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MEETING REVIEW

Reports from a Healthy Community: the 7th Conference on Beneficial Microbes

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ABSTRACT The last two decades have seen an explosion in research about the beneficial microbial communities associated with plants and animals. Initially, this explosion was driven by technological advances that enabled explorations of microbiomes on unprecedented scales. Increasingly, the drive is coming from conceptual advances that are the fruit of research investments into experimental systems to probe the functions of these beneficial microbes and their mechanisms of action. The Conference on Beneficial Microbes has been one of the premiere venues for this research. The 7th Conference on Beneficial Microbes was held from 8 to 11 July 2018 at the University of Wisconsin—Madison Memorial Union. The 308 attendees, representing academia, industry, journals, and funding agencies, participated in an intense 4-day meeting encompassing research frontiers in beneficial microbiology and microbiome science.

KEYWORDS conference, microbiome, microbiota, symbiosis

MEETING HISTORY, FORMAT, AND OVERVIEW

The origin of the Beneficial Microbes conference series was an NIH-sponsored meeting entitled "Beneficial Microbial Workshop," held in Seattle, WA, in October 2001. Attended by 125 investigators, the workshop brought together biologists studying human-microbe interactions, mechanistic interactions in model systems, and the evolution of host-microbe relationships. The workshop led to the first American Society for Microbiology (ASM) Conference on Beneficial Microbes, which was held in 2005 in Lake Tahoe, NV. Beginning in 2008, meetings were held biennially in San Diego, CA; Miami, FL; San Antonio, TX; Washington, DC; and Seattle, WA. Throughout these meetings, the focus was to highlight the most exciting and creative scientific advances in the field and to be forward-looking at emerging systems and technologies. To accomplish these goals, two speakers were invited for each session, with the remaining talks selected from abstracts submitted. Diversity of the topics and speakers has been a priority for the organizing committees. Each meeting has balanced work from human-associated microbiomes, other vertebrate microbiomes, invertebrate microbiomes, plant microbiomes, and environmental microbes. Organizers have endeavored to feature speakers who represent the gender and ethnic diversity of biologists today. For example, at the past two meetings, 50% of the speakers have been women. Although there are a number of scientific conferences for the microbiome field, the diversity of research topics and disciplines represented at the Conferences on Beneficial Microbes sets it apart. The community has voted with its feet, and each of the Beneficial Microbes conferences has been well attended by more than 150 investigators (Fig. 1).

In 2017, ASM ended support for most of its small conferences, including Beneficial

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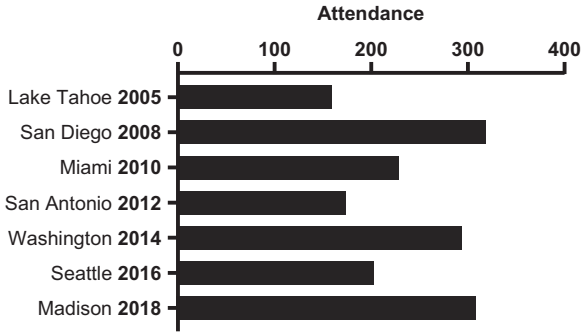

FIG 1 Attendance at the Conference on Beneficial Microbes.

Microbes. The organizing committee, with broad support from the research community, decided to continue the meeting. In an effort to reduce costs, we moved the meeting to an academic venue, on the campus of the University of Wisconsin—Madison. This location was particularly well suited as a home for the Beneficial Microbes meeting because of the university's strong support for research into beneficial microbes and its excellent track record in hosting successful scientific meetings, including the long-running Molecular Genetics of Bacteria and Phages meeting. By holding the meeting on a university campus with a strong conference-organizing staff, we were able to provide more to attendees and at a lower cost than for previous meetings held at downtown hotels. Two key changes included offering on-site conference meals, which provided an informal environment for scientific discussions, and offering a low overall registration fee and a further reduced fee for graduate students. We maintained the previous tradition of excluding speakers from presenting at consecutive conferences, ensuring opportunities for new voices and viewpoints. And we continued the tradition of a conference dance party, this time augmented by local Wisconsin refreshments.

Most importantly, the 7th Conference on Beneficial Microbes maintained its important role in highlighting conceptual innovations and featuring leading experts in our field. The meeting was hosted over 4 days, beginning with an opening keynote presentation on Sunday evening, two full days of scientific sessions, and a final session and closing keynote on Wednesday morning. In addition to the two keynote speakers, an invited session chair for each of the six scientific sessions was asked to host the session and present work from their own laboratory. Of the 46 talks at the meeting, 28 were from trainees selected from submitted abstracts. A total of 134 poster presentations were accepted and exhibited for 2 days, including during the two designated poster sessions. A subset of these posters is discussed below in the relevant themed sessions. We also note that there was a significant social media presence for the meeting, as attendees tweeted using the hashtag #BeneficialMicrobesMtg.

In this article, we review key themes and topics that were presented during the 7th Conference on Beneficial Microbes.

KEYNOTE ADDRESSES AND CONFERENCE THEMES

The conference featured two keynote speakers who focused on the leading edge of our understanding of the microbiome. Eran Elinav from the Weizmann Institute opened the meeting with a far-ranging and forward-looking talk about the human microbiome as a signaling hub impacted by many aspects of human lifestyle. He first considered the role of the microbiome in determining metabolic responses to diet. By using continuous glucose monitoring and daily microbiome profiling of prediabetic subjects eating their standard meals for a week, his group was able to employ machine learning algorithms to develop personalized diets predicted to maintain steady blood sugar levels. These personalized diets, which were largely informed by people's individual microbiome profiles, outperformed those prescribed by trained dieticians using stan-

dardized tables of food glycemic indices (1). Elinav went on to show that the timing as well as the type of food ingested can have profound effects on energy expenditure. Furthermore, disruptions in circadian rhythms through time-shifted light-dark cycles or genetic mutations can lead to altered gut microbiomes that are sufficient to cause excessive weight gain in gnotobiotic mouse models (2, 3). In a third vignette, Elinav shared a mouse model of "yo-yo" dieting in which cycles of fasting and feeding resulted in a persistent alteration of the gut microbiome that caused increasing recalcitrance to the effects of fasting (4). Finally, Elinav shared work showing that the primary effect of hyperglycemia is GLU-2-dependent disruption of intestinal barrier function, which explained the resulting symptoms of increased inflammation and susceptibility to enteric infections (5). Overall, Elinav painted a picture of microbiomes being a critical and highly individual player in human metabolism, highlighting the central role for microbiomes in the future of personalized medicine.

