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BIOTECHNOLOGY PROGRESS

TOPICAL PAPER

Applied Cellular Physiology and Metabolic Engineering

Proceedings from the 3rd International Conference on Microbiome Engineering

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Abstract

The human microbiome has been inextricably linked to multiple facets of human physiology. From an engineering standpoint, the ability to precisely control the composition and activity of the microbiome holds great promise for furthering our understanding of disease etiology and for new avenues of therapeutic and diagnostic agents. While the field of microbiome research is still in its infancy, growing engineering efforts are emerging to enable new studies in the microbiome and to rapidly translate these findings to microbiome-based interventions. At the 3rd International Conference on Microbiome Engineering, leading experts in the field presented state-of-the-art work in microbiome engineering, discussing probiotics, prebiotics, engineered microbes, microbially derived biomolecules, and bacteriophage.

KEYWORDS

bacteriophage, metabolic engineering, microbiome, probiotics, synthetic biology

1 | INTRODUCTION

The microbiome, the collection of microorganisms that inhabit the human body, is extensively associated with various healthy and disease states.[1,2] Although in their infancy, techniques to precisely control the composition or activity of a microbiome would greatly contribute to our understanding of the relationship between host and microbes and potentially present a new avenue for disease intervention and diagnosis. The 3rd International Conference on Microbiome Engineering presented state-of-the-art strategies to engineer microbiomes from leading experts in the field in both academia and industry (Figure 1). Additive approaches in microbiome engineering were presented, including natural and engineered probiotics and microbial consortia as well as microbially-derived small molecules. This 3-day conference in December 2020 spanned broad topics highlighting systems and subtractive based approaches, molecules and metabolism in the microbiome, and microbes as drugs; each expanding on the theme of microbiome-based therapeutics. Each day began with an opening keynote presentation leading into sessions with presenters. In total, there were six scientific sections where 26 invited speakers and poster presenters spoke about work from their own labs and companies during pre-recorded talks with live panel discussions following each section (Table 1). Due to its virtual setting, this conference brought in speakers and attendees from more than a dozen countries. This article is a review of the key themes and topics that were presented during the 3rd Conference.

2 | SYSTEMS AND SUBTRACTION

2.1 | Keynote Speaker: Timothy Lu

The meeting was opened by keynote speaker, Timothy Lu, Professor of Electrical Engineering & Computer Science and Biological Engineering at MIT. Lu began by acknowledging advances in DNA synthesis and sequencing that have been key to understanding and engineering

