information about food intake and lifestyle, as well as sequencing participants' microbiomes, looking for a million single nucleotide polymorphisms (SNPs), and taking glucose readings every 5 minutes. The study made clear that people have widely different glucose responses to the same food, Elinav said. This explains why the current glycemic indices for food, which were developed based on a few individuals' metabolisms, are not predictive for many people. Among the findings is that, for many people, ice cream appears to not cause a detrimental glycemic response/glucose increase.

The Weizmann researchers used their study data to develop a machine learning algorithm for making individualized predictions of glucose response. They found that the predictions made using only the microbiome sequences of people to be nearly as accurate as predictions made using much more data (including genetic data), Elinav told the audience. The institute's work also documented that individuals' microbiomes can change very rapidly in response to diets predicted to be good or bad for them, he said.

A ROLE FOR METABOLOMICS

Metabolomics studies played a role in a number of the advances described and discussed at the workshop. As Patterson summarized, metabolomics allows researchers to observe the communication between microbiota and the host, including small molecule metabolites such as low-activity hormones and host receptors such as the FXR nuclear receptor. The technology also allows the monitoring of functional changes, such as alterations in bile acids, and

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[9] Madan, JC, et al. 2016. Association of Cesarean delivery and formula supplementation with the intestinal microbiome of 6-week-old infants. *JAMA Pediatrics* 170(3):212-219.
[10] See http://nas-sites.org/emergingscience/meetings/interindividual-variability.

changes wrought by exposure to antibiotics and heavy metals. By analyzing metabolomics data collected over time, researchers are beginning to determine how to assess the health of the microbiome.

Neha Garg of the University of California at San Diego (UCSD) described a novel approach her university is undertaking with researchers at the European Molecular Biology Laboratory in Heidelberg, Germany, to consider the metabolome and the microbiome together by correlating the differing microbiome compositions in various body locations with their metabolomes.[11] To do so, they map the mass spectrometry "signatures" from metabolomics with samples of the microbiomes in different regions of the body, such as the armpit, ear, groin, belly button, toenails, and lungs. By spatially mapping the microbiomes and metabolites in the different regions, they are able to show correlations between molecular and bacterial distributions. Their work has associated spatial changes in microbial 16S profiles with changes in metabolites in response to exposures such as those associated with cosmetics, diet, and lifestyle. They also cataloged unique chemical environments on the largely uncontacted Hazda people of north-central Tanzania.

When the UCSD researchers used the technique to study samples of lung tissues from people with cystic fibrosis, they were able to identify the presence of antibiotics, as well as both known metabolites and unknown degradation products. The UCSD researchers also succeeded in demonstrating the existence of bacteria, such as Achromobacter, at locations where there were no signatures of penetration by antibiotics, such as meropenem. The presence of the bacteria in places where antibiotics did not penetrate suggests the potential for developing antibiotic resistance, an issue known to impact cystic fibrosis patients. Garg said that the team also identified the presence of bis(2-ethylhexyl) phthalate in the lung samples they studied, a discovery with as-yet-unknown implications.

Tools to help uncover and decipher the interaction between the host and the gut microbiome, such as those Garg described, hold promise for helping scientists find ways to assess the microbiome's health, Patterson commented. Anne Summers of the University of Georgia pointed out that cataloging information about the microbiota associated with biochemical niches also may prove to have value.

RELEVANCE OF MICROBIOME FINDINGS TO ENVIRONMENTAL HEALTH DECISIONS

The growing knowledge of the microbiome is opening up new possibilities for identifying how environmental exposures may be impacting disease. However, the immediate implications of microbiome findings on decision making about environmental stressors were not identified to a great degree at the workshop. Highlights from several areas of discussion are presented here to shed light on the current thinking about implications.

Applying what scientists are learning about the microbiome and its impacts on health in toxicology risk assessments is challenging, commented Carl Cerniglia of the U.S. Food and Drug Administration (FDA). He said that risk assessors are struggling with how to connect quantitative metrics that describe a disrupted microbiota to clinical impacts.

Some attendees noted that to buttress the limited data linking environmental exposures to changes in microbiota and disease, it would be useful to have more studies to help researchers understand the relationship between exposures and changes in microbiome function. This relationship can be illuminated with metabolomics studies. While it is yet unclear how such data could aid in risk assessments, recent research showing that people's microbiomes can impact their resulting absorbed dose in response to environmental exposures suggests that the microbiome is an important contributor to interindividual variability, said John Vandenberg of the U.S. Environmental Protection Agency's Office of Research and Development. Microbiome research holds great promise for helping to illuminate cases of interindividual variability that have up until now not been well-understood, he observed.