The closing keynote was presented by Jo Handelsman, Director of the Wisconsin Institute for Discovery. In her keynote, Handelsman reflected on her time in the White House Office of Science and Technology Policy and the questions that led to the initiation of the National Microbiome Initiative. Principal among these questions were what defines a "normal" microbiome and how can we assess when a disturbed microbial ecosystem returns to this normal. A key insight into this process was recognizing that the enormous complexity of microbiome research, combined with a lack of governing principles, made this a difficult question to tackle, whether this was in a person treated with antibiotics or in the Gulf of Mexico following the *Deepwater Horizon* oil spill. Handelsman proposed four questions to focus our community on how to move to a functional, predictive understanding of various microbiomes: first, how to predict community response to perturbation; second, how to design systems that are resistant to or recover readily from change; third, how to alter systems that are resistant to or recover readily from change; and fourth, how to reliably establish an invader. Handelsman proceeded to summarize key concepts of the conference in the context of model systems and their power to inform symbiotic mechanisms (6). Handelsman highlighted the diversity of model organisms that were presented at the conference and discussed features that make a successful model system for studying host-microbe associations. Drawing on Ruby's enumeration of valuable characteristics (6), she highlighted the virtues of model systems that are inexpensive and amenable to growth in the laboratory, genetic manipulations, and imaging and for which the microbial associations have known ecological relevance, evolutionary context, and economic importance. With this backdrop, she then presented a new project from her group to study rhizosphere interactions, using a cultured representative from each phylum most common in plant roots (and other prominent microbiomes): *Bacteroides*, *Firmicutes*, and *Proteobacteria*. Using this system, the group is identifying emergent properties and signaling that affect bacterial behavior and plant growth. Overall, Handelsman provided a roadmap to reveal broad governing principles in microbiomes using tractable model systems.

DEVELOPMENTAL IMPACT OF MICROBES

The session Developmental Impact of Microbes emphasized the role of the microbiome in influencing host development, behavior, and health. The opening talk from Margaret McFall-Ngai (University of Hawaii at Manoa, Honolulu, HI) focused on the binary symbiosis between luminous *Vibrio fischeri* and the Hawaiian bobtail squid, *Euprymna scolopes*. McFall-Ngai highlighted comparisons between the squid light organ and the human gastrointestinal tract, noting that epithelial colonization in the juvenile squid takes place across 100 μm, compared to 300 cm in the newborn human. Over this short distance, the host presents obstacles that must be overcome by the colonizing symbionts. McFall-Ngai described biochemical and biophysical challenges to colonization. Biochemical aspects included host processes to present antimicrobial compounds to the bacteria, such as those that prime the bacteria for success inside the host, and the resulting host responses to bacterial products. Host-produced nitric oxide

is presented to colonizing *V. fischeri*, which releases lipopolysaccharide and the peptidoglycan fragment tracheal cytotoxin, which together downregulate host NO production (7). The role of the biophysical environment encountered at the host interface was examined to understand how bacteria entrapped by the host transit to the light organ pore. Characterization of beating cilia on the surface of the light organ revealed that this physical process strongly promotes the collection of the bacteria in the turbulent environment outside the light organ pores (8). The work on the prominent vibrio-squid symbiosis, which has been a model for understanding host development, highlighted how microbe-host interactions are negotiated at many levels, from molecular to tissue-wide forces, and foreshadowed examples of communications in symbioses presented throughout the meeting. For example, at the molecular level, Barbara Pees (Christian Albrechts University, Kiel, Germany) presented a poster entitled "Role of Caenopores in Shaping *Caenorhabditis elegans* Microbial Associations" that described a group of antimicrobial peptides unregulated in the *C. elegans* intestine upon exposure to bacteria that she hypothesizes play a role in shaping the microbiota. At the level of physical forces, Carolina Tropini (Stanford University, Stanford, CA), presented a poster, "Transient Osmotic Perturbation Causes Long-Term Alteration to the Gut Microbiota," on recently published work (9) showing the impacts of osmotic stress on colonic bacterial communities.

In the session chaired by McFall-Ngai, the oral presentations selected from the abstracts explored different developmental processes shaped by microbes, including overall growth, inflammatory states, and the development of behaviors. In her talk entitled "How Facultative Mutualism Evolves: Experimental *Lactobacilli* Evolution in Gnotobiotic Flies," Maria Elena Martino (University of Padua, Padua, Italy) explored the development of a symbiosis. She evolved *Lactobacillus plantarum* NIZO2877 in flies or in culture in host food to ask how *L. plantarum* promotes host growth. The bacterial trait that was selected, both in bacteria that had evolved in the host and in the food alone, was the production of the metabolite *N*-acetyl glutamine, the addition of which was sufficient to promote host growth in the presence of the ancestral strain (10). The theme of bacterially produced metabolites from host diet was also explored by Kazuyuki Kasahara (University of Wisconsin—Madison, Madison, WI) in the talk entitled "Butyrate-Producing Bacteria Modulate Atherosclerosis in a Diet-Dependent Manner." Patients with atherosclerosis have diminished butyrate-producing bacterial genera, such as *Roseburia*, which can digest complex plant polysaccharides (11). Kasashara colonized mice with a low-complexity community that contained or lacked *Roseburia intestinalis*, resulting in marked differences in butyrate production, which corresponded to the capacity to inhibit aortic inflammation and atherosclerosis (12). Other key roles for small molecules were explored in the later session Microbe-Host Interactions at the Molecular Scale.

Continuing on the theme of bacterial regulation of inflammation and turning our attention to the brain, Bethany Rader (Southern Illinois University, Carbondale, IL) gave a talk entitled "Probiotic Treatment of Post-Early Life Traumatic Brain Injury (TBI) and the Impact of TBI on the Intestinal Microbiome in Adolescent Rats." In a rat model of TBI, Rader found that the administration of *Lactobacillus reuteri* reduced the amount of inflammation and the amount of brain tissue lost. Filtered media from the bacteria led to the same phenotype, suggesting that the bacteria produce a compound that signals to the host and affects brain development postinjury. Also addressing the theme of bacterial impacts on brain development, Judith Eisen (University of Oregon, Eugene, OR) spoke regarding "The Microbiota Modulate Zebrafish Behavior and Brain Development." Separated by electrochromic film that can be experimentally modulated to be transparent or opaque, zebrafish in separate tanks either swim separately or exhibit reciprocal social behavior (13). Germfree fish do not engage in normal social behavior and exhibit altered synapse numbers in a specific brain region shown to be important for this behavior. This work demonstrated an elegant use of a model system to interrogate specific microbiota-induced defects in host brain development and behavior.