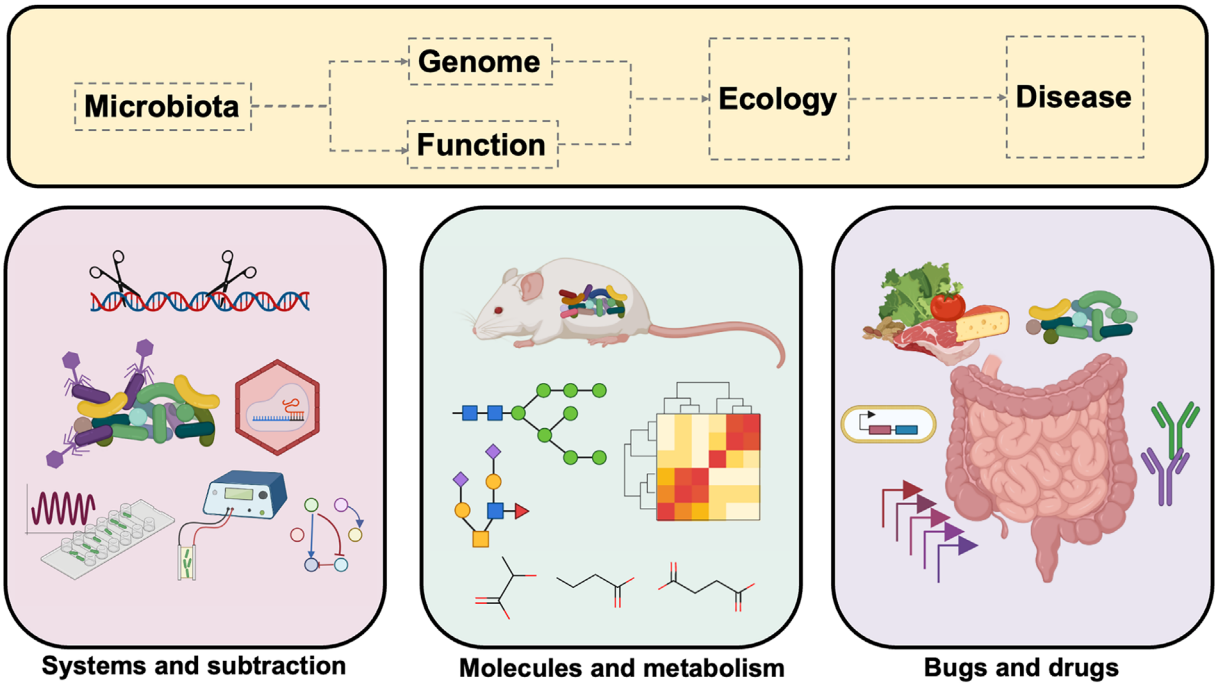

FIGURE 1 Strategies to engineer microbiomes broken down by the three themes of the Third International Conference on Microbiome Engineering. The systems and subtraction session explored subtractive engineering strategies for microbiome manipulation as well as systems-based approaches to understanding microbiota dynamics. The molecules and metabolism session focused on modulating microbe-associated molecules to affect microbial metabolism, interspecies interactions, and host physiology. Bugs and drugs sessions probed microbe–host relationships and how engineered microbes can be utilized in translational approaches to mitigate disease

biological systems. While the microbiome has been proposed to be a key modulator of human health, key limitations exist with regards to the availability of tools to fully understand and manipulate the microbiome. Lu's lab has been working toward using synthetic biology to develop tools to make targeted additions or subtractions to microbial communities. First, he discussed subtractive techniques to remove specific members of the microbiome, largely using bacterial viruses or bacteriophage. The Lu lab developed targeted nuclease-based antimicrobial agents that recognize specific strains using the CRISPR system. His team, led by Citorik and Mimee, packaged the CRISPR-Cas9 system into bacteriophage delivery agents and demonstrated targeted killing of drug-resistant bacteria as well as the selective killing of members in a synthetic community.[3] Next, the lab also developed a yeast-based platform for phage engineering to modulate phage host ranges for several members of the T7 phage family. Although phage-based antimicrobials are a promising, specific treatment option, especially for multi-drug-resistant bacterial infections, bacteria also acquire phage resistance.[4] Taking inspiration from antibody specificity engineering, the Lu lab took an engineering-focused approach to develop 'phagebodies' where they genetically engineered the host-range-determining regions in bacteriophage tail fibers to broaden host range.[5] They identified mutations that allowed the phagebodies to be effective against naturally occurring T3-resistant mutants. Another aspect of Lu's work is geared toward engineering diagnostic bacteria for the gut microbiome for specific biomarker signals including bleeding, inflammation, infection, and cancer. One example is a blood-sensing bacterial strain that utilizes a heme transporter ChuA and a heme-responsive transcriptional repressor HtrR to allow for the expression of a reporter luciferase.[6] The authors took this a step further by building a low-power microelectronic pill for multiple analytes that would allow the signal to be transmitted to an electronic receiver outside of the body.

2.2 | Speaker sessions

The first session began with a presentation from Eligo Bioscience (Paris, France). They are an innovative startup developing a unique approach to engineer the microbiome at the gene level to provide new therapeutic options to patients with microbiome-associated diseases. Aurelie Grienenberger, Chief Business Officer at Eligo, introduced the company's novel modality that packages synthetic therapeutic genes to restore a healthy microbiome without any collateral destruction of commensal bacteria using the Eligobiotic platform, a phage-derived particle engineering platform that harnesses bacteriophages to create phage-derived particles devoid of phage genome. Phage-derived particles deliver an RNA-guided CRISPR-Cas nuclease into bacterial populations of the microbiome, creating targeted lethal DNA double-stranded breaks in targeted strains. Additionally, two platforms that Eligo have developed are the FAME (Function Addition to the Microbiome) platform that uses engineered bacteria to deliver therapeutic payloads, including antigens, nanobodies, and cytokines, via gene cassettes, and the SSAM (Sequence-Specific AntiMicrobial) platform, designed to deliver a nuclease to create sequence-specific

