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Metabolic Resource Allocation in Individual Microbes Determines Ecosystem Interactions and Spatial Dynamics

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SUMMARY

The interspecies exchange of metabolites plays a key role in the spatiotemporal dynamics of microbial communities. This raises the question of whether ecosystem-level behavior of structured communities can be predicted using genome-scale metabolic models for multiple organisms. We developed a modeling framework that integrates dynamic flux balance analysis with diffusion on a lattice and applied it to engineered communities. First, we predicted and experimentally confirmed the species ratio to which a two-species mutualistic consortium converges and the equilibrium composition of a newly engineered three-member community. We next identified a specific spatial arrangement of colonies, which gives rise to what we term the "eclipse dilemma": does a competitor placed between a colony and its cross-feeding partner benefit or hurt growth of the original colony? Our experimentally validated finding that the net outcome is beneficial highlights the complex nature of metabolic interactions in microbial communities while at the same time demonstrating their predictability.

INTRODUCTION

Although often studied alone in well-mixed flasks, most microbial organisms live in multispecies, structured, and highly dynamic consortia (Denef et al., 2010; Dethlefsen et al., 2007; Lozupone et al., 2012; Ramette and Tiedje, 2007; Xavier and Foster, 2007). Interactions of microbes with each other and with the environment play a fundamental role in the evolution and dynamics of these communities. Many of these interactions are mediated by the uptake and excretion of small molecules, produced and degraded by the metabolic network encoded within each organism. In turn, the ensuing spatiotemporal changes of nutrients and by-products in the environment continually modify the conditions sensed by individual cells, causing transient niches and context-dependent interspecies interactions.

Given this complexity, one may ask whether a suitable mathematical modeling framework could help bridge the gap between metabolic strategies of individual species and ecosystem-level dynamics. Such a framework would be a powerful instrument for microbial ecology, with potential impact on research areas as diverse as biogeochemical cycles (Falkowski et al., 2008), the health-balancing role of the human microbiome (Lozupone et al., 2012; Turnbaugh et al., 2007), and synthetic ecology (Klitgord and Segrè, 2011; Park et al., 2011; Shou et al., 2007). Moreover, fundamental questions on the stability (May, 1973; Mougi and Kondoh, 2012) and diversity (Curtis et al., 2002; Gudelj et al., 2010) of microbial ecosystems, the evolution of cooperation (Harcombe, 2010; Xavier and Foster, 2007), and the emergence of multicellularity (Pfeiffer and Bonhoeffer, 2003) lie precisely at the boundary between the metabolic requirements of individual species and the community-level implications of shared resources.

The past decade has seen the emergence of several novel experimental systems for investigating the dynamics of structured microbial consortia. For example, spatial structure was shown to be critical for maintaining diversity in systems with antagonistic interactions, ranging from chemical warfare (Kerr et al., 2002) to predator-prey behavior (Balagaddé et al., 2008), as well as beneficial interactions (Kim et al., 2008). In terms of metabolism, a variety of novel, engineered mutualisms between

codependent strains have been developed (Harcombe, 2010; Hillesland and Stahl, 2010; Shou et al., 2007). These include a laboratory-evolved costly cooperation between *Salmonella enterica* serovar *typhimurium* LT2 and an auxotrophic *Escherichia coli* K12 strain (Harcombe, 2010), which we use as a starting point in the current work.

Although some qualitative results, such as the importance of spatial structure in a two-species system, are consistent with theory on the evolution of cooperation (Sachs et al., 2004), broader and more quantitative predictions such as species ratios or interactions between a larger number of players are unexplored experimentally and computationally. How predictable are consortia compositions in spatially structured environments, and how strongly are they affected by initial species frequencies? Can stable systems be engineered with more than two species? Can interspecies interactions in synthetic microbial consortia emerge as a consequence of individual species solving their own metabolic resource allocation problem?

From a theoretical perspective, these questions bridge multiple distinct scales, from individual intracellular reactions, up to the spatial distributions of multiple species and environmental metabolites (Gudelj et al., 2010; MacLean and Gudelj, 2006). Classical ordinary differential equation (ODE) models have been shown to recapitulate colony diameter and height as a function of time (Kamath and Bungay, 1988; Pipe and Grimson, 2008; Pirt, 1967; Rieck et al., 1973). Agent-based models have successfully shown how colony morphology arises as an emergent property of the behavior of individual cells or clusters of cells (Ben-Jacob et al., 1998; Kreft et al., 1998, 2001; Xavier et al., 2005). However, these approaches typically assume simple interspecies interaction rules rather than computing them based on detailed representations of intracellular biochemical networks.