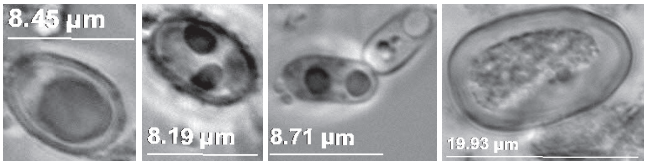

FIG 2 Cyst-stage protozoan parasites from fecal samples. (Courtesy of Bruno Martorelli Di Genova.)

Complementing the work in model systems, Jacquelyn Meisel (University of Maryland, College Park, MD) presented the talk "Characterizing Gut Microbiota Associated with Childhood Diarrhea in the Developing World: the Good, the Bad, and the Ugly." Meisel reported on recent work on the interactions between the microbiome and diarrhea from the large-scale Global Enteric Multicenter Study (GEMS) of Diarrheal Disease (14, 15). With culture-independent approaches, she described taxa that correlated with moderate-to-severe diarrhea, including *Streptococcus* operational taxonomic units (OTUs) that were enriched in disease samples and a *Lactobacillus ruminis* OTU that was significantly associated with the absence of dysentery. Moving from culture-independent to classical microbiology approaches, Meisel is examining genomic variations in isolates from diarrheal cases that cause differing levels of damage to host barrier cells. Additional explorations of human gut microbes came from Laura Knoll (University of Wisconsin—Madison, Madison, WI), who highlighted the emerging understanding of protists as constituents of the microbiome in her talk, "How Does Carriage of Protists Affect the Microbiome and the Development of a Healthy Immune Response." Although approximately 10% of fecal microbiome transplantation samples are rejected for the presence of protists, and although protists are enriched in healthy compared to irritable bowel disease (IBD) patient samples, there is little information as to how the carriage of protists in the microbiome affects the development of a healthy immune response. To address this question, Knoll is examining protists isolated from fecal samples of healthy indigenous people in Venezuela (Fig. 2), including developing a mouse model for colonization and shedding by human-derived protists. This theme of exploring the functional capacity of human-associated microbes was continued in the next session, Host Factors Shaping the Microbiome, and expanded on in the poster presentation by Max Grogan (University of Pennsylvania, Philadelphia, PA), "Investigating the Antagonistic Behavior between Skin Commensal Species Reveals Novel Antimicrobial Metabolites."

HOST FACTORS SHAPING THE MICROBIOME

The talks in the session Host Factors Shaping the Microbiome explored the various ways in which the host can influence microbial colonization and dynamics, whether through underlying genetics or active responses to the microbiome. Elizabeth Grice (University of Pennsylvania, Philadelphia, PA) gave the opening talk for this session and described her laboratory's research exploring the skin microbiome and microbial roles in maintenance and defense of the skin as a physical barrier. Owing to features like continuous epidermal turnover, regions of high oils and salts, and lower temperatures than other body parts, the mammalian skin presents a diverse and dynamic environment. As such, the skin microbiome constitutes a complex ecosystem. Additional perturbations, such as antibiotic treatment, can also shift community composition, leading to colonization by pathogens like *Staphylococcus aureus* (16). To understand this process in more detail, the Grice laboratory developed a culture collection of over 70 isolates that they screened as cocultures, revealing a variety of interactions among members as well as the ability of some isolates to strongly inhibit *S. aureus* isolates from atopic dermatitis and skin and soft tissue infections. The skin microbiome can also enhance the physical barrier of the skin through impacts on host gene expression, including keratinocyte differentiation and epidermal development programs (17), and these impacts on skin integrity are affected by the state of colonization (acute versus chronic). Altogether, these results highlight the dynamic interplay between the skin

and microbiome and suggest the potential therapeutic use of the microbiome to improve skin barrier function and defense. Additional poster presentations explored skin microbiota properties, including the poster from Tiffany Scharschmidt (University of California, San Francisco, San Francisco, CA), entitled "Neonatal Priming Shapes Preferential Capacity for Immune Tolerance to Skin Commensal versus Pathogenic Bacteria."

In talks selected from abstracts, Michael Shapira (University of California, Berkeley, Berkeley, CA) presented work entitled "*C. elegans* as a Model for Studying Genetic Factors Shaping the Gut Microbiota—the Role of TGF-β Signaling." While *C. elegans* has proven to be an important laboratory model, it also persists in the wild in decaying fruit and plant material. In characterizing wild-type *C. elegans* raised in a natural-like environment, Shapira observed that worms have a microbiome distinct from the environment (18), with impacts on composition strongly influenced by host genetics and location. To determine genetic factors that impact microbiome structure, Shapira developed a synthetic community comprised of 30 gut isolates, which identified transforming growth factor β (TGF-β) as a major regulator of the microbiome. Specifically, TGF-β disruption leads to a bloom in *Enterobacter* and shifts its normally beneficial role to a detrimental effect. A poster presentation from Buck Samuel (Baylor College of Medicine, Houston, TX) also leveraged a synthetic *C. elegans* community in "Cultivated Relationships: *C. elegans* Genetic Landscapes That Shape Microbiome Form and Function," in which he developed a high-throughput pipeline to connect bacterial strains to host genetic pathways. The theme of the ways in which host genetics can influence microbiome assembly was continued by Amber Walters (Brigham Young University, Provo, UT) but with an interesting twist. Walters presented her study, entitled "The Microbiota Influences Life History Variation in *Drosophila melanogaster*." It has been well documented that flies from different latitudes exhibit different life history strategies, but it is not known how the microbiome might influence or be affected by such differences. Walters and colleagues found that microbiome compositions were similar between fly populations with shared life history strategies. However, while comparison between germfree and gnotobiotic flies supports some contribution of host genetics to these phenotypes, microbiome swaps between northern and southern fly populations, which represented extremes of life history strategy, could shift the underlying genetic adaptation, thus indicating the ability of the microbiome to override the influence of host genotype.