TABLE 1 Poster presentations

Author	Contact	Title
Lei Dai	SIAT, Chinese Academy of Sciences	Colonization and Resilience of Transplanted Gut Microbiota in Aged Mice
Irina Utkina	University of Toronto	Developing Effective Prebiotics and Probiotic Consortia through Community-Based Metabolic Modeling
Jason Lynch	Massachusetts General Hospital	Development of Designer Probiotics for Targeted Delivery of Immunomodulatory Payloads
John Pisciotta	West Chester University	Effect of Microbiome Manipulation on Withdrawl-like Behavior in Planaria
Liana Merk	Richard Murray's lab at Caltech	Split Activator AND Logic Gate for Inflammation Sensing
Jia Wang	Oak Ridge National Laboratory	Genome-scale Modeling to Elucidate Stable Community Assembly in a Rhizosphere
Nicholas Horvath	Synlogic Therapeutics	*In-silico* Simulation for Predicting Oxalate Distribution in the Body and Strain Activity on Oxalate and its Transit through the Gut for Treatment of Enteric Hyperoxaluria
Ethan Hillman	Purdue University in collaboration with the Pioneer Oil Company	Modulating Microbial Metabolism in Native Microbes Found in Oil Wells to Enhance Oil Recovery, Specifically Targeting Sulfate and Nitrate-reducing Bacteria
Baylee Russell	University of California, San Diego	The Persistent Physiological Changes in a Host Using Engineered Native Bacteria
Sushmita Sudarshan	General Automation Lab Technologies	High-throughput Microbial Solation, Cultivation, and Screening Platform Which Can be used to Investigate Specific Metabolite-producing Bacteria From the Human Gut Microbiome
Mohit Verma	Purdue University	The Death Phase Deriving From Metabolite Utilization in Microbiome Dynamics
Matthew Ostrowski	The University of Michigan	The Unintentional and Intentional Engineering of Probiotics Using Xanthan Gum

antimicrobials. Eligo is using the SSAM platform for three lead indications: prevention of hemolytic uremic syndrome, reducing the rate of antibiotic-resistant infections in patients undergoing organ transplants, and treatment of acne.

This session continued with a speaker from industry, Eric van der Helm, head of Synthetic Biology and Bioinformatics at SNIPR BIOME. SNIPR (Copenhagen, Denmark) uses precision CRISPR killing to modulate human microbiomes by targeting bacteria implicated in infectious diseases, inflammatory disorders, and cancer. The company developed a novel modality, CRISPR-Guided Vectors (CGVs) to selectively and precisely eradicate target bacteria while leaving the rest of the microbial community intact. Upon oral administration of the CGV, it docks onto specific target bacterial strains and expresses a 30-nt CRISPR RNA with homology to the bacterial DNA, forming a complex with the endogenous Cas nuclease and creates a double-stranded break in the bacterial genome, killing the target strain. Once the development candidate has been characterized and selected, its toxicity will be evaluated for Phase 1 clinical trials. Van der Helm presented a case study on SNPR002 that selectively kills *Escherichia coli* strains in multi-drug resistant (MDR) bacterial infections. The synthetically engineered bacteriophage encoded RNA targeting three highly conserved essential genes in MDR *E. coli* strains. SNPR002 resulted in a 4-log reduction in CFU compared to control *E. coli* strains, demonstrating high selectivity, and the bactericidal activity was similar to that of gentamicin, with rapid killing within minutes. The murine gut colonization model also showed a 3-log reduction in CFU in 30 h.

Kicking off the next *Systems and Subtraction* session was Cullen Buie from MIT. In his talk, Buie highlighted an automated high-throughput, microfluidic electroporation platform, which can enable further exploration of the microbial world. Buie suggested that transformation of bacteria is the rate-limiting step in identifying genetically tractable microbial species and is a process amenable to automation. To address this bottleneck, the Buie Lab developed a high-throughput electroporation platform, based on a continuous flow transfection system. The transformation efficiency is a function of the flow rate and the channel geometry. With the continuous-flow system, they were able to achieve efficiencies that were comparable to traditional cuvettes, at very high processing rates.[7] To simplify the electroporation process for biology labs, they built a prototype microfluidic device containing an interface compatible with a liquid handling robot. Despite facing design challenges, this high-throughput electroporation system enables automation of a conventionally manual and laborious step while maintaining high transformation efficiencies. In addition, this system can be leveraged for rapid screening of electroporation conditions for a range of bacterial species, facilitating accelerated development of genetically engineered organisms.

