Metabolic interactions shape a community's phenotype

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Spotlight

Metabolic interactions shape a community's phenotype

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Metabolic interactions between auxotrophs and prototrophs in microbial communities are understudied. Yu *et al*. showed how intracellular as well as intercellular metabolism affects community fitness in the absence and presence of abiotic stress, that is, drugs.

Microbes form complex ecological communities. They react to each other's cues and signals, and they can harm and help one another, for instance by competing for resources or by cross-feeding. The metabolic capacities of microbes reflect their evolutionary history and their current ecological circumstances, since microbial metabolism can affect processes within as well as between members of communities (Figure 1A). Prototrophs are microbes that are metabolically flexible; they can switch between uptake and synthesis of metabolites. Auxotrophs are unable to synthesize specific essential metabolites, often due to the lack of, or mutations within, essential pathways or enzymes. Auxotrophs may therefore rely on essential metabolites produced by other microbes for their growth. It is underexplored how frequently such metabolic dependencies occur in microbial ecosystems, and how this affects ecosystem functioning.

Yu *et al.* [1] took advantage of metabarcoding data of over 12 000 microbial communities that were collected for the Earth Microbiome Project [2] to study the presence and the impact of auxotrophs on the survival of microbial communities. Auxotrophs appear to be omnipresent in these natural communities, as nearly all investigated communities contained auxotrophs. Interestingly, more auxotrophs were identified in host-associated communities than in free-living communities (46% vs. 29%). The authors hypothesize that these differences might be driven by the environment in which host-associated microbes thrive, which is nutritionally rich and thus does not select against auxotrophs, which is in line with other findings on 'genomic streamlining' of microbes associated with hosts [3].

To evaluate the effect of auxotrophs in communities, the authors made use of an elegant synthetic yeast community, fittingly called self-establishing metabolically cooperating communities (SeMeCos) [4]. The authors evaluated the degree of metabolic dependency of prototrophs and auxotrophs in communities; the metabolic exchange was introduced by the presence of auxotrophs. Here, using flux-balance analysis, the authors predicted higher than expected steady-state flux distribution changes in the auxotrophs over the prototrophs. Auxotrophs, compared to prototrophs, seem to rewire their metabolism and increase the metabolic fluxes, leading to the export of metabolites in the environment (Figure 1B). These predictions were corroborated by a highly sensitive targeted metabolomics approach; the presence of auxotrophs in communities changed the levels of extracellular metabolites, which also altered the metabolism of prototrophs. Interestingly, more metabolites were exchanged than those forced to be exchanged, indicating the plasticity of the metabolic capacities of the community members upon metabolic changes in the community.

Host-associated microbes can be frequently exposed to drugs, for instance as members of the human gut microbiome during episodes of antibiotic treatment or as members of the plant microbiome during crop disease management. Interestingly, the authors found that auxotrophs grow better compared to prototrophs when challenged by a selected panel of bioactive drugs. Specifically, when the growth of 40 gut bacterial members was challenged by more than 1000 drugs, auxotrophs generally grew better in the presence of those drugs. This finding was supported by the synthetic SeMeCos yeast model experiments, where the growth of auxotrophs was generally improved over prototrophs in a screening with 900 FDA-approved drugs. Of interest is the robustness of auxotrophs against azole treatment, an antifungal agent to which resistance is rapidly emerging [5].

This raises the interesting question why yeast auxotrophs are better able to deal with drugs. Yu *et al.* found an unanticipated cue in the auxotrophs' increased export of metabolites (as discussed in the preceding text, Figure 1B). Intracellular and extracellular metabolites are connected to each other through molecular transporters. These transporters are thought to be involved in the export of drugs since it has been postulated that these pumps can also export drugs and xenobiotics, including azoles and antifungals, which may lead to the decreased concentration of drugs in the cell. This hypothesis was corroborated by Yu *et al.* by the internal cellular measurements of drugs in the experimental yeast system.

