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Metabolic interactions in microbial communities: untangling the Gordian knot

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Metabolic exchanges are ubiquitous in microbial communities. However, detecting metabolite cross-feedings is difficult due to their intrinsically dynamic nature and the complexity of communities. Thus, while exhaustive description of metabolic networks operating in natural systems is a task for the future, the battle of today is divided between detailed characterizations of small, reduced complexity microbial consortia, and focusing on particular metabolic aspects of natural ecosystems. Detecting metabolic interactions requires methodological blend able to capture species identity, dependencies and the nature of exchanged metabolites. Multiple combinations of diverse techniques, from metagenomics to imaging mass spectrometry, offer solutions to this challenge, each combination being tailored to the community at hand.

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Introduction

Microbial communities are intertwined by metabolic links, whether viewed as narrowly as a pair of symbionts, or as broadly as the earth-wide ecosystem lined up with trophic chains. Understanding metabolic interactions at the global level is thus indispensable in microbial ecology and evolution. However, shifting attention from isolated metabolism of pure cultures to that of microbial communities is challenging and requires new tools and methods. And, as in the case of any complex network, when choosing a focus point in the large web of metabolic interactions, we have to compromise between resolution of detail and coverage.

Seeing microbial metabolism in the community context (as opposed to pure cultures) reveals new phenotypes [1•], helps designing synthetic communities for biotechnology [2,3], and enables cultivating the 'uncultivables' [4]. Accumulating examples of metabolic cross-feeding [5,6•] and evidence from metabolic modeling [7•] create an anticipation of many more to be discovered. Within the broad range of metabolic interactions, here we concentrate primarily on nutrient exchange. We aim to show how studying complex communities is shifting the paradigm of microbial metabolism and what methods and challenges await for those trying to disentangle inter-species connections.

Metabolite exchanges provide group advantage

Multiple studies show that metabolite exchanges form a strategy for group success [6•,8–11]. Metabolic interactions frequently contribute, through division of labor, to the emergent abilities at community level, such as biodegradation [12,13], faster growth [10] or increased virulence [9,14]. Outsourcing metabolic functions to fellow members embeds each pathway in a specialized microenvironment, hence avoiding biochemical conflict [15]. Moreover, under nutrient-poor conditions species can be readily prompted to share metabolites and thus complement each other's biosynthetic capabilities [16,17••,18]. Metabolic specialization can be found even within the same species, for example, filamentous cyanobacteria with specialized heterocyst cells for nitrogen fixation [19].

Despite benefits associated with cross-feeding, its evolution remains controversial, especially in case of metabolic cooperation [20,21]. Emergence and maintenance of metabolic exchanges depends on particular circumstances, such as spatial structure of microbial community, nutrient availability, diffusion constraints and cost effectiveness of concerned biosynthetic processes [22–24]. For example, aggregating or forming a biofilm maximizes efficiency of nutrient transfer and stimulates otherwise thermodynamically unfavorable metabolic processes [25]. In extreme cases, metabolic dependency results in endosymbiotic relationship, a popular solution for hydrogen-producing ciliates that harbor methanogenic archaea for H_2 outflow [26].

Microbial metabolism is plastic and responsive to social cues

Microorganisms can often utilize and secrete a large number of metabolites [27,28]. This plastic network is readily adapted and regulated in response to nutrients, for example, to optimize resource allocation [29,30], but also in response to cues from other microorganisms [31•]. Certain bacterial species can modulate yeast metabolism,

to reduce secretion of toxic ethanol, by deploying chemical signaling [1•]. Transcriptional response of *Streptococcus* species shows metabolic adaptations to other members of community [32].