In contrast, stoichiometric modeling, a class of systems biology methods with roots in metabolic engineering, has been shown to provide testable predictions of metabolic activity at the whole genome scale, with no need for the hundreds of differential equations and kinetic parameters typical of classical kinetic models. One of the most broadly used methods, flux balance analysis (FBA) (Orth et al., 2010) assumes steady state and optimality to predict metabolic rates (fluxes) of all reactions in the cell, including uptake and secretion fluxes, and the amount of microbial growth (Harcombe et al., 2013; McCloskey et al., 2013; Segrè et al., 2002). It is important to keep in mind that the simplifications that make FBA efficient and useful are also among the main reasons for its limitations, including the incapacity to predict intracellular metabolite concentrations, the reliance on a predefined metabolic objective, and the need for prior knowledge of biomass composition. Alternative uses of stoichiometric constrains (e.g., sampling of the feasible space [Bordel et al., 2010]), integration with high-throughput data (Becker and Palsson, 2008; Collins et al., 2012), and thermodynamics or economy-inspired theory (Fleming et al., 2012; De Martino et al., 2012; Reznik et al., 2013; Schuetz et al., 2012) are among the new directions being sought in order to overcome some of these limitations.

Recent efforts have shown how FBA can be extended to model metabolite-mediated interactions between different species in microbial consortia (Klitgord and Segrè, 2011), e.g., by searching for syntrophic compositions (Stolyar et al., 2007), interaction-inducing environments (Klitgord and Segrè, 2010), competition/cooperation balances (Freilich et al., 2011; Wintermute and Silver, 2010), or multilevel optima (Zomorrodi and Maranas, 2012) in multispecies joint stoichiometric models, or by implementing dynamic flux balance modeling of cocultures (Khandelwal et al., 2013; Salimi et al., 2010). Some of these approaches require a priori assumptions on how two species interact, e.g., a tunable ratio of the biomass production rates (Stolyar et al., 2007), a minimal growth rate for each species (Klitgord and Segrè, 2010), or different types of joint or multilevel objective functions (Freilich et al., 2011; Wintermute and Silver, 2010; Zomorrodi and Maranas, 2012). Most importantly, to our knowledge, these approaches have not been extended to multispecies communities in a structured environment, although a single-species model has been previously coupled with reactive transport (Scheibe et al., 2009).

Here, we introduce a multiscale modeling framework that computes ecosystem-level spatiotemporal dynamics based on detailed intracellular metabolic stoichiometry, without any a priori assumption on whether and how different species would interact. Our approach, named Computation of Microbial Ecosystems in Time and Space (COMETS), implements a dynamic FBA algorithm on a lattice, making it possible to track the spatiotemporal dynamics of multiple microbial species in complex environments with complete genome scale resolution. We apply COMETS to the study of a previously built *E. coli*/*S. enterica* synthetic consortium (Harcombe, 2010) and to a new three-member consortium that incorporates *Methylobacterium extorquens* AM1 into the *E. coli*/*S. enterica* system.

RESULTS

From Genome Scale to Ecosystem-Level Spatiotemporal Models

COMETS uses dynamic flux balance analysis (dFBA) (Mahadevan et al., 2002) to perform time-dependent metabolic simulations of microbial ecosystems, bridging the gap between stoichiometric and environmental modeling. Simulations occur on a spatially structured lattice of interacting metabolic subsystems (“boxes”), providing at the same time insight on intracellular metabolic fluxes and on ecosystem-level distributions of microbial populations and nutrients. COMETS incorporates two fundamental steps (Figure 1; Experimental Procedures). The first step, cellular growth, is modeled as an increase of biomass at different spatial locations, using a hybrid kinetic-dFBA algorithm. Each box may contain biomass for an arbitrary number of different species. The second step consists of a finite differences approximation of the diffusion of extracellular nutrients and by-products in the environment, and of the expansion of biomass (see Experimental Procedures). Simple diffusion simulations in absence of growth behave as expected (Figure S1, related to Figure 1). We have incorporated multiple species into COMETS by importing the corresponding stoichiometric models, either from manually curated reconstructions, or from automated pipelines that construct models from annotated genomes and high-throughput data, such as Model SEED (Henry