Two talks in the session highlighted the importance of host immunity proteins in shaping the microbiome but with unexpected insights into their mechanisms of action. Caitlin Murdoch (Duke University, Durham, NC) presented the talk "Microbiota-Induced Serum Amyloid A (Saa) in the Intestine Directs Systemic Neutrophil Function." Murdoch focused on *saa* as one of the most highly induced transcripts following microbiome colonization of germfree zebrafish larvae. Using genetically modified zebrafish in which the *saa* gene was either deleted or overexpressed in the intestinal epithelium, Murdoch and coworkers found that Saa plays both positive and negative roles in neutrophil biology, including the localization of these cells and priming their response to defend against bacteria. *saa* mutants were impaired in their ability to recruit neutrophils to wounds and had an altered distribution in tissues but exhibited improved pathogen clearance. Paralleling these *in vivo* observations, neutrophils isolated from *saa* mutants expressed increased levels of inflammatory mRNAs and had more bactericidal activity. This work showed how microbiota colonization, through induction of intestinal *saa* expression, tempers the systemic inflammatory potential of the innate immune system. Turning to the adaptive immune system, Gregory Donaldson (California Institute of Technology, Pasadena, CA), presented the talk "Indigenous Gut Bacteria Use Immunoglobulin A for Mucosal Colonization." Contrary to the traditional view that antibody production negatively impacts the fitness of host-associated microbes, Donaldson and collaborators found that *Bacteroides fragilis* uses its cell surface capsule to bind host secretory IgA and promote epithelial interactions that result in increased persistence in

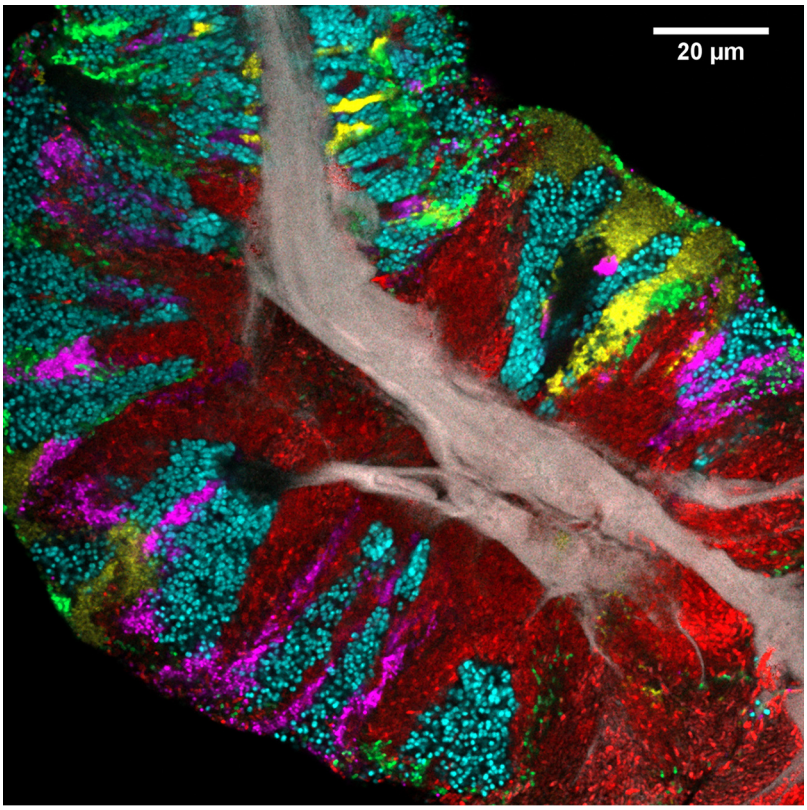

FIG 3 Human tongue dorsum microbial consortium. Each color represents a different genus. Cyan, *Rothia*; red, *Actinomyces*; yellow, *Neisseria*; magenta, *Veillonella*; green, *Streptococcus*; white, host epithelial material. (Courtesy of Steven Wilbert and Gary Borisy; adapted from reference 44.)

the face of competition by related bacteria (19). These findings highlight the complex, and sometimes beneficial, interaction between host immunity and colonizing microbes.

Not just host-encoded factors but also resident microbes play important roles in shaping host-microbe interactions, as illustrated by the talk from Spencer Nyholm (University of Connecticut, Storrs, CT), "The Hawaiian Bobtail Squid as a Model for Studying Defensive Symbioses and Development." In studying the microbiome of the accessory nidamental gland (ANG), a reproductive organ in female cephalopods, including the bobtail squid (*Euprymna scolopes*), he showed that the ANG is packed with a diverse consortium of bacteria (20, 21). The ANG sits adjacent to the oviduct, and as eggs pass by the gland, bacteria are deposited into the developing jelly coat layer. He showed that these bacteria produce antimicrobials that he suggested serve to protect the externally developing embryos from fouling by environmental microbes. These results show how microbiomes of adult hosts can confer benefits on their offspring. To fully appreciate these complex microbial community interactions, Jessica Mark Welch (Marine Biological Laboratory, Woods Hole, MA) shared cutting-edge methodologies in her talk, "Spatial Organization Illuminates Taxon-Taxon Interactions and Host Modulation of Microbiome Structure." Welch presented stunning images of both oral biofilms and gut microbial communities generated using a technique termed CLASI-FISH (Fig. 3). This method is a variation of classical fluorescent *in situ* hybridization (FISH) that uses spectral imaging to distinguish up to 16 different fluorophores and combinatorial labeling to generate a range of spectral signatures, enabling microscopists to distinguish up to 120 different bacteria in a community (22). The application of this imaging method to complex communities has allowed quantification of interactions between microbes that more commonly exist in proximity to each other, revealing new hypotheses about syntrophic relationships (23, 24). Another innovative application of *in situ* hybridization for studying host-bacterium interactions was shared in the poster "Spa-

tiotemporal Expression of Host Genes in Response to Colonization in the *Euprymna scolopes-Vibrio fischeri* Symbiosis," by Tara Essock-Burns (University of Hawaii at Manoa, Honolulu, HI). Here, hybridization chain reaction fluorescent *in situ* hybridization was used to provide detailed spatial patterns of host gene expression in response to colonization. The spatial organization of bacterial communities and host responses was further explored in the next session, Ecology and Evolution of Microbe-Host Interactions.

