Chapter 1

Recent Progress in Engineering Human-Associated Microbiomes

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Abstract

Recent progress in molecular biology and genetics opens up the possibility of engineering a variety of biological systems, from single-cellular to multicellular organisms. The consortia of microbes that reside on the human body, the human-associated microbiota, are particularly interesting as targets for forward engineering and manipulation due to their relevance in health and disease. New technologies in analysis and perturbation of the human microbiota will lead to better diagnostic and therapeutic strategies against diseases of microbial origin or pathogenesis. Here, we discuss recent advances that are bringing us closer to realizing the true potential of an engineered human-associated microbial community.

Key words Microbiome, Microbiota, Synthetic biology, Systems biology, Microbial engineering, Functional metagenomics, Host–microbe interactions

1 Introduction

Of the 100 trillion cells in the human body, 90 % are microbes that naturally inhabit various body sites, including the gastrointestinal tract, nasal and oral cavities, urogenital area, and skin [1]. An individual's colon is home to 10^{11}–10^{12} microbial cells/mL, the greatest density compared to any other microbial habitat characterized to date [2]. Many studies, such as the Human Microbiome Project and MetaHIT, have probed the vast effects of microbiota on human health and disease [1, 3–5]. In addition to metagenomic sequencing [6], traditional methods of studying cells in isolation are important for elucidating molecular bases of microbial activity. However, cells do not exist in single-species cultures in nature. In fact, some species are only culturable in the presence of other microorganisms [7]. This interdependence for survival amongst microbial species in a community attests to the importance of intercellular interactions, both microbe–microbe and host–microbe. Despite the fact that the human microbiota is composed of many individual microbes, these individuals work in concert to

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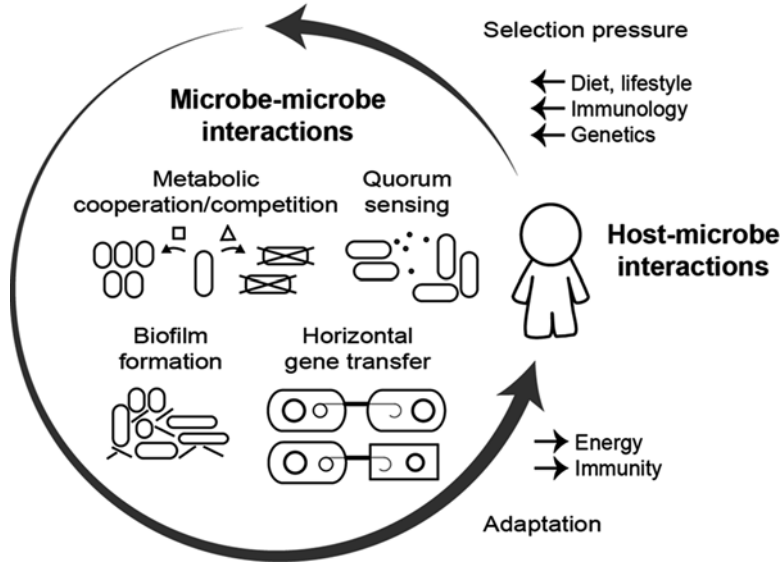

Fig. 1 Engineering human-associated microbiota requires detailed understanding of processes that govern the natural propagation and retention of microbes in the host as well as environmental and adaptive pressures that drive the evolution of cells and communities

perform tasks that rival in complexity to those of more sophisticated multicellular systems. Thus, the human-associated microbiome presents a ripe opportunity for forward engineering to potentially improve human health (Fig. 1). Here, we review recent advances in this area and outline potential avenues for future endeavors.

2 Microbiota, Host, and Disease

Contrary to traditional views, microbes are social organisms that engage with the environment and other organisms in specific ways. Microbes participate in intercellular communication through contact-dependent signaling [8], quorum sensing [9], metabolic cooperation or competition [5], spatiotemporal organization [10], and horizontal gene transfer (HGT) [11]. Human-associated microbes produce by-products that serve as substrates utilized by other resident bacteria [12–14]. For instance, accumulated hydrogen gas from bacterial sugar fermentation is removed by acetogenic, methanogenic, and sulfate-reducing gut bacteria [15]. In contrast to cross-feeding relationships, microbes under stress can release bacteriocins to suppress the growth of competitors [16–18]. If microbes are members of a biofilm community, they benefit from physical protection from the environment, access to nutrients trapped and distributed through channels in the biofilm, development of syntrophic relationships with other members, and the ability to share and acquire genetic traits [19, 20]. Microbial populations also

