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MINIREVIEW

Unraveling interactions in microbial communities - from co-cultures to microbiomes

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Microorganisms do not exist in isolation in the environment. Instead, they form complex communities among themselves as well as with their hosts. Different forms of interactions not only shape the composition of these communities but also define how these communities are established and maintained. The kinds of interaction a bacterium can employ are largely encoded in its genome. This allows us to deploy a genome-scale modeling approach to understand, and ultimately predict, the complex and intertwined relationships in which microorganisms engage. So far, most studies on microbial communities have been focused on synthetic co-cultures and simple communities. However, recent advances in molecular and computational biology now enable bottom up methods to be deployed for complex microbial communities from the environment to provide insight into the intricate and dynamic interactions in which microorganisms are engaged. These methods will be applicable for a wide range of microbial communities involved in industrial processes, as well as understanding, preserving and reconditioning natural microbial communities present in soil, water, and the human microbiome.

Keywords: synthetic communities, system biology, co-cultures, metabolic models

Introduction

Microorganisms in nature rarely exist in isolation and instead are largely found in complex communities. Despite this, most of the scientific research work on microorganisms has been focused on single, isolated strains. Only more recently has the focus been moving towards understanding the roles of microorganisms in communities and their interactions therein. This has been partially fueled by an increasing number of discoveries about the unique metabolic capabilities of microbial communities, including, for example the ability to accumulate, metabolize, and degrade various compounds such as cellulose (Jiménez *et al*., 2014), alkanes (Embree *et al*., 2013), and even plastic (Carson *et al*., 2013), or heavy metal toxins (Maleke *et al*., 2014; Zhou *et al*., 2014). Microbial communities research has recently taken hold in the field of synthetic biology, as compartmentalization of gene circuits into different interacting strains has allowed the development of artificial microbial communities with many desired properties (Brenner *et al*., 2008; Shong *et al*., 2012; De Roy *et al*., 2014). Furthermore, there is a growing awareness of the importance of microbial communities in human health and disease (Clemente *et al*., 2012; Relman, 2013; Chen and Schnabl, 2014; Ding and Schloss, 2014). For example, the composition of the gut community has been shown to have an effect on various pathologies (Zupancic *et al*., 2012; Lazupone *et al*., 2013; Chen *et al*., 2014; Gevers *et al*., 2014) as well as physiological traits such as obesity (Faith *et al*., 2010; Ridaura *et al*., 2013; Walters *et al*., 2014). Many natural communities feature highly complex interactions among their members, with many of them not fully understood. These interactions can be highly dynamic, causing roles of the microorganisms to change within, along with their proportion in the population. Systems biology now offers a possibility to study these complex community interactions in great detail (Zengler and Palsson, 2012). This has been aided by the development of various experimental and computational techniques, such as the ability to identify the composition of a community through high throughput 16S rRNA gene sequencing (Huse *et al*., 2008; Caporaso *et al*., 2010), the ability to sequence and elucidate the genomes of individual community members through a combination of metagenomics and computational binning (Imelfort *et al*., 2014), and the determination of gene expression of each community member using metatranscriptomics (Embree *et al*., 2013). Advances in genome sequencing and annotations have also led to computational models that allow us to simulate various interactions between microorganisms growing in communities (Klitgord and Segre, 2010; Henson and Hanly, 2014). In order to better understand the complexities found in natural communities, many studies make use of simple community models in order to examine various aspects of community life. Even though we are still at a stage where the understanding of natural communities is phenomenological, advances in computational community modeling have provided a path towards shaping and designing communities in a rational way. In this review, we first look at the experimental tools

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that have been developed to aid the study of communities. We then examine five main areas of current research: 1) establishment of relationships during the early stages of a microbial community, 2) cross-strain or cross-species cell-cell communication, 3) different types of interactions occurring in communities, 4) factors affecting stability of a community, and 5) potential industrial applications of microbial communities (Fig. 1). Subsequently, we will discuss the advent of computational community models and examine their implications.

Approaches

The complexity of most communities necessitates the use of various tools for in depth studies. The challenge of working with natural communities lies in the large number of species and strain variables, which have to be elucidated and deciphered. As such, many tools, such as Next-Generation Sequencing (NGS), that deal with measuring the abundance of different microorganisms present as well as their combined metabolic capabilities have been used (Fig. 1). Before sequencing became readily available, community composition had been determined by cultivation approaches (Zengler, 2008). Presently, variations in the 16S rRNA gene are used to classify microorganisms into operational taxonomic units (OTUs) and evaluate OTU abundance and distribution. This has been done traditionally through PCR, cloning, and subsequent sequencing or additionally through quantitative PCR (qPCR). The recent development of NGS technologies and advances in computational biology have led to cost-effective generation of large OTU datasets and the development of 16S rRNA mapping technologies such as QIIME (Caporaso *et al.*, 2010). Bulk DNA sequencing and transcriptomics/proteomics followed by gene annotations have also been used to give researchers a rough understanding of the metabolic capabilities of the community as a whole (Poretsky *et al.*, 2005; Aliaga Goltsman *et al.*, 2009; Marmeisse *et al.*, 2011), while mass spectrometry with or without stable isotope probing has been used to characterize changes in metabolites (Everroad *et al.*, 2012). To unravel the interaction between individual members in a community, an understanding of the role of individual microorganisms within the community was needed. Single-cell DNA sequencing and computational based approaches such as GroopM (Imelfort *et al.*, 2014) have enabled researchers to tease apart the genome sequences of different members within a community. By mapping bulk transcriptomic reads back to the sequenced genomes, researchers are now able to pinpoint the metabolic activity of individual members within the community (Embree *et al.*, 2013).