Georg Gerber, an associate professor at Harvard Medical School, sees the understanding of microbial dynamics as an important step

toward the design of rational therapeutics to manipulate the microbiome. His team developed a novel machine learning method called Microbial Dynamical Systems INference Engine (MDSINE) that allows users to infer dynamical systems models from high-throughput time-series microbiome data and generate precise quantitative predictions about the temporal dynamics and stability of the microbial ecosystems.[8] In this model, Bayesian inference algorithms provide additional functionality, including estimates of error in inferences of dynamical systems parameters and statistical modeling of high-throughput sequencing count-based data over time. They used MDSINE to infer the underlying qualitative network of microbe-microbe interactions from an enteric infection dataset. The resulting network strongly predicted interactions among species, including well-known inhibition of *Clostridium difficile*, providing confirmatory evidence that MDSINE can detect causal interactions from longitudinal microbiota data.[9]

Johan Paulsson from Harvard Medical School spoke about his lab's interest in the question of fluctuations and dynamics in single cells. Every individual chemical reaction within a cell is probabilistic making predictions extremely difficult. Historically, the lab focused on building models and theorems for systems with incomplete information by figuring out how certain properties have global impacts on the cell by means of tracking morphology, growth rate, or gene expression in real-time. More recently, a continuous-culture microfluidic device where individual cells grow and divide in narrow trenches that are fed diffusively by orthogonal flow channels that pushes out the progeny, was adapted to study 700 generations of *Bacillus subtilis* growth while maintaining the same local environment.[10,11] To improve the throughput of the platform they increased the number of trenches to over 1 million, allowing tracking of over 1 million lineages, or up to 1 billion divisions/day which allows users to track morphology, growth rate, or gene expression in real-time.

3 | MOLECULES AND METABOLISM

3.1 | Keynote Speaker: Michael Fischbach

Michael Fischbach, professor at Stanford University, was the keynote speaker for the second session and opened his talk with challenges facing human gut microbiome research. His lab is committed to finding a model microbiome sufficiently large enough to capture all the salient biological functions of the microbiome. Defining a microbiome containing currently genetically tractable microbes would allow for the study of individual microbes and gene knockouts. Fischbach proposes the human microbiota is the largest endocrine organ based on its large output of microbially derived molecules, which are unique modulators of host metabolic and immune responses. To date, there has not been a straightforward way to knock out individual microbial functions in a model system, so there is much left to be discovered about the microbes and the molecules they produce.

A major producer of such modulatory molecules is the second largest group of bacteria in the gut—the anaerobic Firmicutes. Fischbach's team has been able to develop a new genetic system based on Cas9 to engineer a model Firmicutes, *Clostridium sporogenes*.[12,13] With this technology, they were able to produce multiple unmarked deletions in *C. sporogenes*, and are working toward building more novel genetic systems in gut microbes. With these tools, Fischbach and collaborators will evaluate the role of *C. sporogenes* metabolites in host physiology.[13]

Furthermore, Fischbach and his collaborators are working to build high complexity synthetic communities to test hypotheses using defined systems. By systematically reducing initial defined consortia, they have developed a defined microbial system including more than 100 members based on data from the NIH Human Microbiome Project—incorporating strains of the highest prevalence across individual samples. Using their system, a stable consortium can be reproduced to mimic features of native human microbiomes across a wide range of experimental conditions - enabling reproducible standards for future investigations.

One last topic mentioned in Fischbach's talk is the process of in vivo backfilling defined communities to form a more complete model. Starting with germ-free mice containing their stabilized 103-membered community, the group challenged it with undefined human fecal samples for a month before sample collection and metagenomic sequencing. To their surprise, about 80% of their initial community survived the challenge; 15 strains were lost and about 30 non-random new strains were incorporated from the human fecal samples—highlighting unfilled niches in the initial consortia. The group aims to establish a representative community of a human microbiome for use as a research tool and potentially as a therapeutic.