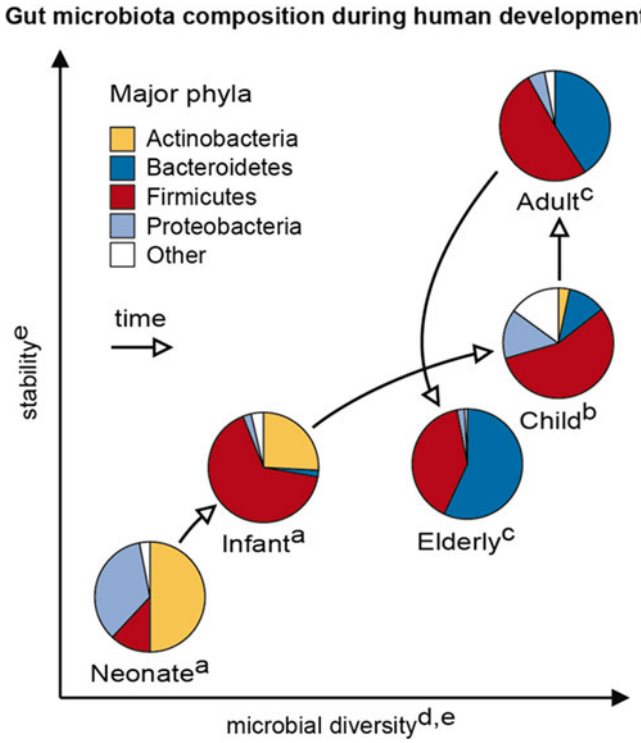

Fig. 2 Composition of the human gut microbiome during development with respect to microbial diversity and population stability. Data compiled from recent studies from the literature: (*a*) Hong 2010 [169]; (*b*) Saulnier 2011 [170]; (*c*) Claesson 2011 [171]; (*d*) Yatsunenko 2012 [172]; (*e*) Spor 2011 [173]

genetically diversify to insure against possible unstable environmental conditions [21, 22]. Moreover, multispecies communities harbor a dynamic gene pool consisting of mobile genetic elements, such as transposons, plasmids, and bacteriophages, which serve as a source of HGT to share beneficial functions with neighbors to preserve community stability [23–26]. Densely populated communities such as the human gut are active sites for gene transfer and reservoirs for antibiotic resistance genes [11, 27–29].

Beyond microbe–microbe interactions, the microbiota coevolves with the host as it develops, driving microbial adaptation [30–33]. Core functions of microbiota benefit the host, such as extraction of otherwise inaccessible nutrients, immune system development, and protection against pathogen colonization [2, 34–37]. Gut microbes are critical in intestinal angiogenesis, epithelial cell maturation, and immunological homeostasis [37–40]. For example, the commensal *Bacteroides fragilis* produces polysaccharide A, which converts host $CD4^+$ T cells into $Foxp3^+$ T_{reg} cells, producing interleukin-10 (IL-10) and inducing mucosal tolerance [41]. Host diet, inflammatory responses, and aging also affect microbial community composition and function [42–45] (Fig. 2). Indeed, aberrations in host genetics, immunology, and diet can lead to

microbiota-associated human diseases. Diet-induced obesity in mice from a high-fat diet is characterized by enhanced energy harvest and an increased *Firmicutes*-to-*Bacteroidetes* ratio [46, 47]. Furthermore, disruptions in the homeostasis between gut microbial antigens and host immunity can invoke allergy and autoimmunity, as in type 1 diabetes and multiple sclerosis [48–50]. It is thought that inflammatory bowel disease (IBD) results from inappropriate immune responses to intestinal bacteria; genes identified in genome-wide association studies highlight the role of a host imbalance between pro-inflammatory and regulatory states [48, 51].

While the host selects for microbial communities that harvest nutrients and prime the immune system, irregular microbiota composition may cause disease (Fig. 3), including IBD [52–54],