When working with synthetic communities, researchers have the benefit of foresight, and have developed methods to distinguish each species prior to co-cultivation. Many researchers have turned to the use of fluorescent reporter proteins due to their ease of use and large number of available colors (Heim *et al.*, 1995). The proportion of individual organisms on solid medium can be easily determined through visual inspection and by fluorescence microscopy, or in the case of liquid cultures, through the use of fluorescence activated cell sorting (FACS) (Gore *et al.*, 2009; Wintermute and Silver, 2010; Hosoda *et al.*, 2011; Hong *et al.*, 2012; Bernstein *et al.*, 2012). Microfluidic devices have also been deployed successfully to study the precise spatial organization of community members and control environmental conditions in synthetic communities (Hong *et al.*, 2012).

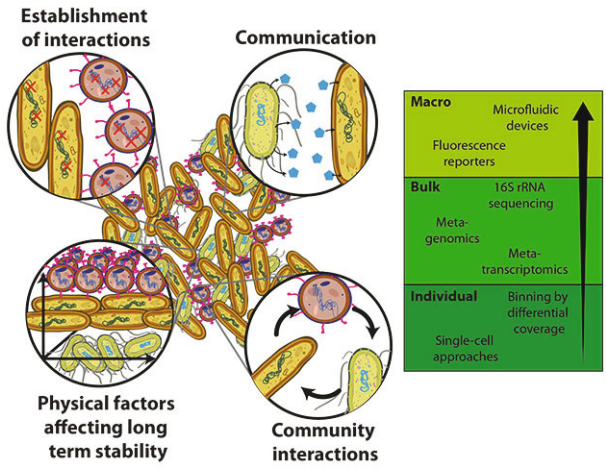

Fig. 1. Unraveling microbial interactions. In order to understand large scale microbial community interactions, many researchers make use of smaller model communities. This allows them to understand specific aspects of community living such as the transcriptomic or genomic modifications that occur during the establishment of interactions, cell-cell communication, various forms of community interactions, as well as other physical factors which affect the long term stability of the community such as spatial heterogeneity. Various tools have also been developed to study communities. These range from macro-scale techniques such as microfluidic devices which can constrain spatial organization of whole communities, down to individual cell scale such as single-cell approaches which allows genome and transcriptome sequencing of individual cells in the community.

Establishing intra- and interspecies interactions

How microbial communities establish themselves and how interactions are formed and maintained is currently an active field of research. An understanding of the genomic or transcriptomic changes occurring in microorganisms during the onset of interspecies interactions is therefore necessary. This becomes especially important for synthetic communities or the introduction of non-native strains into a pre-existing community for industrial applications.

The reaction, interaction, and adaptation of one species to another can happen on various levels (Koide *et al.*, 2009). These adaptations involve changes at the transcriptional or translational level and ultimately are manifested in the genome. Predicting the level and degree of response is key to furthering our understanding of how communities establish themselves. Adaptation can occur in one or multiple organisms at the same time and do not necessarily have to occur at the same level. For example, two different *Geobacter* species that had been adapted to grow in a syntrophic co-culture (Summers *et al.*, 2010) applied different adaptation strategies (Nagarajan *et al.*, 2013). While one species changed

only expression levels of mRNA, the other species underwent genome mutations to accompany the most efficient growth of the co-culture. These different strategies of adaptation occurred very early on in the co-culture and did not change even after the culture evolved over 800 generations.

In others cases, genetic modifications have been shown to be essential for survival in limiting growth conditions as they allow for the formulation of mutualistic interactions. This was demonstrated with a syntrophic co-culture of *Desulfovibrio vulgaris* and *Methanococcus maripaludis* (Hillesland and Stahl, 2010). *Desulfovibrio vulgaris* ferments lactate to produce acetate, carbon dioxide, and hydrogen. Due to the low energy yield of this reaction, continuous product removal is required to drive the reaction and make it thermodynamically feasible. *M. maripaludis* consumes the hydrogen produced by *D. vulgaris* which facilitates the growth of *D. vulgaris*; thus forming a mutualistic relationship. Of the 24 co-cultures originally set up by the authors, four were unable to adapt to this syntrophy and collapsed while the growth rates of the remaining 20 co-cultures fluctuated stochastically and erratically during the initial adaptation phase before eventually stabilizing. This could suggest that at least some of the changes responsible and essential for adaptation to syntrophic interaction of the co-culture were genetic in nature.

