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Causes and consequences of biotic interactions within microbiomes

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An integrative pattern-process-mechanism approach is revealing the roles of biotic interactions in microbiome assembly. Patterns of microbiome diversity observed in metagenomic studies can be partly explained by interaction processes (e.g. competition, facilitation) and underlying molecular or genetic mechanisms (e.g. antibiotic production, nutrient cross-feeding). Exciting opportunities remain to fully understand the significance and generalizability of biotic interactions within microbiomes. Many microbial interactions have been studied by chasing easily quantifiable phenotypes including changes in growth or pigmentation, but it is likely that diverse cryptic interactions occur without obvious growth changes or macroscopic phenotypes. A narrow phylogenetic breadth of well-studied microbes limits our understanding of whether there are conserved genetic or molecular mechanisms of microbial interactions. Biotic interactions can impose strong selective pressures that could shape rates and modes of microbial evolution, but few studies have examined the evolutionary consequences of interactions within microbiomes. Continued exploration of the chemical and genetic mechanisms underlying biotic interactions may provide novel tools to manipulate and manage microbiomes.

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Introduction

Laboratory monocultures have provided the foundational knowledge for much of microbiology. These reduced systems have been essential to minimize complexity and identify fundamental controls of cellular and molecular processes within microbial populations. As a multispecies mindset has swept through microbiology, there is a growing interest in how the biology of individual species changes when interacting with other microbes. Molecular and cellular microbiologists have started adopting a polymicrobial approach to understand how microbial interactions impact cellular processes in target species [1,2]. From simple changes in the growth to more complex changes in physiology and metabolism, neighboring microbes can dramatically alter the biology of microbial species [3]. Interactions may also impact the virulence of pathogens or benefits of commensal microbes [4•,5].

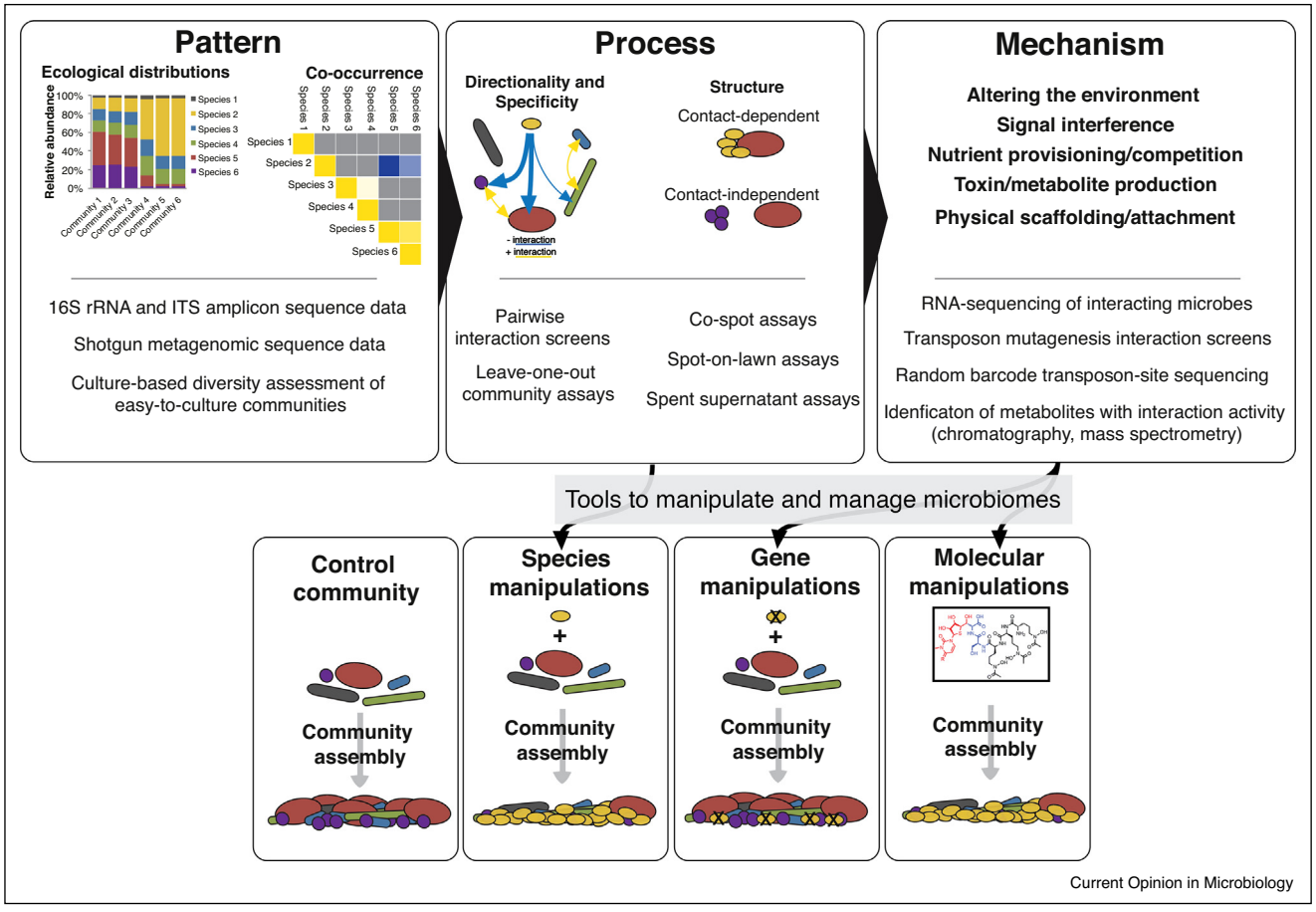
At broader biological scales, microbial ecologists and systems biologists have also been making progress at identifying how microbial interactions can shape the diversity of microbial communities [6,7,8••]. Microbial interactions are often purported to be important in microbiome assembly based on patterns of co-occurrence in sequence datasets [9,10]. Experimental studies of microbial interactions have typically been conducted as pairs of species in high abundance in simplified lab environments. Whether pairwise or multispecies interactions impact the assembly and function of complex microbiomes is still being worked out [11]. It is also unclear how biotic interactions compare to other drivers of microbiome assembly including abiotic selection, ecological drift, and diversification [12].

