MICROBIOLOGY

Bacterial species rarely work together

Competition is prevalent and could be harnessed as an alternative to antibiotics

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Bacteria commonly live in diverse communities where each species can affect the growth and survival of others (*1*, *2*). These species interactions are central to bacterial ecology and have diverse implications for health, agriculture, and industry. To understand and manipulate bacterial communities, it is therefore critical to know how species interact. There is an emerging controversy about the importance of positive interactions, such as cooperation (or mutualism), between bacterial species (*3*–*8*). However, once the standard ecological measures are applied, a clear picture emerges. Negative interactions prevail, and cooperation, where two species both benefit, is typically rare. The prevalence of competition gives hope for bacterial community engineering strategies that seek to eliminate pathogens without the need for antibiotics.

Species affect each other in many ways. Antagonistic interactions such as competition, parasitism, and predation are rife with conflicts. An owl benefits from eating a mouse, whereas the mouse benefits if not eaten. Cooperative interactions, where both parties benefit, are characterized by natural selection on individuals of each species to work together. Plants disperse pollen through the flight of the bumble bee, whereas the bee obtains nectar through the plant's ability to fix atmospheric carbon. Species interactions also define how communities behave as a system, influencing diversity, assembly, stability, and productivity. For example, cooperation (+/+) can destabilize ecological systems by causing dependencies, whereas exploitation (+/–) can be stabilizing because it creates negative feedback between species (*9*). Species interactions are therefore critical to understanding complex communities.

Among interactions, cooperation can seem puzzling (*2*, *10*): Why benefit another over oneself? A vast literature is dedicated to this question, because answering it is central to explaining both complex life and societies (*10*). For bacteria and other microbes, cooperation readily evolves in clonal groups because of kin selection. When cells are genetically identical, natural selection can readily favor one cell investing in others, provided that the total reproduction of the group increases (*2*). However, between species, cooperation is predicted to be much less common because of natural selection for competition and exploitation when different genotypes interact (*1*, *2*, *10*).

In principle, this prediction can be tested using genomic data and computational methods that infer how different bacterial species interact (*2*). However, the gold standard is to culture species alone and together and directly measure their effects on each other. A decade ago, one study did such culturing with environmental bacteria isolated from tree holes (permanent rainwater pools in a beech tree woodland) (*8*). It was concluded that cooperation was uncommon, just as theory predicts. However, it was not clear whether this finding would be robust to different environments and methodologies (*3*). Recent work has provided experimental data on bacterial interactions from a range of environments, including the mammalian gut, nematode gut, phyllosphere (plant leaves and aerial tissue), and soil. How do these new data compare with the tree hole bacteria study? Most studies closely align with the tree hole bacteria data, showing that cooperation is rare (*4*–*7*). However, one concludes instead that positive interactions are common among bacterial species (*3*).

What is the reason for such different conclusions? One study tested a set of 12 bacterial species that colonize the mouse gut [Oligo-Mouse-Microbiota (OMM^{12})] (*4*). Pairwise interactions were quantified in vitro in nutrient-rich media. The data support the prediction that cooperation is rare: Most common was ammensalism (–/0; that is, negative in one direction), followed by neutral (0/0) and competitive (–/–) interactions. Indeed, of the 66 interactions measured, none were cooperative, and just one was commensal (+/0) (see the figure). Another in vitro study of human gut bacteria also found that negative interactions dominate (*7*). There were differences, with many fewer neutral (0/0) and more exploitative (+/–) interactions than in the OMM^{12} study. These shifts in estimates underscore the reality that different studies can yield quite different results, which may be caused by different methodologies as much as real differences between communities. Nevertheless, both of these in vitro studies support the idea that cooperation is rare, with it being absent in the OMM^{12} experiments and accounting for ~2% of interactions in the study of human gut bacteria. This rarity is also supported by in vivo experiments with bacteria associated with nematodes and plants. Pairwise experiments of bacteria from nematode guts revealed that competition was by far the dominant interaction, with cooperation again the rarest at ~3% of interactions (*5*). The plant study was carried out differently: Rather than comparing strains alone and in pairs, single species were removed or added in a diverse community. With this method, only ecological effects in one direction were assessed, but even so, negative interactions again predominated (*6*).

This is where things become less clear. Droplet-based culturing was used to test interactions between 20 species of soil bacteria (*3*). With this method, 190 species interactions across 40 different environments (7600 interactions in total) were surveyed, and >40% of the cases tested contained positive interactions. This result was contrasted against the environmental data from tree hole bacteria (*8*), where the authors of the new study noted that evidence of positive interactions was found in <10% of pairs of bacteria (*3*). With such a shift in the estimates, these data appear to suggest that soil communities are very different to those of tree holes and other environments. However, the soil bacteria study made a subtle but critical change in how "positive interactions" are defined, combining all cases with at least one positive interaction (*3*). This is problematic, because it groups exploitative interactions (+/–), such as parasitism and predation, with cooperation (+/+), which have fundamentally different evolutionary and ecological properties (*2*). Moreover, as a result of the change in definition, the suggestion that the tree hole bacteria study (*8*) had found evidence of positive interactions in <10% of cases becomes inaccurate because that estimate concerned solely cooperation. The importance of this distinction is made clear by looking at the frequency of cooperation proper (+/+) in the soil bacteria data, where it accounts for only 5% of interactions (*3*). This estimate is actually lower than that from the tree hole bacteria study.