Community collapse due to a lack of sufficient genetic modification was also observed in a study by Shou *et al.* (2007) who established a symbiotic relationship between two genetically modified *Saccharomyces cerevisiae* strains containing different amino acid auxotrophies. One strain was auxotrophic for adenine while the other was auxotrophic for lysine. Initial co-cultures were unable to establish a viable mutualistic relationship and quickly died. In order to induce cooperation, additional genetic mutations had to be made in each strain in order to remove feedback inhibition and force overproduction of its partner's corresponding rescue metabolite. Thus, the presence of diverse subpopulations of various genetic mutants within a single species may be essential for the adaptation of this co-culture and survival of the species. Recently, large communities of coexisting subpopulations of the marine cyanobacterium *Prochlorococcus* have been described, suggesting that the stable niche partitioning of species is a dominant factor in nature as well (Kashtan *et al.*, 2014).

Under optimal conditions, a few individuals of the *S. cerevisiae* study with scattered genetic mutations may exhibit cooperative behavior at the expense of a slightly decreased growth rate and are maintained at low proportions in the population. However, when forced into co-culture, cooperation results in an increased fitness of these individuals, causing them to proliferate and dominate the population.

While major genetic changes might be necessary for the formation of some community interactions, studies have shown that interactions can also be established solely by changes of the transcriptome. Hosoda *et al.* (2011) created a simple co-culture using *Escherichia coli* instead of *S. cerevisiae*, and found that co-cultures of two auxotrophic strains (leucine (L^-) and isoleucine (I^-)) were stable without additional genetic modifications required to force each strain to secrete the metabolites required for the symbiotic relationship to exist (Hosoda *et al.*, 2011). In addition, the exchange of metabolites occurred within ten hours after inoculation. The authors reasoned that this was an insufficient time for large scale genetic mutations to occur. This implied that transcriptomic changes were sufficient to cause the overproduction and transfer of nutrients between each other and support the relationship. In a follow up paper, Hosoda and Yomo (2014) discussed how the cells managed to communicate their respective metabolic requirements to their partners. As the cells had managed to establish the symbiotic relationship even when grown without physical contact, this communication had to have occurred through the exchange of small molecules via the medium. The authors concluded that gene knockouts used to generate the auxotrophies caused pathway jams and a buildup of the metabolic precursors upstream of each required amino acid. The authors speculated that these precursors, such as alpha-keto-beta-methylvalerate used in the production of isoleucine, leaked out of the I^- cells and could be taken up by the L^- cells. The L^- cells would subsequently overproduce the required metabolite in order to reduce feedback inhibition in upstream pathways and at a lower than expected metabolic cost.

In another example, Kihara *et al.* (2009) investigated the development of a symbiotic relationship between *E. coli* and the cellular slime mold *Dictyostelium discoideum*. They found that the transcriptomic changes in *E. coli* during the development of the symbiotic relationship were similar to those that occurred in response to the development of biofilms. This suggests that this particular symbiosis resulted from the repurposing of pre-existing genetic circuits and pathways in *E. coli*, triggered by environmental conditions similar to those encountered during biofilm development.

These experiments have provided evidence of both genomic and transcriptomic changes taking place during the establishment of different co-cultures. However, no clear rules have yet been derived and further investigation is needed before we can predict what changes will be beneficial for establishing stable co-cultures.

Communication between interacting partners

The establishment, maintenance, and optimization of co-cultures as well as complex communities rely on communication between the different species. This communication is necessary not only to establish community interactions in the first place but also for the stability and progression of the community. While cells can communicate directly through the exchange of metabolites, it is more common for them to communicate through the use of quorum sensing. Quorum sensing occurs when cells release a small molecule signal whose activity is concentration dependent. This couples the activity of the small molecule signal to the number of cells per space, allowing cells to regulate their activity based on their density as well as coordinate responses to increase its effectiveness (Fuqua *et al.*, 1994; Swift *et al.*, 1996).

The ability to understand the role of quorum sensing is important when studying natural communities. For example, *Pseudomonas aeruginosa* uses quorum sensing for cooperative behavior as well as coordinating virulence. Hence, disrupting quorum sensing using drugs such as azrithro-

mycin seems like a logical intervention for halting virulence. However, Köhler *et al.* (2010) realized that natural populations of *P. aeruginosa* are composed of cooperators (wildtype) and non-virulent cheaters (*lasR* mutants). In natural populations, cheaters experience a fitness advantage over the wildtype, but disruption of quorum sensing by azrithromycin results in a loss of this fitness advantage. While azrithromycin does prevent virulence of *P. aeruginosa* during the course of the treatment, it also enriches the populations of *P. aeruginosa* within the patient for the more virulent wildtype strain. Following the discontinuation of azrithromycin treatment, patients unfortunately experience an increased susceptibility to *P. aeruginosa* infections. Knowledge of different subpopulations of the same species is thus crucial for predicting treatment outcome.