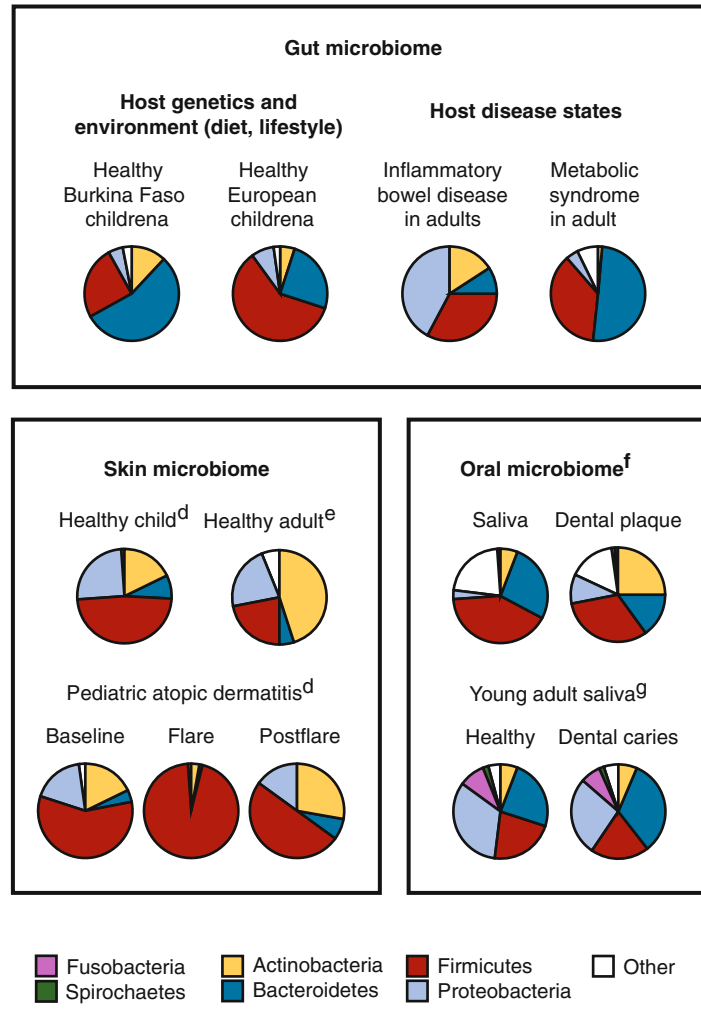

Fig. 3 Changes in the composition of human microbiota during disease states compared to healthy states. Data compiled from recent studies from the literature: (*a*) De Filippo 2010 [174]; (*b*) Peterson 2008 [175]; (*c*) Larsen 2010 [176]; (*d*) Kong 2012 [177]; (*e*) Gao 2012 [178]; (*f*) Keijser 2008 [179]; (*g*) Yang 2012 [180]

lactose intolerance [55, 56], obesity [57, 58], type I diabetes [59], arthritis [60], myocardial infarction severity [61], and opportunistic infections by pathogens such as *Clostridium difficile* and HIV [62–65]. Microbial gut metabolism links host diet not only to body composition and obesity [66] but also to chronic inflammatory states, such as IBD, type 2 diabetes, and cardiovascular disease [67–69]. Intestinal microbes are also important in off-target drug metabolism, rendering digoxin, acetaminophen, and irinotecan less effective or even toxic [70–72]. In the case of irinotecan, a chemotherapeutic used mainly for colon cancer, the drug is metabolized by β-glucuronidases of commensal gut bacteria into a toxic form that damages the intestinal lining and causes severe diarrhea. In the oral cavity, ecological shifts in dental plaque microbiota lead to caries (cavities), gingivitis, and periodontitis [73]. Dental caries arise from acidic environments generated by acidogenic (acid-forming) and aciduric (acid-tolerant) bacteria, which metabolize sugar from the host diet. Translocation of oral bacteria into other tissues results in infections, and cytokines from inflamed gums released into the bloodstream stimulate systemic inflammation. Oral bacteria have been implicated in respiratory [74, 75] and cardiovascular diseases [76–78], though mechanisms remain unclear.

3 Enabling Tools for Engineering the Microbiota

The human-associated microbial community presents a vast reservoir of nonmammalian genetic information that encodes for a variety of functions essential to the mammalian host [79]. Next-generation sequencing technologies have enabled us for the first time to systematically probe the genetic composition of these trillions of microbes that reside on the human body [1]. The ongoing effort by the Human Microbiome Project and MetaHIT to catalog dominant microbial strains from different body sites has generated useful reference genomes for many of the representative species [80]. Metagenomic shot-gun sequencing approaches of whole microbial communities, such as those found in the gut, have yielded near-complete gene catalogs that describe abundance and diversity of genes that contribute to maintenance and metabolism of the microbiota [6].

In order to determine functional relationships between human-associated microbes and their concerted effect in the mammalian host, we rely on functional perturbation of the microbial community. These investigative avenues include genome-scale perturbation assays, specified community reconstitutions, and directed engineering through synthetic biology (Fig. 4). Each approach provides us with a unique angle to attack an otherwise daunting