3.2 | Speaker sessions

To begin the speaker sessions of day two, Yemi Adesokan from Gnubiotics presented on conjugated glycans in the gut and metabolic diseases. Gnubiotics is a Swiss biotech company working to develop and produce conjugated glycans for human health. Gnubiotics is working to discover and produce glycans from human milk oligosaccharides (HMOs) on an industrial scale, due to their roles in reducing antibiotic use, inhibiting the growth of microbial pathogens, and facilitating the restoration of the microbiome after infection.[14–17] Gnubiotics has produced more than 60 conjugated glycans, including sialylated glycans, enabling selective microbiota activity. Their engineered products may support colonization resistance to undesirable microbes, production of short-chain fatty acids (SCFAs) that are reduced in IBD patients, and decrease markers enriched in IBS cohorts. By tuning each glycan, Gnubiotics can increase their structural diversity, modulate the structural composition, and differentiate glycan applications.

Also addressing the theme of innovation and targeted interventions using the microbiome in a predictive way, Angela Marcobal (BCD BioScience, Sacramento, CA, USA) gave a talk on the improvement of probiotic design. BCD focuses on carbohydrates, which are not described in detail or mapped to specific food products, despite being the most abundant biomolecules on the planet and a major

driver of microbiome composition.[18] BCD Bioscience hopes to produce highly specific prebiotics which contain the chemical and physical characteristics necessary for specific targeting of the human gut microbiome species and to fill the knowledge gap regarding low specificity fibers and prebiotics currently used in clinical studies. Such carbohydrate-mediated solutions of the selective microbiome and immune modulation are backed by their creation of the world's first library of oligosaccharides containing over 220 metabolites originating from natural products, incorporating their structural information and functional annotations into microbial biosynthesis parameters for prebiotic optimizations.

Continuing the theme of engineering novel therapeutics, Purna Kashyap (Mayo Clinic, Rochester, Minnesota) gave a talk outlining the roles of tryptamine in modulating gastrointestinal function and how these effects can be leveraged in engineered commensal microorganisms. In a translational study of Irritable Bowel Syndrome (IBS) patients experiencing diarrhea, increased levels of tryptamine were present in their stool.[19] In order to promote mucosal excretion in the gut, Kashyap moved to search for tryptophan decarboxylases present in *Clostridium sporogenes* and *Ruminococcus gnavus* that can produce tryptamine. Through a collaboration with Fischbach and Sonnenburg, a tryptamine overexpression *Bacteroides thetaiotaomicron* strain was constructed.[20] The engineered strain increased fecal water excretion and accelerated gastrointestinal transit in mono-colonized germ-free mice. The biological implication is that tryptamine evoked an increase in mucus secretion and attenuated disease severity predominantly in female mice following DSS administration as a model of inflammatory bowel disease (IBD). Kashyap's future work will include fine-tuning of distribution and density of genetically engineered microbes in the gut as they uncover new mechanisms of additive or synergistic bioactive molecules.

Jan Claesen (Cleveland Clinic Lerner Research Institute, Cleveland, OH) explores small molecule interactions within the human microbiota, focusing on skin microbial communities. His lab's goal is to characterize the mechanisms of small molecules and natural products which mediate interactions within a community. The skin microbiota is a model which allows the dynamic study of intra-species, microbe-host, and interspecies interactions.[21] To investigate community member's influence on each other's behavior and to identify natural products that inhibit pathogens, the Claesen Lab uses *Corynebacterium*, *Cutibacterim*, and *Staphylococcus* as their model systems. Claesen takes two different approaches to identify natural products, an activity-driven approach where they screen bacterial libraries for a specific phenotype or a genomics-driven approach to identify the products of bacterial gene clusters in genetically tractable hosts. The Claesen group was able to use these approaches respectively to identify coproporphyrin—produced by *Cutibacterium acnes* to cause aggregation and biofilm formation in *Staphlococcus aureus*—as well as thiopeptides produced by *C. acnes* as potent antibiotics.[22,23]

Lora Hooper (The University of Texas Southwestern Medical Center/Howard Hughes Medical Institute) presented on metabolic regulation by the microbiome and the circadian clock. Hooper focuses on host–microbe interactions fueled by the communication of microbes with the epithelium through the circadian clock. Two key proteins involved in microbial regulation of host metabolism are the transcription factor NFIL3 and the enzyme HDAC3, both are expressed in intestinal epithelial cells and are regulated by the microbiota and environmental light through circadian clock regulators. Hooper links microbial alteration of the circadian clock through Rev-erbα repression of *Nfil3* expression, causing oscillations. Combined with Hooper's work in elucidating histone deacetylase 3 (*Hdac3*) promotion by the intestinal microbiota, Hooper shows that light and the microbiota come together in the intestine to regulate host lipid absorption.[24] Hooper is continuing this work by determining the specific gut bacteria responsible for this regulation and by designing microbiological interventions for obesity and malnutrition.