Several groups make use of synthetic communities built around quorum sensing circuits. By introducing two different gene circuits into *E. coli*, Basu *et al.* (2004) were able to make one strain a sender and one a receiver. The receiver cells contained a feed-forward loop motif gene circuit such that the production of the inducer signal by the sender cells would result in a transient pulse of green fluorescent protein (GFP). More interestingly, due to the dynamics of the feed-forward loop motif, the receiver cells exhibited spatiotemporal behavior, responding only to nearby sender cells, ignoring distant sender cells. Thus, spatial distributions, as discussed below, have been shown to be a key factor in communities.

In a follow-up study, the authors were able to tweak the receiver circuit, to express GFP at only intermediate levels of the inducer molecule from the sender, while repressing it at both low and high levels (Basu *et al.*, 2005). When sender cells were inoculated on a lawn of receiver cells, the concentration gradient of inducer signal diffusing from the sender resulted in a ring of fluorescence at the optimal concentration. As we allude to later in the chapter, spatial distribution is a critical factor for the survival and function of many communities. By integrating quorum sensing and gene circuits, experiments have managed to recapitulate that phenomenon. Quorum sensing serves as an important way for cells to communicate and coordinate behavior, and a deeper understanding of its application in complex communities will lead to ways to maintain, control, and manipulate communities.

Community interactions

By undergoing different adaptations, cells achieve various forms of interactions that can maximize their chances for survival. In natural communities, microbes interact in a multitude of ways, from the transfer of metabolites to the inhibition of growth through the production of antibiotics. These interactions have either a positive or negative effect on each of its members. In a natural community composed of several species, some or all of these interactions could occur concurrently between different members of the community, leading to increasing complexity as the number of species (and subpopulations) grows. Many of these interactions, such as parasitism, competition, and mutualism, have been studied previously. The development of synthetic communities and simplified models has recently been used to gain insight into the form of interactions.

A form of interaction where one species derives a benefit at the expense of the other is known as parasitism or predation. In order to model a predator-prey system, Balagaddé *et al.* (2008) generated two strains of *E. coli* that communicated via two orthogonal cell-cell communication pathways, LuxI/LuxR and LasI/LasR. The predators induced the expression of the suicide protein ccdB in the prey, resulting in their death. On the other hand, predator cells constitutively produced ccdB protein, requiring the signal from prey cells to induce expression of the antidote protein ccdA. By co-culturing these different strains together, the authors were able to model various ecosystem dynamics such as coexistence and extinction. Furthermore, sustained oscillation could be studied by changing parameters, such as the dilution rate of the chemostat.

An example of parasitism can be found in the context of cheaters and cooperators within a population. Members of a species exhibit cooperativity to produce a public good, such as the production of invertase by yeast which aids in the conversion of sucrose to glucose. This behavior can be exploited by other members in the community that do not contribute to the production of this public good, but still benefit from its presence. Invertase is expressed in the periplasmic space in *S. cerevisiae*, and despite the metabolic costs associated with expressing it, around 99% of the sucrose converted to glucose is lost to the surrounding environment (Celiker and Gore, 2012). This leads to the mutual presence of cooperators and cheaters within many natural populations of yeasts. Gore *et al.* (2009) further explored the dynamics of cheaters and cooperators in natural populations by creating a histidine auxotrophic cooperator strain that produced invertase. They then co-cultured it with a cheater strain that did not produce invertase, making use of histidine levels as a method to control cooperator levels. They found that the strains engaged in what they termed as snowdrift game dynamics, where rare strategies did comparatively better than the common strategy. In wild-type yeasts, however, invertase expression is regulated by the level of glucose, allowing them to have the optimal strategy for success in the snowdrift game. When wildtype yeasts are co-cultured with cheaters, low glucose concentration causes expression of invertase and thus an increased fitness relative to the cheaters. On the other hand, co-culturing wildtype yeast with cooperators results in repression of invertase expression due to high glucose concentration, again increasing fitness relative to the surrounding cells.

Celiker and Gore (2012) also found that cross-species competition selected for cooperative behavior. In the absence of competition and under normal conditions, cooperation results in a high glucose concentration in the environment. Cheaters thus proliferate in the population due to an increased fitness derived from the absence of metabolic costs associated with expression of invertase. However, when *E. coli* is added to the community, it quickly consumes all available glucose, causing cooperating yeast cells to proliferate because they now benefit from a marginal fitness advantage over cheater cells due to locally increased concentrations of

glucose.

One species deriving a benefit from the other without any positive or negative effect on the other organism is known as commensalism. This often occurs when a metabolic byproduct of one species is taken up by the other as a nutrient source. While true commensalism might play a role in natural communities, it is more likely that both species are affected by the interaction. Thus, several studies have looked at one-way crossfeeding mutualism, a situation similar to commensalism where the byproduct of one organism serves as the nutrient source of the other, but where the uptake of the byproduct has a positive effect on the organism producing it due to product toxicity or low reaction energetics. For example, *E. coli* cells grown aerobically on glucose build up acetate, causing feedback inhibition and inhibiting culture yield. Bernstein *et al.* (2012) combined two strains of *E. coli*, one that could break down glucose into acetate (producer), and another that was unable to break down glucose but could feed on acetate from the producer. This co-culture exhibited increased biomass productivity over cultures of either strain. In addition, the authors were able to show even higher biomass productivity when they genetically modified the producer strain to streamline production of acetate, showing the importance of this interaction to the combined growth rate and yield of the community. Several other factors also affect the strength of the one-way crossfeeding mutualism. By creating a mathematical model of a producer and the cross-feeder, Estrela *et al.* (2012) showed that the crossfeeding mutualism is favored by an increase in toxicity and stability of the by-product, both of which serve to increase the importance of the cross-feeder. They also found that mutualistic interaction peaked at intermediate toxicities because at high toxicity, the producer levels were strongly inhibited by by-product formation.