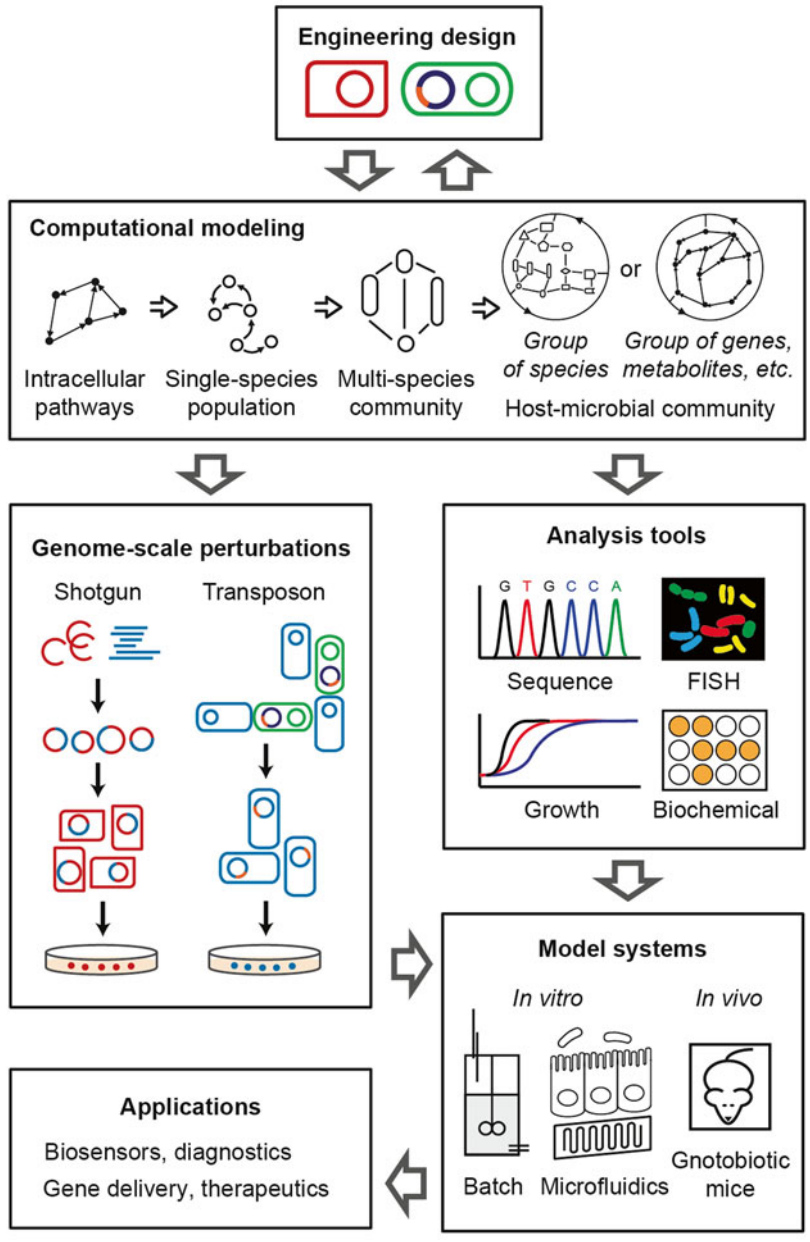

Fig. 4 General approaches to engineer the human microbiome through design, quantitative modeling, genome-scale perturbation, and analysis in in vitro and in vivo models, with the ultimate goal of producing demand-meeting applications to improve sensing, prevention, and treatment of diseases

challenge of de-convolving a highly intertwined set of microbial interactions in a very heterogeneous environment and a difficult-to-manipulate human host. Advances in both in vitro and in vivo host models have thus also facilitated research endeavors in this area, which we discuss in the following sections.

3.1 Challenges of Building New Genetic System

Approaches to study the function of human-associated microbes by genetic manipulation rely on several fundamental capabilities, which are often the largest practical barriers to manipulate microbes genetically. First, individual microbes need to be isolated and cultured in the laboratory. Because microbes have a myriad of physiologies and require different nutritional supplement for growth, different media compositions and growth conditions need to be laboriously tested by trial and error to isolate and culture each microbe. These microbial culturing techniques date back to the times of Louis Pasteur and are still the dominant approach today. More recent microbial cultivation techniques use microfluidics and droplet technologies to enable the discovery of synergistic interactions between natural microbes that allow otherwise "unculturable" organisms to be grown in laboratory conditions [7, 81, 82].

Upon successful microbial cultivation, the next limiting step of microbial genetic manipulation is the transformation of foreign DNA into cells. The passage of foreign DNA (e.g., plasmids, recombinant fragments) into the cell requires overcoming the physical barriers presented by the cell wall or membrane. This task is accomplished in nature through processes such as transduction by phage, conjugation and mating, or natural competency and DNA uptake [83, 84]. Numerous laboratory techniques have been developed for microbial transformation including electroporation [85], biolistics [86], sonication [87], and chemical or heat disruption [88]. Electroporation, the most common of the laboratory transformation techniques, relies on high-voltage electrocution of the bacterial sample that is thought to transiently induce pores on the cell membrane (hence "electroporation") that then enable extracellular DNA to diffuse into the cell. Various protocols for electroporation of human-associated microbes have been described and are good starting points for developing genetic systems in these microbes [89, 90].

Upon transformation of DNA into the cell, the DNA needs to either stably propagate intracellularly or integrate into the microbial host genome through recombination or other integration strategies. Inside the cell, stable propagation of episomal DNA such as plasmids requires DNA replication machinery that is compatible with the foreign DNA [83]. Additionally, cells often use methylation and DNA modification and restriction systems to discern foreign versus host DNA through a primitive defensive mechanism that fights against viruses or other invading genetic elements. Nonetheless, these promiscuous genetic elements can often be used as a way to integrate foreign DNA into the chromosome and are often used for large-scale functional genomics [91].

Taking all these parameters into consideration, we have summarized (Fig. 5) the current genetic tractability of human-associated microbes with respect to culturability, availability of full genome sequences, transfection methods, and expression and manipulation systems. Expansion of these basic genetic tools is crucial for future functional studies of human microbiota.