Aleksandar Kostic (Harvard Medical School/Joslin Diabetes Center) presented the gut microbiome as a regulator of systemic adenosine homeostasis impacting immune tolerance. Beginning with investigations of SCFA production by lactate utilizing bacteria and effects on muscle capacity, Kostic's work in human-microbe symbiosis has extended now to microbial production of adenosine and its effects on central metabolism and immune tolerance in the host. His lab uses a model microbiome, known as the altered Schaedler flora (ASF), composed of eight diverse organisms introduced into gnotobiotic mouse models that replicate many of the functions of a complex microbiome.[25] By screening microbe supernatants for tolerogenic immunomodulators (TNFα, IL-10, TGFβ, and Treg), Kostic observed a division in labor among ASF members in immune regulation where *Parabacteroides goldsteinii* (ASF519), particularly through altering adenosine signaling, induced tolerogenic phenotypes by promoting IL-10 and inducing Treg progeny. Furthermore, Kostic showed that a dextran sulfate sodium (DSS) mouse model was orally gavaged with ASF519 and was protected against colitis and disease severity was substantially decreased with ASF519 administration in both a collagen-induced arthritis mouse model and in an insulitis-diabetes mouse model.

The session on molecules and metabolism ended with a talk from Dylan Dodd (Stanford, Stanford, CA, USA) on anaerobic metabolism. His work highlights the production of circulating host metabolites from anaerobic respiration, specifically through the highly conserved Rnf complex. In an anaerobic defined media with all 20 amino acids, *C. sporogenes* produced many metabolites such as aromatic amino acid derivatives, amines, short-chain and branched-chain fatty acids. Gnotobiotic and mono-colonized murine models were used to determine which amino acid metabolites of *C. sporogenes* gain access into host circulation. Dodd found metabolites known to be present in many human samples such as phenylpropionate and indolepropionate, among others, were present only in the mono-colonized feces and plasma when *C. sporogenes* was present. Furthermore, after investigation of the amino acid metabolism of anaerobic bacteria, Dodd identified gene clusters important in ATP production, including studies that elucidated the importance of the Rnf complex in anaerobic ATP generation from amino acid substrates.