Co-cultures have been used to simulate interactions such as parasitism, competition, or mutualism. However, natural communities are often very complex and can contain multiple members engaging in different interactions with different members of the community at different times. Elucidating different forms of interactions and their underlying mechanisms is therefore paramount when we begin to decipher the many intertwined layers of interactions found in natural communities.

Physical factors and long-term community stability

Not only are intracellular, intercellular, and interspecies interactions important for the long term stability and survival of a community, but physical factors such as spatial organization also determine its survival and function.

Spatial organization

One of the most important factors for the establishment and stability of a community is the spatial organization of its members. Communities in nature often achieve and maintain a defined spatial structure. This can be mimicked in the laboratory by growth on solid substrates or through the establishment of biofilms which constrain cellular movement. Many synthetic communities have been developed to look into the effects of spatial organization on community trajectory and steady state. Kerr *et al.* (2002) demonstrated this using three strains of *E. coli*, a colicinogenic strain (C), a colicin resistant strain (R), and a colicin sensitive strain (S), which is killed by the C strain . Colicin is a highly effective class of bacterial toxins, which can be produced by some strains of *E. coli* and are toxic to others. Production of colicin carries an inherent metabolic cost, while resistance to colicin involves repression of specific outer membrane receptors which the toxin target, forfeiting the ability to sense or uptake key nutrients. Both the production and the resistance results in a decreased growth rate relative to the wild type, with colicin production having a larger metabolic burden than resistance. This system mimicked the game of rock-paper-scissors, where R was outcompeted by S, S was killed by C, and C was correspondingly outcompeted by R. When grown in a well-mixed environment, C quickly caused the extinction of S, and was then gradually outcompeted by R. On the other hand, when grown on a plate which provided a heterogenous environment where only local interactions occurred, all three strains were able to survive, and continuously exhibited an effect which the authors termed 'chasing'. This was due to colonies on the plate invading other colonies to which they were vulnerable, but at the same time being invaded by other colonies to which it was vulnerable.

Kim *et al.* (2008) also looked at the effects of spatial structure using three different species of bacteria: *Azotobacter vinelandii*, *Bacillus licheniformis*, and *Paenibacillus curdlanolyticus*. In this community, each species performs a vital role. *A. vinelandii* fixes nitrogen into amino acids under aerobic conditions, while *P. curdlanolyticus* excretes cellulases which cleave carboxymethyl cellulase, the only carbon source available to the community, to produce glucose. Lastly, *B. licheniformis* is necessary for reducing antibiotic pressure by degrading penicillin G. The authors found that in a well-mixed environment such as liquid media, regardless of nutrient availability or antibiotic levels, the culture was unstable resulting in the extinction in at least 2 of 3 community members. However, when the community was cultured in a microfluidic device in order to spatially localize the species, they were able to form a stable community. Additionally, the use of a microfluidic device allowed them to view the effects of distance on the dynamics of the system. They found that decreasing the separation of the species, which mimics a well-mixed environment, or increasing the separation of just one of the species result in an overall decline in population size.

The effect of spatial structure on the community is not merely unidirectional, and several communities have been shown to exhibit self-organizing capabilities. While creating a community consisting of wildtype *E. coli* and a knockout mutant which made use of acetate, Bernstein *et al.*, noted that when the wildtype *E. coli* was genetically modified to enhance acetate production, the community exhibited an emergent property of self-organizing into a laminated biofilm, where the acetate consuming strain localized itself at the biofilm air interface (Bernstein *et al.*, 2012). While the acetate producer could respire anaerobically, and in fact probably did so in order to increase acetate yield, the acetate-consuming strain required oxygen for energy produc-

tion. The formation of a laminated biofilm allowed for the best division of labor.

In addition to organization for access to oxygen and various nutrients, some communities have also been shown to self-organize in order to maximize positive and minimize negative interactions. Momeni *et al.*, made use of three genetically modified yeast strains, one of which was lysine auxotrophic but supplied alanine (R), another was alanine auxotrophic but supplied lysine (G), and the last was lysine auxotrophic but unable to supply alanine (C) (Momeni *et al.*, 2013). R and G alone were able to form a mutualistic relationship, but C was a cheater that was able to survive in the presence of G, but alone would be unable to sustain the relationship. When the environment was well-mixed, the main competition was between C and R as both made use of G for lysine production. The steady state community proportions were dependent on which strain had a better competitive adaptation for the uptake of lysine, and could result in either C or R dominating the culture. However, when the culture was grown on a plate and hence was spatially constrained, the cooperation between R and G allowed them to self-organize to exclude cheaters from colonies. This was due to the fitness advantage that cooperators in very close proximity attained over the rest of the cells, a phenomenon termed partner fidelity feedback. This phenomenon results in colonies made out of just cooperating partners to grow much faster and at the expense of any other colonies which contained cheaters.