To recognize which data and information are important for decision making, Ivan Rusyn of Texas A&M University believes that, once a critical mass of data has been collected, the community will have to grapple with determining what underlies the distributions of variability seen across individuals. The data may show that variables such as genetics, nutrition, life stage, and microbiome are partially or largely overlapping. Careful analysis of the available data will be required to allow researchers to recognize what data and information is really important for decision making, he stressed. Along the same lines, Linda Birnbaum of the National Institute of Environmental Health Sciences pointed out that the microbiome and environmental chemicals are just aspects of the full picture of what impacts our health, in addition to our lifestyles, socioeconomic status, and exposure to infectious agents. "We need to get better at putting it all together," she observed, commenting that pattern recognition may aid with this in the future.

As researchers attempt to combine data in line with Birnbaum's suggestion, they should also consider trying to find ways to document how the microbiome may play a role in the dose of environmental contaminants to which cells are exposed inside the body and the effect on the resulting hazard, Vandenberg observed. This may aid risk assessors in efforts to incorporate the microbiome into risk assessments, he predicted.

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[11] Bouslimani, A, et al. 2015. Molecular cartography of the human skin surface in 3D. *Proceedings of the National Academy of Sciences of the United States of America* 122(17):E212-E219.

LOOKING FORWARD: RESEARCH NEEDS AND STRATEGIES

Obtaining more human microbiome data. Ana Navas-Acien of Johns Hopkins University and other speakers and attendees noted that long-term epidemiology studies offer a potential opportunity for collecting data that show microbiome changes over time and as a resource for further study. Family cohort studies may also provide valuable information, Navas-Acien suggested. Germaine Buck Louis of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development added that samples from twins and other multiple birth groups may prove useful. Birnbaum advised researchers to make efforts to collect information that can both identify and shed light on the impact of changes to the microbiome during critical windows of susceptibility, such as early life. New initiatives such as the Children's Health Exposure Analysis Resource (CHEAR) and the Biological Database Tool Kit (BDTK) may benefit from incorporating the microbiome into sample collection if they are not already doing so, suggested Carol Mattingly of North Carolina State University.[12,13]

Participants discussed the lack of agreed upon methodologies for processing and preserving samples collected during epidemiology studies to ensure the samples are useful. Lita Proctor of the National Human Genome Research Institute suggested that the best way to ensure that any collected samples would prove useful is to know what questions the samples are intended to answer, but others argued that banking various types of samples would be useful because it is yet unknown what issues may be investigated in the future. It is not yet clear what kind of data—metabolites, DNA, protein—or samples (e.g., fecal samples) will prove most useful. Rusyn suggested that an effort be made to identify what types of samples, data, and information are likely to be most interesting and informative while factoring in what material is bankable and amenable to long-term follow up. This could help determine which methodologies to create.

Expanding beyond the gut microbiome. A recent analysis of the microbiome research conducted to date documents that the vast majority has focused on the gut microbiome.[14] This highlights the need to ramp up research on other areas of the body with microbiome communities, said Proctor. The analysis also suggests that tools and resources have received disproportionately fewer resources than other types of research. The field will benefit from improvements to in vitro models, ex vivo animal models, and gnotobiotic models, which only harbor known strains of bacteria and other microorganisms, pointed out Patterson, Helmut Zarbl of Rutgers University, and other workshop attendees.

Mathematical modeling. Mathematical models may help shed light on the microbiome's importance and impact. Joel Schwartz of the T.H. Chan Harvard School of Public Health pointed out that statistical methods exist for assessing variability in response to exposures and that these methods can be used to help gauge the impact of the microbiome. Machine learning algorithms such as the ones Elinav discussed offer a means to combine available data to help determine how much of the variability in response to certain exposures may relate to variability in the microbiome, Schwartz said. If the microbiome modifies a response to an environmental toxicant by, for example, metabolizing it or changing its functional form, information about the mechanism involved may not be necessary to factor the microbiome's impact into a risk assessment, he said. The key information for a risk assessor is the distribution of how much the microbiome modifies the response to the toxicant, he argued. This is true whether the microbiome is either modifying or mediating the environmental response, he said. Some participants described the difficulties of obtaining National Institutes of Health (NIH) funds for modeling in general. Agencies that have made notable investments in mathematical modeling related to the microbiome include the National Science Foundation and the U.S. Departments of Defense, Energy, and Health and Human Services, Proctor said. Summers noted that such models also can provide an important and testable means for hypothesis testing. In this way, mathematical models can help tease out what components of the data already being collected are really significant, pointed out William Farland of Colorado State University. Such models may also help amass evidence of situations where the microbiome explains species differences in toxicity that previously have been observed, said Rusyn.