ECOLOGY AND EVOLUTION OF MICROBE-HOST INTERACTIONS

The session Ecology and Evolution of Microbe-Host Interactions highlighted the ecological constraints on host-microbe systems, from the geographic availability of partners to the resource provisioning that occurs within individual host-microbe partnerships. The opening speaker, Kathryn Jones (Florida State University, Tallahassee, FL), began the session by describing one of the longest-studied host-microbe interactions, the relationship between rhizobial bacteria and leguminous plants. This endosymbiotic association is tightly regulated and limited to specific bacteria that are recruited to plant roots. In response to bacterial signals, the hosts differentiate to create nodules that house the bacteria and create the conditions conducive for them to fix nitrogen for use by the plant. This reciprocal manipulation by bacterium and host is necessary for successful establishment of the symbiosis. To understand these processes in more detail, Jones uses a *Medicago truncatula* (alfalfa)-*Sinorhizobium meliloti* model. When associated with *S. meliloti* in the laboratory, *M. truncatula* grows on media without nitrogen, providing a great tool to study their interactions. Symbiotic bacteria communicate to *M. truncatula* via lipochitooligosaccharides called Nod (nodulation) factors. Bacteria then travel down an infection thread and are endocytosed by the host, leading to nodule formation and the differentiation of bacteria within the nodule. By screening mutants of *S. meliloti*, Jones identified the key feature of the microbe-derived polysaccharide succinoglycan that is necessary for the establishment of symbiosis with *M. truncatula* (25). *S. meliloti* succinoglycan-deficient mutants are unable to invade *M. truncatula*. Jones further showed that bacterial processing of this succinoglycan modulates the bacterium-host interaction. For example, certain bacterial mutants deficient in infection thread formation produce large quantities of a high-molecular-weight form of succinoglycan that cannot be cleaved by bacterial glycanases, suggesting that the cleaved, low-molecular-weight form is needed for the interaction. However, glycanase cleavage is not completely required because strains deficient in glycanase production are still able to invade *M. truncatula* and establish a functional, albeit less efficient, symbiosis.

Ryan Melnyk (University of British Columbia, Vancouver, BC, Canada) also explored bacterial symbioses in his work, "Evolutionary Origins of Bacterial Commensalism and Pathogenesis in the Plant Rhizosphere." Using a comparative genomics analysis of plant-associated *Pseudomonas* spp., Melnyk found evidence that lateral gene transfer is responsible for the transition of strains between pathogenic and growth-promoting phenotypes (26). The theme of the evolution of plant symbioses was also explored in a poster presentation by Jean-Michel Ané (University of Wisconsin—Madison, Madison, WI), entitled "Evolution and Engineering of Symbioses between Plants and Beneficial Microbes." Expanding these evolutionary questions to insect systems, Miguel Medina Munoz (West Virginia University, Morgantown, WV) talked about a genetic approach to explore the interaction between tsetse flies and members of the *Sodalis* genus in "Quorum Sensing: Tipping the Scales in Symbiosis." The *Sodalis glossinidius* symbiont is widespread in tsetse fly tissues, residing intracellularly and extracellularly, but is mainly localized to the gut. Interestingly, a progenitor-like strain of *Sodalis praecaptivus* uses quorum sensing to moderate itself during artificial infection of a weevil. It produces a toxin at low cell density during the establishment phase of infection, which is then downregulated at high cell density, promoting persistence (27). *S. praecaptivus* is also able to colonize tsetse flies through a mechanism that requires quorum sensing, suggesting that quorum sensing regulation of host colonization is likely a conserved

trait. On the topic of connecting natural diversity to evolving host interactions, Ella Rotman (Northwestern University, Chicago, IL) and Katherine Bultman (University of Wisconsin—Madison, Madison, WI) presented a poster on surprising diversity in symbiotic biofilm regulation, entitled "Distinct Biofilm Regulatory Strategies across the *Vibrio fischeri* Evolutionary Tree" (28).

Additional talks in the session explored ways in which nutrients or host-associated chemicals shape host-microbe associations. Aspen Reese (Harvard University, Cambridge, MA) presented data in her talk, "Microbial Nitrogen Limitation in the Mammalian Large Intestine," supporting the hypothesis that the intestinal tracts of dozens of mammals surveyed are intrinsically nitrogen limited. A key source of amino-acid-derived nitrogen is the secreted mucin that helps protect the intestinal epithelium from microbial encroachment. Reese presented data supporting a model in which, during states of lower rates microbial colonization (e.g., after antibiotic treatment), the host secretes less mucus because it has fewer microbes from which it needs protection as well as a lower microbial demand for host-supplied nitrogen. The concept that host secretions, containing sources of nitrogen from amino acids and amino sugars, is a "set point" for microbial colonization levels has important implications for how homeostasis between host and microbe is achieved in the gut and other habitats (29). Kellyanne Duncan (Brown University, Providence, RI) also explored bacterial interactions with mucus in her talk, "Bacterial Communities within the Intestinal Mucus Layer Exhibit Distinct Spatial Variation in Composition and Diversity." In the mammalian colon, secreted mucus forms two distinct layers: an adherent gel-like and sterile inner layer and a looser outer layer where microbes can colonize (30). The work presented by Duncan used laser capture microdissection (LCM), followed by 16S rRNA community analysis, to probe microbial communities on a fine spatial scale to describe microbial heterogeneity within the colonic mucus layers. Angus Chandler (University of California, Berkeley, Berkeley, CA) shared his work, "Bacteria-by-Ethanol Interactions Impact *Drosophila melanogaster* Fitness and Physiology." Most previous work has focused on impacts of ethanol vapor on flies, whereas Chandler investigated how ethanol ingestion impacts flies and how these effects are mediated by the animal's microbiome. Chandler observed that dietary ethanol leads to a dose-dependent decrease of life span in germfree flies, while conventionally reared flies have stable life spans at low and moderate ethanol concentrations. Chandler found that ethanol also alters some immune markers but only in conventionally reared flies (31), which may parallel findings in humans that ethanol-induced inflammation and alcoholic liver disease are influenced by the microbiome. Reed Stubbendieck (University of Wisconsin—Madison, Madison, WI) presented "Bacterial Competition Mediated by Siderophore Production among the Human Nasal Microbiota." Some members of the prominent *Actinobacteria* genus *Corynebacterium* have the ability to suppress growth by members of the prominent *Firmicutes* genus *Staphylococcus*. By sequencing the genomes of *Corynebacterium* isolates with strong and weak inhibition of *Staphylococcus epidermidis*, Stubbendieck and his collaborators identified a siderophore biosynthetic gene cluster (BGC) that is unique to the *Corynebacterium* strains with strong inhibitory activity and confirmed that these strains produce siderophore activity *in vitro*. Supplementing *Corynebacterium-Staphylococcus* competition cocultures with extra iron relieved growth inhibition by siderophore-producing *Corynebacterium*. Finally, *in vivo* expression of the siderophore BGC was confirmed using metatranscriptomic data from the Human Microbiome Project, suggesting that available iron is a critical nutrient in the nasal microbiome (32).