Spatial organization in natural communities is often achieved through the formation of biofilms. These allow for efficient division of labor and spatial separation of strains. It stands to reason that in order to introduce synthetic communities into nature or to influence existing natural communities, the ability to form, maintain, and control biofilms will be crucial. Hong *et al.* (2012) developed a genetic circuit which they could introduce into cells in order to control formation and dispersal of biofilms. Initial colonizer cells would create a biofilm within a microfluidic device, which could then be quickly invaded by a second cell type, called disperser cells, to form a dual-species community. These disperser cells could then be induced by a chemical signal to completely displace the initial colonizer cells, and at a second chemical signal, to disperse the biofilm.

Cell densities

In addition to spatial organization, the seeding proportions of different members of the community are also important for determining the trajectory and viability of the community. In an attempt to construct a binary interaction between two yeast strains with different amino acid auxotrophies, Shou *et al.* (2007) made use of metabolomics to determine the amino acid release dynamics of each strain of yeast. They noticed that one strain only secreted the amino acid essential for the growth of its partner strain shortly prior to cell death. This delay in release meant that a specific range of initial seeding densities of the two strains was essential for the community to be viable. This was a result recapitulated by Hu *et al.* (2010) using a co-culture consisting of two strains of *E. coli* which were each innately resistant to either kanamycin or ampicillin and conditionally resistant to the other antibiotic depending on a quorum sensing signal produced by its partner.

Similar to the bidirectionality of spatial organization and community viability, final population composition and densities are also affected by the community. Hu *et al.* (2010) made an interesting observation that the final strain proportions and community densities were consistent, regardless of inoculation proportions and densities. As population proportions are in turn linked to a community's metabolic capabilities, the ability to accurately control the proportion of community members will allow us to control the community's metabolic capabilities. This control will be highly beneficial when attempting to manipulate existing natural communities or perform a complex function using synthetic co-cultures and communities. Kerner *et al.* (2012) developed a synthetic co-culture which consisted of a tryptophan auxotrophic and a tyrosine auxotrophic *E. coli* strain. Each strain had additional genetic modifications under control of inducible promoters, which strengthened the mutualistic relationship; a *yddG* gene in the tryptophan auxotroph increased export of tyrosine, and overexpression of *trpEDfbr* in the tyrosine auxotroph increased tryptophan biosynthesis. By controlling the amount of induction and thus the strength of the mutualism, the authors were able to tune growth rate and population ratios within the consortium.

Microbial co-cultures and communities for industrial application

Since microbial communities play a role in many parts of our lives, the ultimate goal is to rationally design and control co-cultures and communities for various medical, industrial, and environmental applications. Even without an in depth knowledge of individual community members, the form of interactions they are engaged in, or a mechanistic understanding of their interactions, microbial communities have been deployed for centuries in food production (Ercolini *et al.*, 2003; Kim and Chun, 2005) or wastewater treatment (Lay-Son and Drakides, 2008; Morgan-Sagastume *et al.*, 2008). However, the use of systems approaches and the development of new technologies for community microbiology has greatly accelerated knowledge about these communities and opened new avenues for rational interrogation and process improvement.

Microbial communities have been deployed for several industrial uses. They have been widely used for the production of biofuels from lignocellulosic material in both a one-step (Minty *et al.*, 2013) or two-step process (Eiteman *et al.*, 2008; Lin *et al.*, 2011). They have also been used for biomining (Dopson and Lindstrom, 1999) and bioremediation (Canstein and Kelly, 2002) and have been shown to be beneficial for the production of natural products such as vitamin C precursor 2-KGA (Ma *et al.*, 2011, 2014; Du *et al.*, 2013; Ding *et al.*, 2014), and might even prove to be an indispensable method in the process of natural product discovery (Schroeckh *et al.*, 2009; Nützmann *et al.*, 2011; Ola *et al.*, 2013).

Community modeling

Most detailed studies related to community interactions have been performed using artificial co-cultures. The transition to rational design and manipulation of more diverse communities has so far proven to be out of reach due to the sheer complexity of interactions within many natural communities. On the other hand, computers have proven to be highly adept at keeping track of vast numbers of parameters and interactions, performing millions of calculations per second. The use of computational models has transformed many fields (Buizza *et al.*, 1993; Ferziger *et al.*, 1997); they provide the only realistic means of calculating and predicting these complex phenomena. Computational models will likely prove invaluable to the continued pursuit of research on microbial communities.