The relationship between transporters and metabolite transport remains opaque and is not always that straightforward [6]. It would be interesting to investigate the changes in abundance of an extended set of metabolites, including specialized (secondary) metabolites that can increasingly be characterized using computational metabolomics approaches. Mounting evidence shows that these can also impact

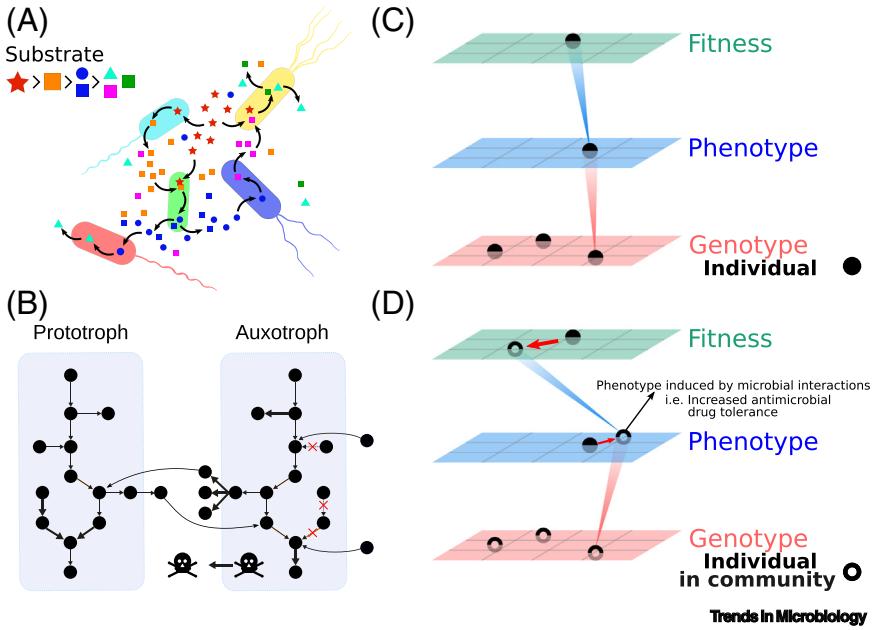

Figure 1. Microbial interactions can alter ecosystem functioning. (A) Illustration of interactions of metabolic interactions between microbes. Metabolic substrate (star) is utilized in stepwise processes, where the by-product of the prototroph (light blue microbe) provides the substrate to the other members of the community. Among the community members there are different degrees of competition, interdependency, and auxotrophy. (B) Cartoon of an auxotroph and a prototroph. Note the auxotroph's increased export of metabolites, and the enriched exometabolites that can benefit the prototrophs. The rewiring of auxotroph metabolism may lead to the excretion of the intracellular toxic compounds (e.g., drugs). (C) The genotype–phenotype–fitness map of a microbe in the absence of an ecological community. (D) The genotype–phenotype–fitness map of a microbe in the presence of a microbial community. Intracommunity interactions alter the phenotype and fitness of the microbe (i.e., increased antimicrobial drug tolerance).

the functioning of multidrug-resistance efflux pumps [7]; furthermore, changes in primary metabolic pathways could also indirectly contribute to changes in specialized metabolic pathways.

Importantly, the community benefits are reciprocal. Auxotrophs benefit from prototrophic resources, but vice versa, the increased levels of metabolic export of auxotrophs also benefits prototrophs. In the presence of auxotrophs the authors observed a positive effect on drug tolerance in prototrophs. The increased efflux of basic building blocks for cell growth by auxotrophs is thought to lead to drug tolerance in prototrophs. This was confirmed by nutrient supplementation, which markedly increased growth in the presence of azoles in wild-type cells. Interspecies community drug-tolerance is also found in other settings [8,9], which emphasizes the importance of intercommunity metabolism in the context of drug tolerance.

Concluding remarks

Yu *et al.* convincingly demonstrate that microbial interactions alter the metabolic capacities of the community members, and consequently the genotype–phenotype–fitness map in communities can be viewed only in an ecological context. Cooperative interactions are generally assumed to destabilize the ecological networks [10]. The fact that auxotrophs, which rely on such cooperative interactions, are overwhelmingly present in natural communities may also point to the importance of environmental selection of such community members. Likely, both cooperation and competition occur in such environments, and the type of interaction may actually depend on specific (abiotic) environmental factors.

Microbial interactions were also shown to alter the sensitivity to drugs. This has not only consequences for the functioning of microbes in the community (Figure 1C,D), but also potentially alters the selective pressures and genotypic trajectories of the evolution of drug resistance. Evolutionary trajectories towards drug resistance may be more or less probable depending on the presence of (particular) community members.

All in all, this work highlights that the contribution of the metabolism to fitness goes beyond its catabolic role, as fitness within a microbial community is determined by the intracellular as well as intercellular metabolism.

Declaration of interests

No interests are declared.

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