SOCIAL INTERACTIONS AND MICROBIAL TRANSMISSION

The session Social Interactions and Microbial Transmission spanned many spatial and temporal scales of microbial transmission, from the immediate movements of microbes in the oral cavity, to the movement of microbes across host populations in a fish tank or a coral reef, to host adaptations to their resident microbes over evolutionary time scales. Ilana Brito (Cornell University, Ithaca, NY) chaired the session and spoke about unpublished work on the epidemiology of the human microbiome explored in

a network of 5 villages on an island in Fiji. Using extensive metagenomic data, collected from three body sites of each individual at multiple time points, combined with anthropological data on social networks, she showed that patterns of microbiome sharing could be discerned within households, between spouses, and between mothers and their children, even when the children were grown and living in separate households. Brito reported that similar to supershedders of pathogens, such as Typhoid Mary, individuals could be microbiome supersharers, a status that was not simply explained by their connectedness in social networks. The talk captured the excitement of the emerging frontiers of studying microbiome transmission in social interactions. The work set the stage for the rest of the session by highlighting the need for comparative studies across different host systems, studies using model systems amenable to manipulation, and studies involving social scientists and ethicists to consider the causes and consequences of being able to predict social interactions from microbiome data.

In the subsequent talks selected from submitted abstracts, we heard about new mechanisms mediating microbial transmission from Abhishek Shrivastava (Harvard University, Cambridge, MA). In his talk, entitled "Cargo Transport Shapes the Spatial Organization of a Microbial Community," Shrivastava showed how gliding bacteria like the human oral bacterium *Capnocytophaga gingivalis* use a type IX secretion system-based apparatus to move in a screw-like manner across surfaces or other cells. Strikingly, he showed how nonmotile bacteria like *Prevotella oris* could "hitch" rides on the corkscrewing *C. gingivalis* cells, resulting in redistributions of cells into nonuniform distributions or islands (33). Islands of microbes were also evoked in the subsequent three talks that covered the transmission of host-associated microbes in aquatic systems. We heard from Clotilde Bongrand (University of Hawaii at Manoa, Honolulu, HI), in her talk, "Achieving a Multistrain Symbiosis: Strain Dominance or Sharing in the Host," about strain variation in *Vibrio fischeri* symbionts of the juvenile Hawaiian bobtail squid light organ. Whereas some of these strains, termed "S" for "sharing," would coexist together in the same squid, other "D," or "dominant," strains would dominate the colonization. In head-to-head experimental competitions, this dominance was achieved by the D strains colonizing the host faster and persisted even if the inoculations were heavily skewed to favor the S strains (34). A similar story of the importance of immigration was relayed by Cathy Robinson (University of Oregon, Eugene, OR) in her talk, "Experimental Evolution of a Bacterial Symbiont to its Vertebrate Host Reveals a Primary Role for Immigration in Host Adaptation." Robinson showed that experimentally evolved *Aeromonas veronii* would outcompete ancestral strains for dominance in gnotobiotic larval zebrafish by immigrating into the intestines more quickly. Sequencing of the genomes of the evolved isolates revealed multiple mutations in a diguanylate cyclase gene that she showed functions in regulating bacterial swimming speed. Alison Gould (California Academy of Sciences, San Francisco, CA) described work in natural populations of coral reef cardinalfish in her talk, entitled "Shedding Light on Symbioses: Lessons from a Bioluminescent Vertebrate-Microbe Association." In this elegant study, she used genetic mapping of both hosts and their luminescent symbionts. The patterns of genetic variation in the hosts showed no clear geographic structure and were consistent with admixture, whereas the symbiont genetic variation was geographically structured, consistent with the fish acquiring their symbionts during larval dispersal and then maintaining these symbionts during adulthood through continual seeding of their adopted home reefs (35). More evidence for microbiome transmission came from the poster of David Kang (Cornell University, Ithaca, NY), "Back to Nature: Assembly of the Gut Microbiome in Natural Populations of *Drosophila*," which provided evidence for microbial transmission dynamics shaping the microbiomes of wild-caught fruit flies.

Extending the idea of mutualist transmission from life span to evolutionary time scales, Hongjie Li (University of Wisconsin—Madison, Madison, WI) talked about "Convergent Evolution of Complex Structures for Ant-Bacterial Defensive Symbiosis in Fungus-Farming Ants." Through phylogenetic reconstructions, ancient amber fossils, and electron micrograph analysis of the exoskeletal structures of extant ants (Fig. 4), he

FIG 4 A queen ant imaged under an electron microscope is covered in black dots, each of which is a pocket capable of housing and supporting symbiotic bacteria. (Courtesy of Richard Noll and Hongjie Li.)

showed that fungus-farming ants have evolved defensive symbioses with bacteria at least three independent times (36). The session closed with a talk from Rosa Krajmalnik-Brown (Arizona State University, Tempe, AZ), "2-Year Follow-Up Study Reveals Consistent Benefits of Microbiota Transfer Therapy on Autism and Gut Symptoms." In this study, which involved a comprehensive microbiome-oriented therapy, including fecal microbiome transplants, pediatric patients with autism spectrum disorder (ASD) who received the treatment were found to have increased fecal microbiome diversity and improved gastrointestinal and behavioral symptoms, as reported by parents and assessed by an independent evaluator. Implicit in these findings is the possibility that reduced microbial transmission during childhood could be a risk factor for the development of ASD.

MICROBE-HOST INTERACTIONS AT THE MOLECULAR SCALE

The session Microbe-Host Interactions at the Molecular Scale highlighted the major advances in understanding the chemical biology of host-microbe partnerships. The opening talk for this session, "Deciphering the Human Microbiota with Chemistry," was presented by Emily Balskus (Harvard University, Cambridge, MA). A major goal of the Balskus laboratory is to determine the mechanisms underlying the gut microbiota's influence on human biology, with a focus on how chemicals produced by the microbiome mediate this interaction. While many microbiome metabolites have been identified, a major limitation is that most of this microbial chemistry has not been linked back to specific microbiome members. Balskus highlighted the example of choline, a dietary compound that is metabolized by a variety of microbiota to produce trimethylamine (TMA), which is absorbed by the host and further metabolized. Genome mining identified a choline TMA-lyase enzyme, CutC, from anaerobic gut microbes that contributes to this metabolism. Elegant gnotobiotic mouse experiments performed in collaboration with Federico Rey demonstrated the importance of CutC in choline

metabolism and suggested the possibility of altering host metabolism by targeting microbial chemistry using small-molecule inhibitors of key pathways (37). In closing her talk, she noted that the vast pool of genes from metagenomes that encode enzymes of unknown function are also likely a source of underappreciated microbiome metabolite activity. As a proof of concept, she highlighted an example of a gene of unknown function that is found at high copy numbers in the gut. Purification of this enzyme confirmed a predicted function based on comparative genomic analyses (38), pointing to the utility of chemically guided functional profiling to discover new enzymes and metabolites from microbiomes that influence host physiology.