4 | BUGS AND DRUGS

4.1 | Keynote Speaker: Justin Sonnenburg

The final day of the conference opened with keynote speaker Justin Sonnenburg, Associate Professor of Microbiology & Immunology at Stanford University. The Sonnenburg Lab is working toward understanding the basic mechanisms that underlie microbe–host interactions with the application of systems-based approaches partnered with genetic tools to examine perturbations in the intestinal environment. While many actuators can be used to study microbial dynamics associated with intestinal changes, such as fecal microbiota transplant or supplementation with prebiotics or small molecule drugs, his lab has a particular interest in the effect of diet on the microbiota and, in turn, the impact of these changes on the host. To investigate the dynamic behavior of the microbiome in response to changing stimuli and establish the basic principles that govern colonization, Sonnenburg's lab has developed strategies to image the localization of a broad range of constituent microbiota members.[26] Using a high-throughput strategy, they identified strong constitutively expressing promoters from bacteriophage capable of driving predictable levels of gene expression. These promoters were applied to drive fluorescent protein expression at different levels to provide unique fluorescent signatures to multiple members of the *Bacteroides* genus. He also discussed work done to create a privileged niche for predictable engraftment of bacterial species.[27] By using prebiotic strategies to introduce polysaccharides accessible to only a small population of species, he sought to create a privileged niche to facilitate their engraftment. By administering porphyran, a polysaccharide found in seaweed, *Bacteroides ovatus* naturally containing a porphyran utilization locus as well as *Bacteroides theta* heterologously expressing the gene cluster were able to stably engraft in the microbiome, not observed without the specialized diet. In his final vignette, Sonnenburg discussed some of his work regarding the evolution of the microbiome in the modern day.[28–30] To examine if industrialization spurred changes in the microbiome, his lab examined the differential microbiome compositions between traditional Hadza hunter-gatherers and individuals from industrialized nations.[29] Analysis of the compositions of the microbiome revealed that across geography, traditional populations tended to have a similar microbiome composition to one another that significantly varied from the microbiome observed in several industrialized populations. Corroborating this work, Jha et al also found that among Nepalese citizens, lifestyle differences correlated with significant changes in the composition of the taxa of the gut microbiome.[30] From these studies, Sonnenburg has garnered an interest in these VANISH (Volatile and/or Associated Negatively with Industrialized Societies of Humans) taxa, or the microbes that are lost as populations transition from rural to urban lifestyles. They propose that as diets shift to reflect that of a western industrialized nation, the loss of microbiota accessible carbohydrates is leading to a depletion of microbiota members and their associated metabolites.[31] Microbial metabolic products may in turn be important for the pathogenesis of different immune disorders and other gut-associated diseases. To address these questions, recent work in the Sonnenburg Lab sees many collaborations through the Center for Human Microbiome Studies at Stanford University. Collaborations have focused on using a reverse-translational approach, where a dietary intervention (including high-fiber and high-fermented interventions) is administered and the impact on the microbiome is observed to understand how beneficial alterations to the microbiome might be achieved.[32]

4.2 | Speaker sessions

Lauren Popov from Novome Biotechnologies, Inc. (San Francisco, CA, USA), a biotechnology company engineering living medicines for chronic diseases, started off day three of presentations with their technology platform that allows controllable colonization of the gut with engineered commensal bacteria. Their first targets are diseases of metabolism with the goal of modifying gut metabolic profile by breaking down specific gut derived metabolites that are linked to chronic disease. Novome is utilizing its proprietary platform in its lead preclinical program in hyperoxaluria, which is focused on the development of a therapeutic strain of bacteria that degrades oxalate to prevent the formation of kidney stones. Enteric hyperoxaluria is caused by hyperabsorption of dietary oxalate leading to kidney stones and is generally accompanied by bowel disease or bariatric surgery. There are limited treatment options for these patients and dietary treatments have limited efficacy. Popov discussed in vivo preclinical data, where rats fed a diet rich in oxalate and low in calcium were induced with hyperoxaluria and a one-time dose of the engineered oxalate-degrading strain alongside a daily administration of porphyran to aid with strain engraftment showed a 30%–50% reduction in urine oxalate levels, well above the 20% reduction believed to be relevant for a benefit to patients. Beyond hyperoxaluria, Novome is applying their platform to a range of diseases, including IBS.

Mark Charbonneau of Synlogic (Massachusetts, USA) spoke about the 'synthetic biotics' that the company is developing for the treatment of enteric hyperoxaluria during this speaker session. Synlogic's goals are to create a new class of medicine based on therapeutic bacteria with programmable clinical benefits. Their synthetic biotic medicines mainly focus on metabolic diseases, in particular the consumption of toxic metabolites in the gastrointestinal tract. Their enteric hyperoxaluria program is built off of their phenylketonuria program, which demonstrated the ability of engineered *E. coli* to consume toxic metabolites in the gastrointestinal tract. The engineered strain is designed to only express therapeutic payloads under anaerobic inducible promoters and as a thymidine auxotroph to control growth, regulate expression, and safety. When oxalate is fed to mice, the engineered strain lowered urinary oxalate excretion and could be recovered in feces after oral administration. Further testing to validate if its activity is sufficient to deliver a clinical benefit to enteric hyperoxaluria patients is under way with In Silico Simulations (ISS) of strain activity in the human gastrointestinal tract. Their ISS model simulates strain activity in the human gut with the administration of oral oxalate, its endogenous production, and its excretion into blood and urine.