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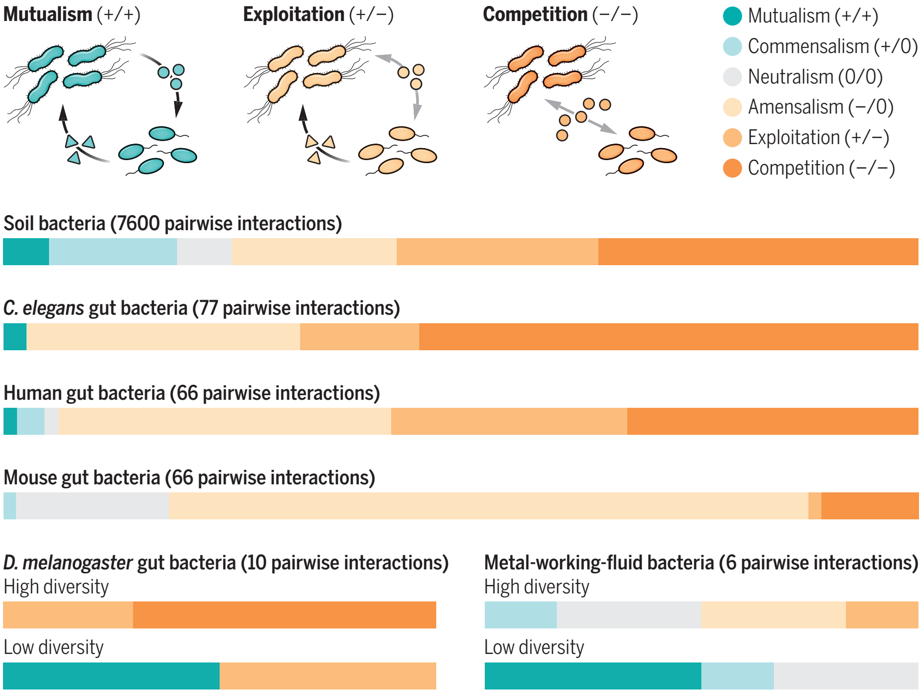
Bacterial interactions in different communities

There are diverse interactions between bacterial species in a community, including mutualism (+/+), exploitation (+/–), and competition (–/–). How bacteria interact is important for community stability and composition. Analyses of the ecological interactions between bacterial species in soil (*3*), *Caenorhabditis elegans* gut (*5*), human gut (*7*), mouse gut (*4*), *Drosophila melanogaster* gut (*13*), and metal-working fluid (*12*) reveal that antagonistic interactions such as competition and exploitation dominate, whereas mutualism (cooperation) is rare. Examples of mutualism are seen in some studies when pairs of species are cultured ("low diversity") (bottom), but these disappear in cultures at more natural levels of diversity ("high diversity").



Whichever definition of positive ecological interactions is used, negative interactions are much more common than positive ones in the soil bacteria study. Such statistics do not mean, of course, that cooperation never evolves between bacterial species. There is evidence that cooperation has evolved between bacterial species of the human gut (*11*), even if it is generally uncommon. Moreover, one study found several cases where pairs of species grew better together than alone in toxic metal-working fluid (*12*). This study is important because it shows that the likelihood of bacterial species working together can greatly increase in certain environments. In this case, low species diversity and the challenges associated with growing in a harsh environment were found to be crucial. When nutrients or additional species were added, the cooperative interactions were lost (*12*) (see the figure). Consistent with the importance of diversity, a study of bacteria in the *Drosophila melanogaster* gut also found several positive interactions when species were cultured in pairs, but these nearly all shifted to negative when species diversity was increased to more natural levels (*13*). Indeed, the original tree hole bacteria study also found that the impacts of negative interactions increased with species diversity (*8*), presumably because the presence of more species increases the competition for limiting nutrients (*12*).

There is a need for further surveys of species and environments to understand the general characteristics of bacterial communities. Nevertheless, the emerging pattern is that cooperation is relatively rare, with negative interactions predominating. Another important pattern in the data is variability in the strength of ecological interactions, with many being relatively weak (*3*, *4*). Like negative interactions, weak interactions are predicted by evolutionary models: Strong competition between species is predicted to drive the extinction of one species or the evolution of niche separation (character displacement) that weakens the competition (*2*). However, these weakly negative interactions also present something of a conundrum. Many bacteria use powerful antibacterial toxins against one another, which create strong negative interactions (*1*). How are these observations reconciled? Antibacterial toxins often target members of the same species, which vie for the same nutrients and locations. Many strongly negative interactions, therefore, take place between different genotypes (strains) of one species, which are not captured by most ecological surveys that focus on different species. These intraspecific interactions deserve more attention and are needed for any complete picture of bacterial community ecology.

The emerging patterns in bacterial ecology have implications for those that seek to manipulate and engineer microbial communities for human benefit. Negative and weak interactions can be desirable from a community engineering standpoint because both can promote stability (*9*). Indeed, a key feature of the human gut microbiome is its relative stability, which allows it to recover from perturbations, such as a course of antibiotics (*9*). Negative interactions can also drive priority effects, where late-arriving species are unable to grow because of the effects of early arriving species (*6*). Competition and exploitation, therefore, can make the addition of new species challenging. This effect is well known from studies of probiotic use, where "beneficial" bacterial species often fail to colonize the gut (*14*). However, such colonization resistance can also be a benefit when incoming bacteria are harmful. With rising levels of drug resistance in pathogenic bacteria, alternatives to antibiotics are urgently needed. After the success of fecal transplants for the treatment of *Clostridium difficile*, there is great interest in finding bacteria that both colonize the human microbiome and compete strongly with pathogens (*15*). If bacterial community ecology can be mastered, the hope is that these competitive species can be introduced as a prophylactic, or even as a treatment, to eliminate pathogens. The way out of the crisis in antibiotic resistance may rest upon both the prevalence and power of bacterial competition. ■

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