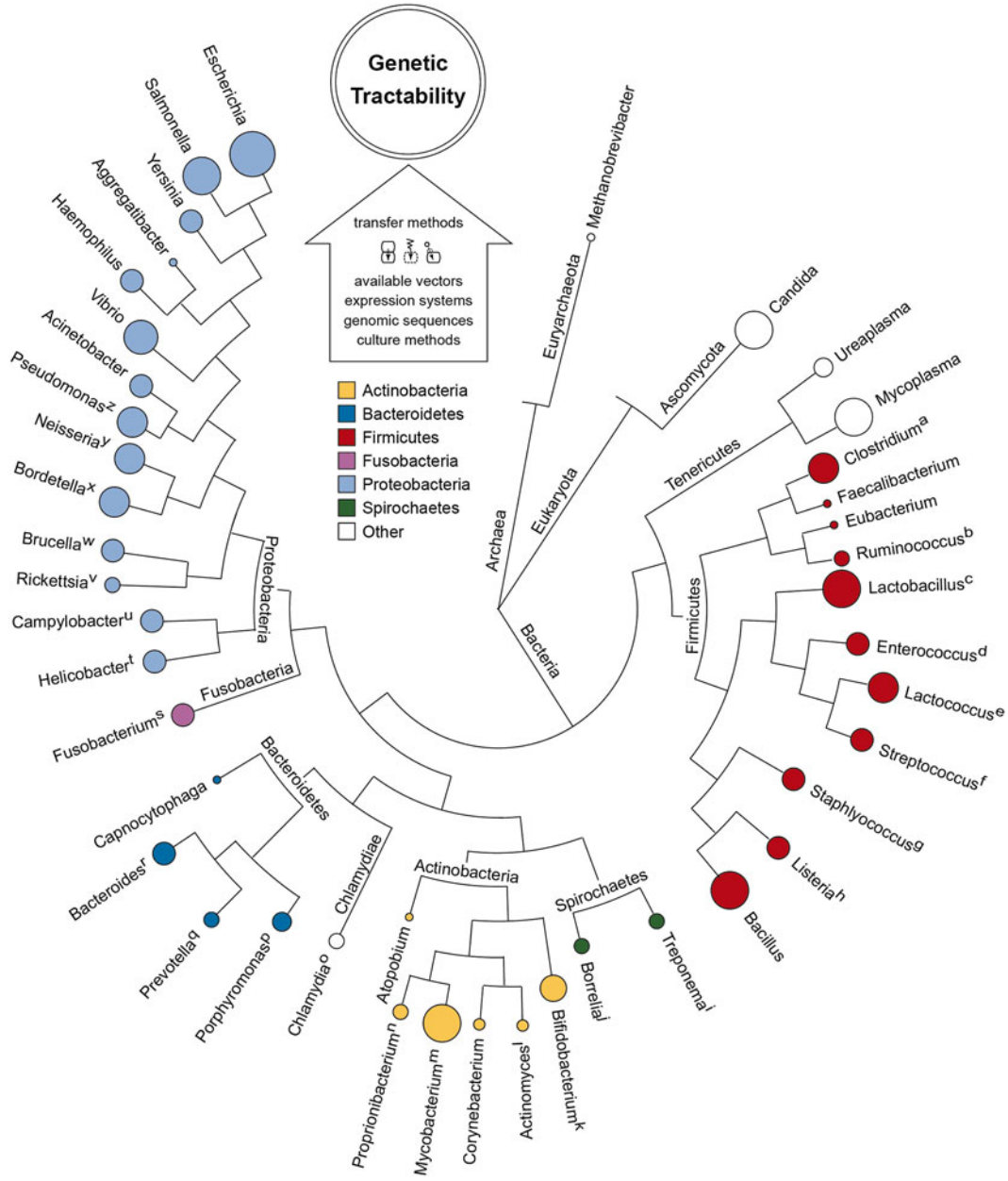

Fig. 5 Genetic tractability of abundant or relevant human-associated microbial genera, evaluated by the availability of means to introduce genetic material (e.g., transformation, conjugation, or transduction), vectors, expression systems, completed genomic sequences, and culturing methods. *Circles* of increasing sizes indicate greater genetic tractability. Protocols and demonstrated methods for genetic manipulation are listed as follows: (*a*) Clostridium: Phillips-Jones 1995, Jennert 2000, Young 1999, Bouillaut 2011 [181–184]; (*b*) Ruminococcus: Cocconcelli 1992 [185]; (*c*) Lactobacillus: van Pijkeren 2012, Ljungh 2009, Damelin 2010, Sorvig 2005, Thompson 1996, Lizier 2010[107, 186–190]; (*d*) Enterococcus: Shepard 1995 [191]; (*e*) Lactococcus: Holo 1995, van Pijkeren 2012 [107, 192]; (*f*) Streptococcus: McLaughlin 1995, Biswas 2008 [193, 194]; (*g*) Staphlyococcus: Lee 1995 [195]; (*h*) Listeria: Alexander 1990 [196]; (*i*) Treponema: Kuramitsu 2005 [197]; (*j*) Borrelia: Hyde 2011, Rosa 1999 [198, 199]; (*k*) Bifidobacterium: Mayo 2010 [200];

3.2 Genome-Scale Perturbations

Genome-scale perturbations are a class of genetic approaches that disrupt or perturb the expression of functional genes that contribute to relevant phenotypes by individual microbes. To dissect the function of different genes in the cell, we have relied heavily on the use of transposons, which are selfish genetic elements that can splice into and out of different locations of chromosomal DNA, thereby disrupting the coding sequence [92]. This classical approach, known as transposon mutagenesis, has allowed us to isolate many genetic mutants whose disrupted genes give rise to interesting phenotypes that reflect the importance of those genes to its physiology. Next-generation DNA sequencing has now enabled multiplexed genotyping of pools of transposon mutants by using molecular barcodes that then can be applied to measure the effect of genome-scale perturbations in different environmental conditions. For example, techniques such as insertion sequencing (INSeq) [93] utilize the inverted repeat recognition of the Himar transposase, which is one nucleotide change away from the restriction site for type II restriction enzyme MmeI, to generate paired 16–17 bp flanking genomic sequences around the transposon that can be sequenced in pools. Thus, the defined insertion location of every transposon in the library can be determined. By sequencing this pooled mutant library pre- and posttreatment with any number of environmental perturbations, one can probe the effects of different gene disruptions on the physiology of the cell in a multiplexed fashion. Similar techniques using other transposon systems such as transposon sequencing (Tn-seq) [94], high-throughput insertion tracking by deep sequencing (HITS) [95], and transposon-directed insertion-site sequencing (TraDIS) [96] have also been developed.