Continuing on the theme of connecting microbial physiologies and metagenomics, Zakee Sabree (The Ohio State University, Columbus, OH) presented work, entitled "Modeling Host-Microbial Interactions at the Gut Interface," focusing on the omnivorous cockroach *Periplaneta americana*. Culturing of endemic and abundant members of the cockroach gut community recovered significant bacterial diversity, especially among members of the *Bacteroidetes*, from which several new *Bacteroides*, *Dysgonomonas*, *Paludibacter*, and *Parabacteroides* species were isolated. Sabree's results expand the set of polysaccharide-digestive functions conferred by mutualistic microbes, with striking parallels to the mammalian digestive tract. In another example of connecting metabolites and genomes, Kali Pruss (Stanford University, Palo Alto, CA) presented data from experiments aimed at testing the hypothesis that toxin-mediated inflammation benefits toxigenic *Clostridium difficile* during infection. She used transcriptome sequencing (RNA-seq) and metabolomics to measure differences in the physiological responses of members of a synthetic community, built in germfree mice, to colonization by toxigenic or nontoxigenic *C. difficile* and a series of laboratory-adapted strains that vary in the amounts of inflammation that they elicit. The results presented support the conclusion that fully virulent, toxigenic *C. difficile* elicits significant alterations in the responses of other community members, which may help it sustain colonization during infection. Noting the importance of small molecules in microbe-host interactions, the talk from Fariba Assadi-Porter (University of Wisconsin—Madison, Madison, WI), "Novel Tools for *In Vivo* Functional Analysis of Host-Microbiome Interactions," focused on technology to analyze metabolite exchange. The work focused on assessing *in vivo* microbial metabolism in hibernating ground squirrels. Hibernation is a period of fasting, but squirrels do go through oscillations of arousal and torpor. Assadi-Porter described nuclear magnetic resonance (NMR)-based assays for *in vivo* measurements of the metabolism of carbohydrates such as inulin. By measuring labeled inulin (which is metabolized only by the microbial partners), Assadi-Porter observed microbiome- and season-dependent changes in short-chain fatty acids derived from inulin.

Moving from community-wide characterization of metabolisms to that of individual strains, Robert Glowacki (University of Michigan, Ann Arbor, MI) presented work entitled "Ribose Metabolism in *Bacteroides thetaiotaomicron* Plays an Important Role *In Vivo* in a Diet-Dependent Manner and May Represent a Nutrient Niche." Beginning with a gene cluster first identified in *B. thetaiotaomicron*, Glowacki discovered certain functions that are essential for growth on free ribose or certain covalently linked forms (pyrimidine nucleosides and RNA). Bacteria lacking this cluster exhibit a competitive fitness defect *in vivo* in a diet-dependent fashion. The most relevant sources of biologically available ribose that drive this fitness effect are still unknown. However, comparative genomic analysis of dozens of *Bacteroidetes* genomes revealed a family of 70 partially homologous but highly diversified gene clusters containing the same core ribose utilization functions as *B. thetaiotaomicron*. Each is equipped with a different enzyme repertoire that may release ribose from a variety of different biological molecules. These findings highlight the importance of competition for available nutrients in the gut microbiome and other ecosystems and the genetic malleability of systems to adapt to the same molecule present in different forms. A second talk on *Bacteroides* in this session, by David Clarke (University College Cork, Cork, Ireland), also highlighted the metabolic innovations of this important inhabitant of the mammalian intestine. In this talk, entitled "The Production of Glycine Lipids Is an Important Fitness Determinant in

Bacteroides thetaiotaomicron," Clarke described the discovery of two conserved and adjacent *Bacteroides* genes, *glsA* and *glsB*. The genes encode acyltransferases that are both required for the production of glycine lipids, and heterologous expression in *Escherichia coli* was sufficient for glycine lipid synthesis. Deletion of the *glsB* gene in *B. thetaiotaomicron* revealed that glycine lipids are important for the membrane composition of both lipids and proteins. Further characterization of the mutant suggested that these lipid and protein modifications are important for *B. thetaiotaomicron* to withstand environmental stresses and to colonize the mammalian gut (39). A final theme of the session, explored by Vania Pankievicz (University of Wisconsin—Madison, Madison, WI), was host molecular mechanisms that support symbiont growth. Pankievicz presented work on maize plants, which are notoriously nitrogen limited, resulting in financially and environmentally costly fertilization. She described an indigenous giant maize from the Sierra Mixe region of Mexico that reaches heights of 15 to 18 ft but grows without nitrogen fertilizer. A striking feature of this giant maize is that it produces about 3 to 4 times the number of aerial roots compared to modern maize varieties. Immediately after rainfall, these aerial roots start producing a large amount of mucilage, within which they measure significant amounts of nitrogenase activity (Fig. 5). Using culturing and metagenomics, it was shown that the maize mucilage was enriched in nitrogen-fixing bacterial taxa and that giant maize required both mucilage production and bacteria within the mucilage for proper nitrogen fixation (40).

ENGINEERING BENEFICIAL MICROBES

The session Engineering Beneficial Microbes explored many dimensions of the promises and challenges of manipulating host-associated microbes and microbial communities. Robert Britton (Baylor College of Medicine, Houston, TX) started off the session with a talk entitled "Engineered Microbe for the Intestinal Delivery of Interleukin-22," which highlighted multiple hurdles that his group has overcome to adopt synthetic biology approaches to a non-laboratory-adapted bacterium, *L. reuteri*. To engineer *L. reuteri* to express the therapeutic protein interleukin-22 (IL-22), Britton had to optimize every dimension of the protein's expression in bacteria, from codon usage to secretion sequence. This group showed that the bacteria produce biologically active IL-22 in human intestinal organoids, but additional factors will need to be considered before introducing this engineered probiotic into humans, including the potency of the organism and its biocontainment.