Our goal in this review is to illustrate how an integration of both cellular/molecular and ecological/evolutionary approaches is providing a greater understanding of the causes and consequences of microbial interactions. Many comprehensive reviews have already described the diversity of interactions between different groups of microbes [13,14], specific methodologies or informatic approaches to study microbial interactions [10], and mechanisms of interactions [15]. Here we highlight challenges and opportunities in the study of microbial interactions that span from the community to the cellular scale. Much of our own research has focused on interactions in relatively simple fermented food microbiomes and has generally been focused on bacterial-fungal interactions [16–22]. But the themes we discuss are broadly applicable to microbial interactions in many environments.

Studying microbial interactions in a pattern-process-mechanism framework

An integrative approach to identify both the causes and consequences of microbial interactions has recently emerged [16,23••,24,25]. These studies tend to use a three phase approach: pattern, process, and mechanism (Figure 1). In the pattern phase, metagenomic surveys of

Figure 1



A pattern-process-mechanism framework to study the causes and consequences of biotic interactions within microbiomes. Microbiome sequence data can highlight ecological patterns that may be explained by biotic interactions. *In vitro* experiments with cultures can determine the processes that drive outcomes of microbial interactions. A variety of genetic and '-omics' approaches can identify genes and molecules that mediate interactions. Processes and mechanisms can provide tools for manipulating microbiomes. For example, specific metabolites identified as mediators of interactions can be purified and added to microbiomes to shift *in situ* composition.

in situ microbial communities are used to discover patterns in community composition that might be explained by interactions. In the process phase, experimental microbial communities are used to determine the processes shaping the outcomes of microbial interactions. In the mechanism phase, transcriptomic, metabolomic, and high-throughput mutant screens are used to identify genetic and molecular mechanisms underlying microbial interactions. With these mechanistic insights, it is possible to return to *in situ* communities to manage and manipulate microbiome diversity through microbial interactions.