Discovering metabolic interactions

Meta-omics analyses guide interaction discovery

Meta-omics technologies are culture independent and scalable in space/time. Metagenomics is a particularly powerful tool for discerning species identity and for detecting patterns of interspecies associations. These in turn can generate verifiable hypotheses about metabolic (and other) interactions between community members. Genotyping of associated microbes can reveal their functional palettes [33] and task distribution among community members [12]. For example, individual genomes of a co-aggregated pair of archaea showed that one of the symbionts is dependent on another for lipid, cofactor, amino acid, and nucleotide biosynthesis [34]. Following a specific community over time can also reveal metabolic dependencies as one species dynamically responds to change in abundance of the other, as shown in an activated sludge community [35]. Overlaying taxonomic data with other information, such as spatial distribution and geochemical profiles [36] or specific enzymatic function [37], can deepen insight into community co-metabolism. Beyond individual communities, metagenomics has allowed the identification of species co-occurrence structure across different habitats/samples [38,39] — associations that hint at interspecies interactions [7•,40].

Transcriptomics and proteomics are commonly used to complement metagenomics, to deduce what genome encoded metabolic potential is being used [41••,42]. For instance, analysis of transcriptional patterns in co-culture of a marine bacterium and a diatom, as well as ocean samples, pinpointed cross-feeding of 2,3-dihydroxypropane-1-sulfonate, a new link in marine microbial food web [41••]. Metabolic applications of meta-proteomics are more commonly used for relatively simple systems — it was used to demonstrate metabolic adjustments made by three species comprising a model oral biofilm [43] or to show how the presence/absence of *Aggregatibacter actinomycetemcomitans* modulates metabolism of other bacteria in a 10-species biofilm [44]. Although not distinguishing between species, these results give a sense of the complexity and scale of metabolic adjustments that happen in 'real-world' communities. On a larger scale, meta-proteomics, in combination with meta-genomics, allowed proposing differential flow of nitrogen, sulfur and hydrogen among the abundant taxa of marine microbial communities in response to oxygen availability [45].

Isotope labeling for tracing community-scale pathways

Tracing of isotope labeled substrates, a standard approach in pathway discovery, can also be adapted to reveal flow of metabolites in microbial consortia. Although this is the most conclusive method for showing metabolite exchange, the major challenge is to distinguish labeling fingerprints of different populations. To do so, one can use an artificially expressed reporter protein [46], species-specific peptides [47], or detect labeled DNA or RNA in conjunction with metagenomics analysis [48]. To give some examples, ^{13}C labeling served to experimentally prove bacterial feeding on fungal exudates [49], to suggest a chain of toluene degraders in methanogenic enrichment culture [13] and to identify key naphthalene-degrading bacteria *in situ* [50].

Imaging community structure — clues from the neighbors

Efficient mass transfer between organisms is a prerequisite of successful metabolic interaction, therefore it is not uncommon for microbial partners to form tight aggregates and develop special structures that facilitate metabolite exchange. Microscopic detection of these structures can be a powerful tool in identifying interacting microorganisms. Illustrative is an example of nanotubes formed by cross-feeding *Escherichia coli* auxotrophs [51] or variety of formations in acid mine drainage community, such as cytoplasmic bridges, pili, and 'synaps like connections' [52].

Fluorescence *in situ* hybridization (FISH) based methods reveal spatial distribution of interacting partners, for instance showing stratification and co-aggregation patterns in biofilms [53] or bacterial groups attached to phytoplankton host [54]. In addition to resolving spatial structure, imaging, for example, based on fluorescent dyes, can be used to assess general metabolic state of community members [55,56•].

Exploration using metabolomics

Mass spectrometry (MS) based methods can detect a broad spectrum of compounds and are being developed rapidly. This technique has a wide range of modifications, varying in application from a single cell to multiple colonies on a petri dish (reviewed by Watrous *et al.* [57]). Interestingly, MS can be used in an imaging set-up to study metabolic interactions [58]. The potential of imaging-MS unfolded, for example, in a study of chemical interactions on actinomycete bacteria, showing interactions through spectra of secondary metabolites [59•]. Application of MS to microbial interactions is, however, currently limited by various challenges in data analysis and compound identification [1•,59•,60,61]. Other methods that can facilitate interrogation of metabolic space of the community are reviewed by Maurice *et al.* [56•] and Wessel *et al.* [62•].

Metabolomics alone usually does not provide sufficient resolution to pinpoint exchanged molecules. Elucidating cross-feeding in a complex nutritional environment is possible only in combination with other techniques such