Sean Leonard (University of Texas at Austin, Austin, TX) continued the theme of engineering nonmodel bacteria in his talk, entitled "Symbiont-Mediated RNAi in *Apis mellifera* with Engineered Gut Microbiota." He described a new set of genetic tools for modifying *Proteobacteria* from honeybees and showed how symbionts could be engineered with consequences for the host (41). Travis Wiles (University of Oregon, Eugene, OR) also presented work on engineering nonmodel bacteria from the zebrafish intestine in his talk, "Bacterial Motility and Chemotaxis Promote Stable Intestinal Colonization and Host Inflammation." Studying a resident *Vibrio* species, he used genetic switches (42) to toggle on and off the trait of bacterial motility during the colonization process and showed that this bacterium requires motility within the intestine to resist expulsion by the flushing forces of peristalsis (Fig. 6). Furthermore, he showed that motile, but not nonmotile, *Vibrio* species in the intestine induce host inflammatory responses, suggesting that motility is an engineerable trait for modulating bacterial persistence and immunostimulatory activity. Bookending the session was a second talk on *L. reuteri* from Laura Alexander (University of Wisconsin—Madison, Madison, WI), "Exploiting Prophage-Mediated Lysis for Biotherapeutic Release by *Lactobacillus reuteri*," which showed that lysis by naturally occurring prophages in this probiotic bacterium could be harnessed to deliver biotherapeutic molecules. Not only does this approach alleviate the need to optimize signal peptides to enhance the secretion of recombinant proteins, phage-mediated lysis also contributes to biological containment of the recombinant probiotic. In collaboration with Bernd Schabl, it was demonstrated that phage-mediated delivery of IL-22 by *L. reuteri* alleviated alcohol-induced liver

FIG 5 Aerial roots of Sierra Mixe maize with secreted mucilage. (Courtesy of Jean-Michel Ané.)

disease in mice, which exemplified the potential of this approach to deliver therapeutics *in situ*.

A second theme of the session was the importance of considering microbial communities when engineering beneficial microbes. In his talk, entitled "Introducing THOR, a Model Microbiome for Genetic Dissection of Community Phenotypes," Gabriel Lozano (Yale University, New Haven, CT, and University of Wisconsin—Madison, Madison, WI) presented his work on a simple rhizosphere model community that is amenable to thorough genetic analyses of the emergent properties of the system that influence the ecology of each member. Thomas Mansell (Iowa State University, Ames, IA), in his talk, "Engineered Substrate Usage Allows Prebiotic Control of Microbial Community Population and Gene Expression," also explored the capacity to manipulate microbial communities via prebiotics. By engineering the well-characterized *E. coli* Nissle strain, currently used as a probiotic in humans, to break down human milk oligosaccharides, he showed how community membership could then be manipulated by the availability of these glycans (43). The poster session presented additional uses of

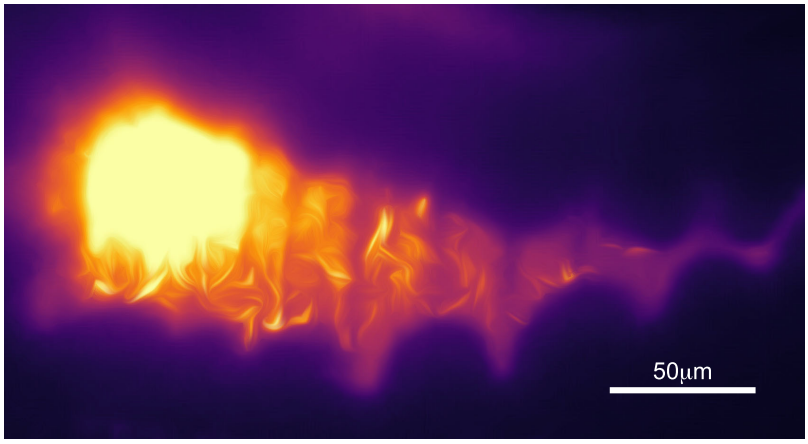

FIG 6 *Vibrio cholerae* swimming in zebrafish intestine. Depicted is a stylized image of a highly motile *Vibrio cholerae* symbiont (amber) within the larval zebrafish intestine (dark, convoluted silhouette). The swim trajectories of individual bacterial cells appear as swirls and streaks as they amass within an anterior region of the intestine against peristaltic flow. (Courtesy of Travis Wiles.)

E. coli Nissle. In a second presentation, Mansell showed how *E. coli* Nissle could be engineered to produce and sense butyrate and improve *C. elegans* fecundity, while Alexander Barron (University of Connecticut, Storrs, CT) showed that *E. coli* Nissle colonizing *Drosophila melanogaster* intestines could protect against reactive oxygen species stress. Finally, Michael Zimmermann's (Yale University, New Haven, CT) talk, "Disentangling Host and Microbiome Contributions to Drug Pharmacokinetics and Toxicity," combined bacterial genetics, gnotobiology, and pharmacokinetic modeling to uncover the contributions of specific microbiota members to drug metabolism. This talk echoed many themes of the meeting about the molecular mechanisms of microbe-host interactions and the specific contexts in which they matter.

CLOSING NOTES AND OUTLOOK FOR THE FUTURE

A major question, highlighted in Handelsman's keynote address and running throughout the 7th Conference on Beneficial Microbes, was the definition of healthy microbial communities. Salient defining features include communities with high diversity, high productivity, and resilience to perturbation. All of these features are also apt descriptors of the community of scientists studying beneficial microbes who gathered in Madison this past summer. Undeterred by ASM's withdrawal of support, the community rallied. Private foundations such as the Burroughs Wellcome Fund and the Gordon and Betty Moore Foundation continued their generous support of the conference, and representatives from the burgeoning microbiome sciences industry stepped up to participate. Top scientists in the field accepted invitations as keynote speakers and session chairs, and investigators submitted their best work for presentations, allowing us to put together an outstanding scientific program with representatives from all academic ranks, from graduate students to full professors. The new format at a university campus allowed us to hold an even more interactive and financially accessible meeting than in the past. Strong attendance, exciting work presented, and establishment of partnerships with industry groups all suggest that the Conference on Beneficial Microbes has successfully adapted and has a promising future. Mark your calendars for the 8th Conference on Beneficial Microbes in Madison, WI, 12 to 16 July 2020.

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