A variety of ecological patterns within microbiomes could potentially be explained by microbial interactions. For example, highly abundant microbial taxa may inhibit other microbes or may benefit from positive interactions with community members. Conversely, rare species may be inhibited by other community members. In our own work and in studies of the human microbiome, the abundance of species within microbiomes may be partly explained by biotic interactions [16,18,25]. Using the cheese rind model system, we determined that an abundant *Staphylococcus* species is dominant across cheese rinds because its growth is promoted by a widespread fungus [16]. Broader patterns of community composition, including the distribution of higher taxonomic (e.g. Gammaproteobacteria) or functional (e.g. yeasts versus filamentous fungi) groups may also be a starting point for understanding contributions of interactions in microbial communities.

The process discovery phase is often focused on identifying a phenotype associated with microbial interactions in a community. These studies typically first use interaction screens that attempt to identify the directionality

(negative, positive, neutral), magnitude, and specificity of interactions between community members. This work can quickly pinpoint the ecological process that may explain the previously observed patterns. In our example of the cheese rind *Staphylococcus* species, we observed that specific facilitation of the dominant *Staphylococcus* species, not inhibition of the subdominant species, explained why one *Staphylococcus* was more abundant than others within the cheese community [16]. Many of these studies use interaction growth assays or macroscopic phenotypes that identify interactions (e.g. zones of inhibition, co-spot cross-feeding, etc.). One challenge with these approaches is creating relevant ecological conditions that reflect *in vitro* environments where microbes interact [19]. Microscopy has also played an important role in identifying structural phenomena, including how microbial cells of two or more species sense one another or interact in physical space [26].

With a process phenotype in hand, genetic screens and various '-omics' approaches can be used to pinpoint genetic or molecular mechanisms underlying the process. Dissection of interaction mechanisms is typically done using a pairwise approach. Numerous studies have employed RNA-sequencing to pinpoint potential genomic regions that are differentially expressed when comparing growth alone and growth with a neighboring microbe [16,17,20,27•,28–32]. In our *Staphylococcus* cheese example, we were able to pinpoint strong differential expression of genes related to iron uptake in the presence of a specific fungus, suggesting that iron or siderophore provisioning was facilitating the interaction [16]. Transposon mutagenesis has also proved to be a powerful tool to dissect microbial interactions. If an easy to score colony or pigment phenotype is involved, mutant libraries of one interacting partner can be constructed and screened against another species to identify those genes that are important determinants of interaction outcomes [17,33,34]. When a phenotype is not available, transposon mutagenesis paired with high-throughput sequencing approaches can be used to determine interaction-relevant genes in mutant pools [35••]. Limited genetic tractability of the microbial community may constrain the use of transposon mutagenesis and other mechanistic approaches, but ongoing work to develop genetic tools in non-model microbes may help remove some of these barriers [36,37]. Various metabolomic approaches have become useful tools to identify chemical mediators of molecular interactions. Targeted identification of fractions with activities related to the interaction (inhibition, stimulation, etc.) can be isolated and identified using chromatographic and mass spectrometric approaches [38–42].

Beyond changes in growth

Traditional efforts to elucidate processes underlying microbial interactions are largely limited to measuring changes in growth or obvious phenotypes (color change, morphology, etc). Much of our own work has focused on these easy-to-quantify phenotypes because they clearly matter for the fitness and physiology of the interacting microbes. But these approaches can mask important biological insights due to the assumption that the nature of interactions is fixed and binary. In at least some cases, important biological changes may occur without dramatically changing growth. For example, changes in biofilm production or virulence of bacterial pathogens induced by other microbes could have limited impact on total colony forming units. Moving beyond changes in growth requires innovation in the ways we measure such cryptic microbial interactions.

Transcriptome sequencing can reveal major biological shifts induced by interspecies microbial interactions, irrespective of growth changes. For example, a recent study used a dual RNA-seq approach to understand the transcriptomic changes occurring between the biocontrol rhizobacterium *Lysobacter capsici* AZ78 and the soilborne phytopathogenic oomycete *Phytophthora infestans* [27•]. Growth of *Lysobacter capsici* did not change in the presence of *P. infestans*, but the bacterium did experience major transcriptional reprogramming. Genes involved with attachment to the oomycete, degradation of oomycete cell walls, and antibiotic production were all highly upregulated in the presence of *P. infestans*. Cryptic interactions can also be productively dissected using metabolomic approaches. Studies have demonstrated that the presence of a microbial partner can activate a greater variety of molecules in their secondary metabolome that may be useful in drug discovery for other organisms [43]. High-throughput metabolomic screens of microbial interactions that are not constrained by specific phenotypes may reveal previously unrecognized metabolic cross-talk between microbial species.