From a theoretical perspective, classical ordinary differential equation (ODE) models have been shown to recapitulate interactions such as mutualism, commensalism, neutralism, and predator-prey relationships. For example, modeling the stability of plasmids in recombinant microorganisms, competition between two microorganisms for an inhibitory substrate in a biofilm, or the colony diameter and height as function of time have been successfully investigated (Dunn *et al.*, 2003; MacLean and Gudelj, 2006; Gudelj *et al.*, 2010). However, these approaches typically assume simple interspecies interaction rules, and also require knowledge of hundreds of differential equations and kinetic parameters typical of classical kinetic models.

New developments on the reconstruction of community networks have paved the way towards a model of ecosystem dynamics (Fig. 2). Recently, constraint-based modeling has become a powerful tool for interrogating biological networks. Constraint-based modeling approaches using genome-scale metabolic networks have provided significant mechanistic insights into the genotype-phenotype relationship and microbial physiology of single bacterial species (Edwards *et al.*, 2002; Schellenberger *et al.*, 2011). The main method of constraint-based modeling, flux balance analysis (FBA), analyzes network capabilities under a steady-state assumption. FBA is based on linear optimization of an objective function, which often is biomass formation (Schellenberger *et al.*, 2011). Different resources for metabolic reconstructions and software tools have been developed. Additionally, there are many databases, software tools, and solvers for FBA available (Raman and Chandra, 2009). The use of systems biology has allowed complex community interactions to be described using metabolic models, and models are now able to predict the outcome of community alterations and the effects of perturbations.

One of the earliest attempts at extending the constraint-based modeling approach to microbial communities was made by Vallino in 2003 using thermodynamic constraints. This simple model consisted of just twelve lumped reactions, each accounting for the community-wide contribution to processes such as nitrogen source uptake, CO_2-fixation, and biomass synthesis, yet achieved relative success in simulating a marine phytoplankton bloom (Vallino, 2003).

Stolyar *et al.* (2007) went on to expand community models to the genome-scale, investigating the mutualistic interaction between the sulfate-reducing *Desulfovibrio vulgaris* and the methanogenic *Methanococcus maripaludis*. The predicted metabolite fluxes showed high concordance with experimental data, confirming the viability of computational modeling for microbial communities. However, because constraint-based modeling at the time could only account for a single objective function, the authors considered the species as interdependent, and represented the objective function as the maximization of the sum of each species' biomass production fluxes, weighted by the experimentally observed population ratios. This limitation was later rectified by OptCom, an algorithm, which allowed the optimization of multiple objective functions (Zomorrodi and Maranas, 2012). Nagarajan *et al.* (2013) applied this algorithm for the modeling of direct interspecies electron transfer (DIET) between *Geobacter metallireducens* and *Geobacter sulfurreducens*.

Extensions were also made to FBA. One widely used extension has been the development of dynamic FBA. This algorithm was first implemented for a single *E. coli* strain model by Mahadevan *et al.* in 2002. Zhuang *et al.* (2011) implemented dynamic FBA into the Dynamic Multi-species Metabolic Modeling (DyMMM) framework. By solving the FBA problems representing each organism separately, the authors were able to model batch-growth of the community more accurately, as well as recapitulate competition and cross-feeding interactions. A similar approach has now been utilized for the modeling of industrially relevant processes, such as ethanol production by microbial communities growing on single sugars (Hanly and Henson 2011; Hanly *et al.*, 2012) as well as mixed substrates (Hanly and Henson, 2013). Even more recently, by incorporating diffusion via a finite diffe-

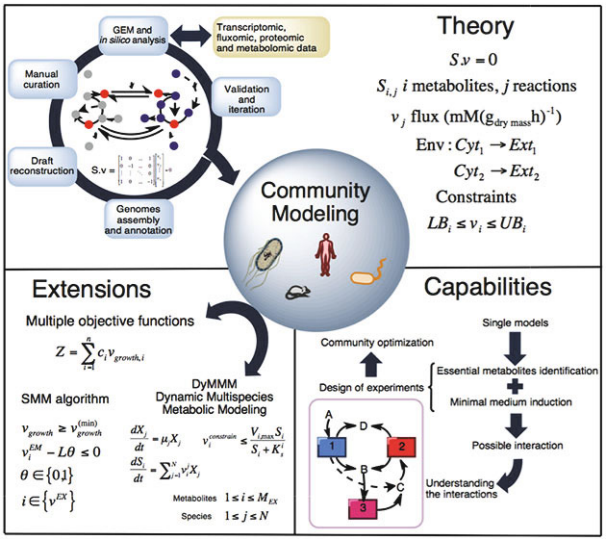

Fig. 2. Predictive and rational design of communities. Computational modeling of an organism starts with the creation of a draft reconstruction, manual refinement, conversion from reconstruction to mathematical model (GEM), and model evaluation through validation by experimental data. Various extensions to the traditional FBA method have been developed for use with communities. These have been used to predict possible community interactions and guide community optimization. Data from both natural communities and model communities has been used to better understand community structure, metabolic capabilities, and activity.

rence approximation, Harcome *et al.* (2014) have added an extra dimension to this method. This allows the model to recapitulate spatial effects which have repeatedly been shown to be important in community structure, leading to the prediction of both spatial and temporal changes in two and three-membered communities.