In addition to transposon-based systems, shotgun expression libraries have been useful in discovering functional DNA elements in genomic or metagenomic DNA. Shotgun expression libraries rely on physical shearing or restriction digestion of a donor DNA source into smaller DNA fragments that are then cloned into a gene expression vector and transformed into a host strain for functional analysis. A library of metagenomic DNA samples can for example be extracted from an environment and cloned into plasmids that are then expressed in *E. coli*. Selection and sequencing of the *E. coli* population for heterologous DNA that enable new function lead to discovery of novel gene elements that perform a particular

Fig. 5 (continued) (*l*) Actinomyces: Yeung 1994 [201]; (*m*) Mycobacterium: Parish 2009, Sassetti 2001 [202, 203]; (*n*) Proprionibacterium: Luijk 2002 [204]; (*o*) Chlamydia: Binet 2009 [205]; (*p*) Porphyromonas: Belanger 2007 [206]; (*q*) Prevotella: Flint 2000, Salyers 1992 [207, 208]; (*r*) Bacteroides: Salyers 1999, Smith 1995, Bacic 2008 [209–211]; (*s*) Fusobacterium: Haake 2006 [212]; (*t*) Helicobacter: Taylor 1992, Segal 1995 [213, 214]; (*u*) Camplyobacter: Taylor 1992 [214]; (*v*) Rickettsia: Rachek 2000 [215]; (*w*) Brucella: McQuiston 1995 [216]; (*x*) Bordetella: Scarlato 1996 [217]; (*y*) Neisseria: O'Dwyer 2005, Bogdon 2002, Genco 1984 [218–220]; (*z*) Pseudomonas: Dennis 1995 [221]

activity. This approach can easily identify activities such as antibiotic resistance [97] but have yielded less success with other functions.

Towards forward engineering of human-associated microbes, new genome engineering tools such as trackable multiplex recombineering (TRMR) [98, 99] and multiplex automated genome engineering (MAGE) enable efficient, site-specific modification of the genome [100–103]. TRMR combines double-stranded homologous recombination [104] and molecular barcodes synthesized from DNA microarrays to generate populations of mutants that are trackable by microarray or sequencing. MAGE relies on introduction of pools of single-stranded oligonucleotides that target defined locations of the genome to introduce regulatory mutations [102] or coding modifications [105]. These and other recombineering technologies are now being developed for a variety of other organisms including gram-negative bacteria [106], lactic acid bacteria [107], *Pseudomonas syringae* [108], and *Mycobacterium tuberculosis* [109], and are likely to be very useful for engineering human-associated microbes.

3.3 Reconstituted Communities

The community of microbes that make up the human microbiome can be considered a "pseudo-organ" of its own. These microbes interact with one another and the mammalian host in potentially highly complex ways that may be difficult to decipher even with tractable genetic systems [110]. A direct approach to study these interactions is to build reconstituted communities of microbes derived from monoculture isolates in defined combinations. This de novo reconstitution approach to build synthetic communities has significant advantages over attempts to deconvolute natural communities. Reconstituted synthetic consortium presents a tractable level of complexity in terms of number of interacting microbial species that can be tracked by sequencing and predicted with quantitative models. In one such study, researchers inoculated germ-free mice with ten representative strains of the human microbiota [111]. The mice were then fed with defined diets of macronutrients consisting of proteins, fats, polysaccharides, and sugars. By tracking the abundance of the ten-member microbial consortium using high-throughput sequencing, the researchers could predict over 60 % of the variation in species abundance as a result of diet perturbations. This avenue of investigation presents a viable approach to study the human microbiome and ways to analyze synthetically engineered microbiota.

Engineered microbes have been utilized to reconstitute synthetic communities to investigate the role of metabolic exchange. One such important metabolic exchange is that of amino acids, as they are the essential constituents of proteins. Various syntrophic cross-feeding communities have been described using auxotrophic *E. coli* and yeast strains that require different amino acid supplementation for growth [112–114]. In these syntrophic

systems, metabolites that are exchanged across different biosynthetic pathways promote more syntrophic growth than those that are exchanged along the same pathway, which also relates to the cost of biosynthesis of the amino acid metabolites. Amino acid exchange is likely a large player in driving metabolism of microbial communities as a substantial fraction of all microbes are missing biosynthesis of various metabolites and thus require growth on more rich and complex substrates that are found in the gut [115].