Toward a phylogenetically-diverse understanding of mechanisms of interactions

Our current understanding of the mechanisms underlying microbial interactions is phylogenetically fragmented and sparse. As noted earlier, most studies that have determined the genetic or chemical mediators of microbial interactions have generally focused on two interacting species and rarely explore how one target species responds to the presence of a diverse range of other species or vice versa. The limited phylogenetic breadth stems from a strong focus on plant and animal pathogens.

We predict that there are conserved mechanisms that control the outcomes of microbial interactions at broad phylogenetic levels. Many interaction-relevant traits are conserved at genus, family, and higher taxonomic levels in some microbes [44,45]. For example, many cheese rind Gammaproteobacteria are motile and can swim along the liquid layer of fungal hyphae to spread across the cheese surface [17]. In one bacterial species we found that

flagellar biosynthesis was essential for this interaction to occur. It is likely that motility-related genes are important across motile Gammaproteobacteria when interacting with the physical networks created by filamentous fungi.

High-throughput sequencing screens of many pairwise combinations of a broad range of microbes will help fill the sparse sampling of interaction mechanisms. Transcriptomic profiling is likely the best place to start given the relative ease of preparing samples and the ability to apply it to many microbial combinations where genetic manipulation may not be possible. A few studies have included multiple species in pairwise transcriptomic interaction studies and have observed general responses of one microbial group to another. Some studies of bacterial-fungal interactions have illustrated general fungal responses to the presence of bacteria including growth arrest, defense, and toxin production [28,32].

High-throughput mutagenesis approaches are also illustrating the conservation of genes necessary for pairwise microbial interactions. In a recent study of the cheese rind system, Morin *et al.* paired RNA-sequencing with random barcode transposon sequencing to identify genes that play roles in interactions between *Escherichia coli* and three phylogenetically diverse cheese rind microbial species [35••]. In the presence of all three species, various genes involved with biofilm formation and response to toxic stress were associated with negative fitness in the presence of the neighbor. These data suggest that production of biofilms and dealing with toxins produced by neighbors are important genetic requirements for interactions in cheese rinds.

Impacts of biotic interactions on microbial evolution

Most studies of microbial interactions across the pattern-process-mechanism continuum have focused on short-term interaction outcomes. Studies typically operate on the scale of a few days where the interacting microbes will undergo just a few generations. But many microbial interactions can occur over longer timescales. For example, microbes that colonize and co-exist on human skin surfaces may interact with each other over the lifetime of the human host. In our fermented food systems, microbial populations often recirculate within a fermented food system as they are serially passaged from one batch of food to the next [22]. These longer-term interaction dynamics may have important consequences for the phenotypic and genomic evolution of the interacting microbes. Experimental evolution studies have demonstrated that microbial traits — including metabolism, stress resistance, and virulence — can evolve rapidly in serially passaged populations [46,47]. How these traits evolve under different biotic selection pressures has not been thoroughly explored.

Based on evolutionary theory and observational studies of macroorganisms, biotic interactions could impact microbial evolution in several different ways [48•,49]. Microbial interactions could inhibit phenotypic and genetic evolution of a target microbe by reducing population sizes and decreasing the potential genetic variation for selection. Interactions could also promote adaptation when they cause a target microbe to shift niches or when interacting microbes provide novel ecological opportunities.

There are very few studies that have explicitly manipulated biotic interactions in microbial experimental evolution studies. Most studies to date have focused on *Pseudomonas* as target species and have demonstrated that competition by another species can both inhibit [48•,50] and stimulate microbial evolution [51,52]. These synthetic systems are excellent testing grounds for the role of biotic interactions in microbial evolution, but without natural analogs, it is difficult to directly link results of multispecies experimental evolution to patterns of diversity in naturally forming microbiomes. For example, are specific biotic environments and community compositions correlated with the abundance of microbial phenotypes or genotypes?