Standard test systems and animal studies. Determining ways to show the microbiome's contribution to how animals respond to exposure to environmental chemicals via standard toxicology tests, such as 14-day, 90-day, and cancer bioassay tests will be important, Rusyn commented. An idea raised by Jonathan Arias of NIH is the potential for humanizing animal models in light of ethical constraints on the kinds of experiments possible with humans. Patterson said that he and his colleagues have considered humanizing the microbiome in humanized mice, but thus far they have

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[12] See http://www.niehs.nih.gov/research/supported/exposure/chear.
[13] Sankar, P, et al. 2010. BDTK (Biological Database Tool Kit). *Biomirror* 1(6):63-66.
[14] Stulberg, E, et al. 2016. An assessment of US microbiome research. *Nature Microbiology* 1:15015.

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only replaced mouse microbiomes with single monocultures of bacteria. These kinds of test systems can show the ability of bacteria to metabolize particular pools of metabolites, such as bile acids, he said. Redinbo noted that even if a humanized microbiome could be established in a test animal, it would still not be interacting with a human immune system.

Reference compounds. Because the research conducted to date has made clear that many disparate groups of microbial communities can work together to perform the same general functions. Redinbo suggested the creation of a standard set of compounds to use for animal testing, or perhaps even for humans. This would allow researchers to understand how the microbiomes of animals compare across studies. Cataloguing the spectra of resulting biomarkers and metabolites may also help scientists gain a better understanding of microbial health, he said. Along the same lines, the recently published assessment of U.S. microbiome research pointed out the need for sample and data collection protocol standards, Proctor said. Many organizations whose work was summarized in the assessment reported a need for standard reference materials, such as reference microbial genomes and "mock community" metagenome sequences, to facilitate comparisons between different laboratories and for longitudinal studies. Because of issues with the comparability of microbiome measurements, including sample collection, extraction techniques, the use of technologies including mass spectrometry, and data analysis and interpretation, the National Institute of Standards and Technology (NIST) is planning to hold a workshop on standards related to the microbiome in September 2016.[15]

Garg described her group's efforts to encourage the sharing of metabolomics mass spectrometry data through the Global Natural Products Social Molecular Networking (GNPS) website. The GNPS dataset is focused on the microbiome, and Garg said the network has 8,700 users from more than 100 countries.[16] This is important because finding a biological context for the microbes and molecules associated with an environment can be like finding a needle in a haystack, she observed. "We hope that spatial mapping serves like a metal detector" to assist researchers trying to find a biological context that helps explain why bacteria may be found in a specific environment, Garg said. One example is Garg and her colleagues discovered bacteria found on skin is associated with skin degradation.

While perhaps less relevant to the theme of this particular workshop, several other research areas discussed at the workshop included:

- Studying other microbes. The human microbiome also includes yeasts and viruses, as well as parasites, Wilson pointed out. These have been the subject of much less study than bacteria. The number of viruses and viral particles such as phages and plasmids in human microbiomes is an order of magnitude higher than the number of bacteria, Proctor noted. Similarly, Summers and Cerniglia pointed out the scarcity of funding for the study of anaerobic bacteria, including those that are believed to help protect us from pathogens.
- Antibiotic resistance. The human microbiome is a hotspot for horizontal gene transfer, whereby bacteria share genes that confer benefits such as resistance, and farm animals are the most frequent human source, Proctor commented.
- Habitat-related microbiome research. The value of habitat-related microbiome research that some federal agencies, such as the U.S. Department of Agriculture (USDA) and the U.S. Department of Energy conducted was pointed out. Isabel Walls of the USDA noted that foodborne exposures are an important component of environmental exposures. Redinbo believes it is also important to study the impact of environmental chemicals on microbes associated with plants. The USDA is beginning an initiative on the phytobiome— the microbial community associated with plants.

To better leverage funds, Proctor suggested that the formation of consortia and collaborations be encouraged in addition to the creation of mechanisms for sharing data, cohorts, and experiments among agencies to reduce costs and duplication and get the most out of each study. In addition, agencies and members of initiatives will be well-served by focusing on common needs, protocols, and methods of data analysis. Communication will be critical to facilitate this, Farland observed. Data mining may also help those conducting research now and in the future to be more aware of what related findings may exist, Proctor said. Linda Wennerberg of the National Aeronautics and Space Administration pointed out that massive national and international databases including samples exist that could be utilized. Garg urged attendees to deposit data into the GNPS website dataset. Resha Puzrath of the Navy and Marine Corps Public Health Center suggested that a method for matching available data with experts who analyze the data could be developed using an approach similar to Craigslist.

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[15] See http://www.nist.gov/mml/microbiome-standards.cfm.
[16] See http://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp.

Zarbl summarized the meeting by concluding that the potential for loss of diversity in modern humanity's microbiomes is a serious issue for us all. While acknowledging the need to move forward in strategic fashion given that "we can't do everything," Zarbl urged that even without certainty about which data and samples to collect, it is important to begin collecting samples to enable microbiome study as soon as possible.

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