Shannon Sirk, assistant professor in Bioengineering at University of Illinois at Urbana Champaign, is establishing a platform for in situ delivery of biological therapeutics by engineered commensal bacteria. Their approach aims to accommodate rapid responses to emerging threats in human and animal disease and to expand the therapeutic reach of locally delivered bacterially-produced biotherapeutics. Her biological therapeutic of choice are therapeutic antibody fragments, that mimic the Fc domain by creating a small peptide in the inert linkage between the variable light and variable heavy chains. By adding this function back into the antibody fragments, the half-life can be prolonged, increasing the therapeutic impact by having it exist in circulation longer and reaching distant body sites.[33] Other modifications such as circularizing disulfide bonds and acidifying mutations have been also added to improve their function while retaining their ability to bind their targets. Sirk's system of modified antibody fragments can engage in receptor-mediated transcytosis, some can even trancytose more efficiently than native IgG.

Continuing on the theme, Sarkis Mazmanian, Professor of Microbiology at Caltech, discussed the gut microbiome-brain connection in Parkinson's disease. In a mouse model of Parkinson's that overexpresses α-synuclein (αSyn), a team from the Mazmanian Lab demonstrated that motor deficit and GI symptoms in the animal were dependent on the gut microbiome. They showed that mice with microbiota from patients with Parkinson's demonstrated increased motor deficits compared to mice with microbiota from healthy control subjects, suggesting that specific microbes or lack thereof contribute to disease symptoms in genetically susceptible hosts.[34] To understand potential triggers by the microbiome, they looked at bacterial amyloids, similar to mammalian amyloids shown to nucleate and initiate the process of neural protein, α-synuclein aggregation. They saw an enrichment of *E. coli* and other curli-producing bacteria (curli fibers are bacterial amyloids with structural similarity to misfolded α-synuclein) in stool samples from patients with Parkinson's disease. For potential therapeutic interventions for Parkinson's, they investigated epigallocatechin gallate (EGCG), a plant-derived, dietary polyphenol that physically inhibits amyloid formation, including αSyn aggregation. They demonstrated that oral treatment of *E. coli* mono-colonized transgenic mice with EGCG reduced αSyn aggregation in the brain and improved both motor and gastrointestinal defects exacerbated by curli-producing *E. coli*.[35]

Mark Smith, CEO of Finch Therapeutics, discussed their efforts aiming to reverse the incidence of many diseases by restoring the structure and function of the microbiome. Finch's lead candidate CP101, an investigational microbiome therapeutic, targets recurrent *C. difficile* infection by reestablishing a functional microbiome. In their clinical trials with CP101, they observed the restoration of species that compete with *C. difficile* for the metabolism of primary bile acids, which are typically absent in individuals susceptible to *C. difficile* infection. In their clinical studies, patients on CP101 showed significant improvement in sustained clinical remission compared to the antibiotic-only group. Their second-generation therapeutic strategy includes the selection of individual strains designed to engage targeted pathways to treat disease, including ulcerative colitis (UC) and gastrointestinal symptoms in children with autism spectrum disorder. Clinical data from trials for the treatment of UC revealed super-donors that drove patients toward remission and Finch is analyzing data to identify microbes linked to positive patient outcomes. Finch is developing a therapeutic that combines both the full spectrum scaffold from CP101 and an individual strain targeting the oxytocin pathway in autism.

To close the last day of speakers, Peter Turnbaugh, associate professor of Microbiology and Immunology at the University of California in San Francisco, is evaluating the feasibility of using engineered bacteriophage to edit the genomes of bacterial members in the human gut microbiome. His group endeavors to understand host-associated microbes, specifically their molecular mechanisms, to improve medicinal practices associated with the microbiome. Due to the complex nature of microbe–host interactions, coupled with uncertainty in the causal roles which many species play in affecting human health, the group has turned toward strategies to eliminate or edit the microbiome in a targeted way. To achieve this specificity, they have been harnessing bacteriophage as a targeted antimicrobial agent and a delivery mechanism for genetic engineering machinery. Turnbaugh demonstrated the use of bacteriophage, M13 to deliver a minimal Cas9 and an sfGFP targeting guide to *E. coli* engrafted in the murine microbiome and knock out sfGFP expression.[36] In the future, Turnbaugh plans to generalize this system by expanding to other gut bacteria.

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AUTHOR CONTRIBUTIONS

Sandra McClure: Writing – original draft (equal); writing – review and editing (equal). **Fatima Enam:** Writing – original draft (equal); writing – review and editing (equal). **Jack H Arnold:** Writing – original draft (supporting); writing – review and editing (supporting).

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