Applications of microbial interactions

Identifying the causes and consequences of microbial interactions provides insights into the basic biology of how microbiomes assemble. Microbial interactions also provide opportunities to manipulate and manage microbiomes in medicine, agriculture, industry, and natural systems [13,53,54]. When microbial taxa that have desired impacts on microbiome composition via interactions are identified, these taxa can be developed as probiotics for inoculation into systems. Metabolites that mediate interactions may serve as drugs that could be used to manipulate microbiome composition. Predictable control of microbiomes through manipulating microbial interactions is still difficult and limited, but there are a few promising areas of microbial interaction application.

Biocontrol agents have been deployed in plant agriculture to reduce plant pathogens [55]. A variety of microbes have been developed to directly inhibit the growth of pathogenic microbes, but many biocontrol agents are notorious for varying success across different agricultural systems [56,57]. The effectiveness of biocontrol strains that are designed to reduce the abundance of a specific undesirable microbe may be dependent on how that undesirable microbe interacts with other community members. Recent efforts to understand pathogens and biocontrol agents in the context of multispecies plant communities [57–59] may ultimately provide a pattern-process-mechanism framework that can make biocontrol more predictable.

The human microbiome may also be manipulated and managed by taking microbial interactions into account. In

the human gut microbiome, *Bacillus subtilis* species have been recently demonstrated to inhibit the growth of *Staphylococcus aureus* by disrupting an essential quorum sensing system [23••]. Direct application of *Bacillus* probiotics or the chemical mediator of the *Bacillus–Staphylococcus* interaction may provide a novel tool to reduce *S. aureus* carriage in susceptible populations. As with biocontrol in agriculture, managing one pairwise microbial interaction across highly divergent human microbiomes will likely result in different outcomes [60].

Microbial interactions have also been used in the design and management of fermented food microbial communities [61]. Interactions have been typically used in a very coarse-grained way. In surface-ripened cheeses, yeasts and filamentous fungi are widely recognized for their ability to promote the growth of bacterial species, but specific mechanisms that drive these interactions are still unknown. Increasing the pH of the cheese curd and release of free amino acids are two possible explanations [16,21,35••,62,63], but other mechanisms likely mediate these interactions. Finer-scale control of the aesthetics and functions of microbial communities in food may ultimately be possible with a growing understanding of the processes and mechanisms that drive microbial interactions. For example, enhanced cheese rind pigmentation due to the production of coproporphyrin III could be controlled using specific combinations of bacteria and fungi that induce this pigment production [20].

Conclusions

The multiscale pattern-process-mechanism framework has potential to provide a more comprehensive understanding of the causes and consequences of microbial interactions within microbiomes. In this limited space, we tried to review some excellent studies that use this integrative approach as well as our own work from the cheese rind system. We acknowledge that this approach presents challenges and limitations. It may not be possible to culture dominant community members, making it difficult to go beyond patterns in metagenomic sequence data. Genetic tools may be currently unavailable to dissect mechanisms of microbial interactions. In some systems, there is considerable work on the ends of the pattern-process-mechanism continuum, but with limited connections in the intermediate process space. For example, many correlative metagenomic studies have pinpointed potential interaction networks within communities, but have not actually tested whether they occur in communities. Likewise, many mechanistic studies have described in detail the molecular and cellular biology of potential microbial interactions in isolation, but have not shown that they occur in microbiomes or impact community structure.

Continued collaborations between microbial ecologists and molecular and cellular microbiologists will help bridge gaps in the microbial interaction space. It is generally difficult for one lab to be good at all the methods needed to move from pattern to mechanism. Ecological labs that discover an interesting microbial interaction can pair with microbial geneticists or chemists to identify mechanisms of interactions. Labs with expertise in characterizing mechanisms of interactions can pair with microbial ecology and metagenomic labs to place an interaction mechanism into an ecological context. These continued collaborations in microbiome science will help discover unknown microbial interactions that may play essential roles in driving microbiome assembly.

Conflict of interest statement

Nothing declared.

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