3.4 Microbial Engineering Through Synthetic Biology

New approaches are now utilizing synthetic biology to engineer human-associated microbiota to improve health and metabolism as well as to monitor and fight diseases. These efforts focus on developing genetic circuits that actuate in an engineered host cell such as *E. coli* that can sense and respond to changes to its environment and in the presence of particular pathogens. For example, to detect the human opportunistic pathogen *Pseudomonas aeruginosa*, which often causes chronic cystic fibrosis infections and colonizes the gastrointestinal tract, *E. coli* was engineered to detect the small diffusible molecule that is excreted by *P. aeruginosa* through the quorum sensing pathway [116]. An engineered synthetic circuit was placed in nonpathogenic *E. coli*, which when placed in the presence of high-density *P. aeruginosa* triggered a self-lysis program that released a narrow-spectrum bacteriocin that specifically killed the *P. aeruginosa* strain. Similar strategies have also been demonstrated to detect and respond to *Vibrio cholera* infection using engineered *E. coli* that sense autoinducer-1 (AI1) molecules from *V. cholera* quorum sensing pathway [117]. These strategies appear to yield improved survival rates against microbial pathogenesis in murine models [117]. Quorum sensing systems, which normally help microbes detect local cell density, have been further enhanced to improve robustness and performance to enable coupled short-range and long-range feedback circuits that enable microbial communication across large distances in an engineered community.

Other microbes have been successfully engineered to perform specific functions on human-associated surfaces such as the mucosal layer of the gut epithelium. Numerous diseases that occur along the intestinal tract are targets of such engineered approaches. For example, the probiotic strain *Lactococcus lactis* has been engineered to secrete recombinant human IL-10 in the gastrointestinal tract to reduce colitis [118, 119]. Other future applications of engineered probiotics include enhancing catabolism of nutrients (e.g., lactose and gluten), modulation of the immune system, and removal of pathogens by selective toxin release [116].

3.5 In Vitro Host Models

To probe and engineer the human-associated microbial community, various in vitro models have been developed, ranging from traditional batch culturing in chemostats to microfluidic systems that incorporate host cells. Single-vessel chemostats inoculated

with fecal samples from healthy individuals have helped identify HGT [120] and selective bacterial colonization on different carbohydrate substrates [121, 122]. A multichamber continuous culture system mimicking spatial, nutritional, and pH properties of different GI tract regions can be used to investigate stabilization dynamics [123–125]. Similarly, the constant-depth film fermenter resembles oral biofilm [126] and has enabled studies on biofilm formation, antibiotic resistance [126], and HGT in a multispecies oral community [127, 128]. To incorporate mammalian cells in studying host–microbial interactions, organ-on-a-chip microfluidic devices have been recently used. In one version of such a system, a gut-on-a-chip device, the microfluidic channel is coated with extracellular matrix and lined by human intestinal epithelial (Caco-2) cells. This system mimics intestinal flow and peristaltic motion, recapitulates columnar epithelium polarization and intestinal villi formation, and supports the growth of commensal *Lactobacillus rhamnosus GG* [129]. These microdevices offer an opportunity to investigate host–microbiota interactions in a well-controlled manner and in physiologically relevant conditions.

Inoculating with native microbiota samples provides a method to overcome the un-cultivability of many microbes as well as to study collective activity and discover novel functions without a priori knowledge of community composition. However, starting with a predefined microbial community allows a controlled setting better suited for testing engineered systems. In one study analyzing the dynamics of a community representing the four main gut phyla in a chemostat, the authors propose that intrinsic microbial interactions, rather than host selective pressure, play a role in the observed colonization pattern, which was similar to what has been documented in the human gut [130]. Similar models have been developed for oral microbiota studies. The use of predefined oral microbial inocula has helped elucidate metabolic cooperation in batch culture [12] and community development in saliva-conditioned flow cells [131].

3.6 In Vivo Host Models

In order to move into in vivo animal models that more closely represent the physiology of the human host environment, researchers have extensively utilized murine models including germ-free, gnotobiotic, and conventionally raised mice. Gnotobiotic animals are born in aseptic conditions and reared in a sterile environment where they are exposed only to known microbial species; technically, germ-free mice are a type of gnotobiotic mice that have not been exposed to any microbes. Similar to in vitro systems, mice can be inoculated with either a natural microbiota sample or a predefined microbial community. Fecal samples, as well as oral swab and saliva samples, can then be collected from gnotobiotic mice for biochemical analysis and species quantification of gut and oral cavity microbiota. In vivo models have been used to study the

transmission of antibiotic resistance in the mouse gut [132, 133] and colonization resistance in the oral cavity [134]. Furthermore, the choice of the inoculum donor offers opportunities to compare different host selection pressures and microbial community responses. Microbiota can be transplanted from conventionally raised to germ-free animals of not only the same species but also interspecies, as in human microbiota into mouse, called humanized gnotobiotic mice [134]. In one study, transplants from zebrafish gut microbiota into germ-free mice and mouse gut microbiota into germ-free zebrafish revealed that the resulting community conformed to the native host composition, demonstrating host selection [135].

Altering host diet, environment, or genetic background can also enable studies in host–microbiota interactions. One method to gain insight into the role of microbial communities in disease is to utilize mice with recapitulated pathologies. For example, IL-10$^{-/-}$, ob$^{-/-}$, apoE$^{-/-}$, and TLR2$^{-/-}$ or TLR5$^{-/-}$ mice are models for colitis, obesity, hypercholesterolemia, and metabolic syndrome, respectively [46, 136–139]. To generate antigen- or pathogen-specific phenotypes, mice can be infected with *Salmonella typhimurium* to study colitis [140] or *Citrobacter rodentium* as a model for attaching and effacing pathogens, such as enterohemorrhagic *E. coli* [141, 142]. Furthermore, murine models with chemically induced inflammation can be tools to study chronic mucosal inflammation; dextran sodium sulfate (DSS) can induce ulcerative colitis, and trinitrobenzene sulfonic acid (TNBS) can stimulate Crohn's disease [143]. To investigate oral microbiota, there are periodontal disease [144] and oral infection models [145, 146]; gnotobiotic rodents can also be fed a high-sucrose cariogenic diet to promote plaque formation.