Recently, progress has been made towards predictive modeling of microbial communities. Tzamali *et al.* (2009) used a network-based method for simulating a long-term evolution experiment of *E. coli* strains grown on ten different carbon sources. By setting rules which allow interactions to form only between strains with different metabolic capabilities, the authors determined the diversity that would theoretically emerge from a population of single gene deletion mutants of *E. coli*.

In 2010, Klitgord and Segre further expanded the predictive capabilities to pairwise combinations of different model organisms. The authors studied combinations of seven bacterial species: *E. coli, Helicobacter pylori*, *Salmonella typhimurium*, *Bacillus subtilis*, *Shewanella oneidensis*, *Methylobacterium extorquens*, and *Methanosarcina barkeri*. Based on a set of metabolites essential for growth of two microorganisms, the authors developed an algorithm which identified an interaction-induction minimum medium. The medium is designed to sustain the growth of a pair of organisms, but not either of the two individually. Using this medium allows researchers to design experiments for the study of commensalism or mutualism in the pairwise combinations.

Future directions and conclusions

While a multitude of experimental tools now exist to study bacterial communities, the complexity and vast number of factors involved in even simple communities necessitate the construction and use of computational models for rational design or prediction of community trajectories.

Mathematical and computational models based on ordinary differential equations (ODEs) have long been used in the modeling of ecological phenomena (McCook, 1994), but they suffer from limitations such as the need for kinetic parameters. The advent of constraint-based modeling circumvents many of these limitations for community modeling - making detailed insights into microbial communities and predicting their responses to perturbations a reality. Many new methods and algorithms have been developed ever since the first genome-scale model of an artificial two-member co-culture was described in 2007 (Stolyar *et al.*, 2007).

Although great progress has been made over the last few years, certain limitations still exist that hinder the widespread use of computational models for microbiology research. Community modeling efforts rely on reconstructions for single microorganisms. Out of over 30,000 sequenced archaeal, bacterial, and eukaryotic genomes available (NCBI Reference Sequence Database), only 78 curated genome-scale models have been reconstructed (NCBI, 2014; Bordbar *et al.*, 2014). The availability of high quality reconstructions currently represents the main bottleneck in the process of community modeling. Furthermore, population diversity dictates manual curation of existing models to match different realities (Monk *et al.*, 2013; Kashtan *et al.*, 2014). Automatic reconstruction tools, such as ModelSEED (Overbeek *et al.*, 2005) have become available to aid the genome-scale reconstruction process; however, these tools still require extensive manual curation to improve accuracy. These tools will improve over time when more highly curated models become available, in particular of microorganisms containing unique metabolic capabilities. One could argue that current metabolic models provide only a partial representation of the vast metabolic diversity present in different microorganisms. The fact that the majority of microorganisms are so far not culturable (Zengler, 2009) and their metabolic capabilities are therefore difficult to elucidate, further stymies the development of true environmental community modeling. Furthermore, we are still lacking knowledge about gene functions, even in model organisms. For example, around 30% (1,336) of the genes in *E. coli*, arguably the best studied microorganism, are considered y-genes of unknown function. Many of the organisms sequenced in natural habitats (e.g., by metagenomics) have poor homology to their cultivated counterparts, potentially resulting in altered metabolic capabilities (Tettelin *et al.*, 2005). Genome-scale reconstructions can be hampered by an insufficient breadth of biochemical data. This is especially true for lesser-studied microorganisms with poorly understood biochemistry. A concerted effort of both experimental and computational approaches could provide this knowledge and would undoubtedly lead to a plethora of discoveries.

Recent advances in experimentally derived genome annotations have resulted in reconstructions with broader scope (Cho *et al.*, 2009; Qiu *et al.*, 2010; Latif *et al.*, 2013). These next generation reconstructions integrating both metabolism and expression (transcription and translation) have led to an improved mechanistic understanding of transcription, translation, and protein modification machinery (O'Brien *et al.*, 2013). The development of eukaryotic genome-scale models such as RECON1 (Duarte *et al.*, 2007) has also spawned models for eukaryote-prokaryote interaction. These models were deployed to simulate pathogenicity (human cell/*Mycobacterium tuberculosis*) (Bordbar *et al.*, 2010), interactions between host (mouse) and a gut bacterium (*Bacteroides thetaiotaomicron*) (Heinken *et al.*, 2013), as well as the bulk metabolic capabilities of both host and gut microbiome (Sridharan *et al.*, 2014).

Substantial progress has been made towards predictive modeling of microbial communities as well as host/microbe interactions. The potential applications of these models are as diverse as the microbial niches on the planet, spanning from industrial settings to the natural environment. Recently, the human microbiome has become of particular interest. Understanding the complex relationship of bacteria and fungi with the human body and their role in health and disease could ultimately lead to tailored interventions and new kinds of pharmaceuticals. We envision that predictive modeling of host/microbe interaction and insight into the intertwined relationship will play a major role in the rational design of microbes and drugs to improve human life.

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