Germ-free mice inoculated with defined microbes are informative models for analyzing microbial colonization and metabolic adaptation [147]. For example, resident bacteria and probiotic strains adapt their substrate utilization: in the presence of *Bifidobacterium longum*, *Bifidobacterium animalis*, or *Lactobacillus casei*, *Bacteroides thetaiotaomicron* diversified its carbohydrate utilization by shifting metabolism from mucosal glycans to dietary plant polysaccharides [148]. Furthermore, the effect of different diets on microbial community composition can be studied, as in gnotobiotic mice inoculated with ten sequenced gut bacterial species and fed with various levels of casein, cornstarch, sucrose, and corn oil to represent protein, polysaccharide, sugar, and fat content in the diet, respectively [111].

3.7 Computational Frameworks for Human Microbiomics

Over the past several decades, a large number of theoretical and quantitative models have been developed to describe the cell and its behavior. Constrain-based models are used to describe metabolism of individual cells using stoichiometric representation

of metabolic reactions and optimization constraints [149]. Approaches such as flux balance analysis (FBA) enable the analysis of metabolism under steady-state assumptions by linear optimization solution methods. These methods are now being scaled to ecosystems of cells. Recent developments using multi-level objective optimization [150] and dynamic systems [151] enable the modeling of synthetic ecosystems of three or more members. Using metagenomic data of the gut microbiome, Greenblum et al. generated a community-level metabolic reconstruction network of the microbiota and discovered topological variations that are associated with obesity and IBD, giving rise to low diversity and differences in community composition [152]. For models that account for systems dynamics, population abundance and metabolite concentrations can be solved independently through different FBA models that are iterated at each time step. This approach called dynamic multi-species metabolic modeling (DMMM) can capture scenarios of resource competition, leading to the identification of limiting metabolites [153]. Other complementary models include elementary mode analysis (EMA) [154] that enables quantitative analysis of microbial ecosystems in a multicellular fashion.

4 Perspectives

Reframing the microbiota community as a core set of genes, not a core set of species, opens a new front to the microbiome engineering design space. In a metagenomic study of 154 individuals, no single-gut bacterial phylotype was detected at an abundant frequency amongst all the samples, a finding that is consistent with the idea that the core human gut microbiome may not be best defined by prominent species but by abundantly shared genes and functions [155]. We propose that manipulation at the gene, genome, and ultimately metagenome level offers the ability for precise multicellular engineering of desirable traits in human-associated microbiota. Besides controlled perturbations of the microbiome to advance our understanding of host–microbiota interactions, metagenome-scale tools enable novel developments in diagnostics and therapeutics.

From biosensors on the skin to reporters in the gut, there are several opportunities in monitoring the health and disease status of the human host, such as sensing nutritional deficiencies, immune imbalances, environmental toxins, or invading pathogens. Prophylactic and therapeutic avenues for human microbiome engineering include modifying community composition, tuning metabolic activity, mediating microbe–microbe relationships, and modulating host–microbe interactions. Two current microbiota-associated treatments

have shown clinical efficacy: (1) fecal transplants for recurrent *Clostridium difficile* infection [156] and (2) probiotics for pouchitis, which is inflammation of the ileal pouch that is created after surgical removal of the colon in ulcerative colitis patients [157–159]. The main challenge is transmission of undesirable agents from donor feces to the recipient gut in fecal transplants and native colonization resistance that would impair infiltration and growth of new species in probiotics [160, 161]. Nevertheless, these successful approaches demonstrate the potential benefits of leveraging natural microorganisms and entire microbial communities.

In fact, coupling organismal and functional gene-level approaches would be a powerful way to engineer the native microbiota. Microbiome engineering enables multiscale system design for the synthesis of nutrients and vitamins, enhanced digestion of gluten and lactose, decreased acidity of the oral cavity, targeted elimination of multidrug-resistant pathogens, and microbial modulation of the host immune system. As vehicles for drug delivery, commensal bacteria designed to secrete heterologous genes have been explored for treating cancer [162–164], diabetes [165], HIV [166], and IBD [118]. For example, IL-10 has immunomodulatory effects in IBD but requires localized delivery at the intestinal lining to avoid the toxic side effects and low efficacy of systemic IL-10 injection. Ingestion of modified *Lactococcus lactis* that secrete recombinant IL-10 is safe and effective in animal models and has been promising in human clinical trials for IBD [119, 167].

Finally, besides addressing clinical safety and efficacy criteria for FDA regulatory approval [168], overall safety precautions are critical considerations to minimize unintentional risks in releasing genetically modified material into the natural environment. Rational design, such as creating auxotrophic microbes [119], for robust stability, non-pathogenicity, and containment of recombinant genetic systems will be essential in microbiome engineering.

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