Antibiotics and the art of bacterial war

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> "He will win who knows when to fight and when not to fight."
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> – Sun Tzu, The Art of War, *circa* 500 BC (1)

Microbes are remarkably social. They live in complex, interdependent communities where they share and exchange a variety of beneficial compounds ranging from cell–cell signals to iron-scavenging siderophores to digestive enzymes. Cooperation, particularly between cells of a single genotype (2), is fundamental for how cells survive and grow. However, life is not always so amicable. Microbes are regularly confronted by other genotypes armed to the teeth with weapons including secreted toxins, domesticated viruses, and even poisoned spears. Some microbial toxins have been exploited for decades to produce clinical antibiotics. However, several papers have argued that many antibiotics at their low, ecologically relevant concentrations are, in fact, friendly signals that coordinate community functions (3). A study in PNAS by Abrudan et al. (4) challenges this view, focusing on 13 natural bacterial strains from the most famous genus of antibiotic producers, *Streptomyces*. The authors seek to firmly reestablish antibiotic production and regulation in terms of the logic of attack and defense.

Streptomyces strains are well known to inhibit and kill one another, but what determines the strength and impact of these attacks? To address this question, Abrudan et al. (4) studied how antibiotic inhibition from one strain changes when a second strain is nearby. All strains were studied under two conditions: "asocial" and "social" (Fig. 1*B*). The asocial experiments evaluated the baseline tendency of one strain to harm others. This was done by simply growing a focal strain alone on agar for a few days before adding a target strain on top (in soft agar) and asking whether this second strain could grow. The social experiments were different in that the focal strain was grown next to a "modifier" strain from the start, before a third target strain was added, again to look for inhibition by the focal strain. The authors could now ask whether passive interactions became aggressive when another (modifier) strain was nearby, and also whether initially aggressive interactions were affected by the presence of these potential competitors (Fig. 1*B*).

When initially grown alone, a focal strain, on average, inhibited about a third of the other strains. However, what was interesting is that most of the passive interactions could be rendered aggressive by growing the focal strain next to at least one of the other strains. Moreover, the inductions were frequently self-harming, meaning the strain inducing the production of (presumably) antibiotics in another strain could also be harmed by this production. What about the cases where a strain blindly launched an attack when it was alone (Fig. 1*B*, *Bottom*)? Many of these interactions could be rendered noninhibitory by growing the focal strain alongside another strain, likely indicating that this second strain suppressed or degraded the focal strain's antibiotics. The experiments were initially done in high-nutrient media, but the authors repeated their experiments in a low-nutrient, "soil" media and found that the induction of attacks was even more common, whereas suppression was less common. The authors then used their data to parameterize a simulation model of bacterial competition and showed that, in high-nutrient conditions, diversity could be maintained by some bacteria protecting their neighbors from assault, consistent with recent work (5).

The work by Abrudan et al. (4) builds on previous work (6), showing that the competition between coexisting streptomycetes is strongly dependent on neighboring bacteria. Moreover, the data make a lot of sense in terms of bacterial warfare. Not only do originally passive bacteria initiate an attack when faced with a competitor, but strains can also limit incoming attacks from others. A great strength of the Abrudan et al. study is its large scale; every combination of isolates was considered in each of their three roles (focal strain, target strain, and modifier strain), yielding over 2 thousand combinations and a broad ecological overview of the interactions among these strains. However, the flip side is that we cannot be sure of the compounds responsible for the observed interactions. This is where the work is complemented by another important recent study that used spatial mass spectrometry to identify *Streptomyces* compounds that increased when a competing strain was nearby. Antibiotics were indeed seen to be up-regulated (Fig. 1), but so were a diversity of other compounds, particularly siderophores that are involved in mining iron from the surrounding environment (7).

It is clear, then, that *Streptomyces* strongly regulate their secretions based on the presence of other strains, but how do they detect these nonself cells? One way is to sense

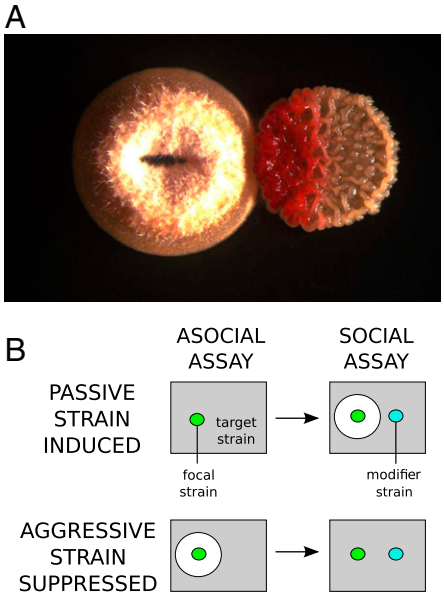

Fig. 1. The activation and suppression of chemical warfare in *Streptomyces*. (*A*) Interactions between colonies of actinomycete bacteria. *Streptomyces coelicolor* (right) responds to an adjacent colony by initiating production of the red-pigmented antibiotics prodiginine and actinorhodin. Image credit to Matthew Traxler; see ref. 7 for more details. (*B*) Two key experimental outcomes seen in Abrudan et al. (4). The authors studied how well a focal strain (green) inhibited an overlain target strain (gray box) when growing in the absence, or presence, of a modifier strain (blue). Strains that were passive when growing alone often became inhibitory when a modifier strain was added (*Top*). By contrast, strains that were aggressive when alone frequently had this aggression suppressed by the presence of a modifier strain (*Bottom*).

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specific compounds released by others; in this way, a bacterium can potentially sense a variety of competitors and even respond differentially to them. Examples include responses to competitor quorum-sensing molecules (8) as well as responses to the cellular components of others, such as cell wall fragments shed by Gram-positive bacteria (9). Another strategy is for a bacterium to use its own physiological state to sense competition directly and thereby respond to other strains. We recently termed such detection of competition-induced harm "competition sensing"—a physiological response that detects harm caused by other cells and that evolved, at least in part, for that purpose (10). The well-known bacterial stress responses allow cells to do just this. We found that bacteria frequently up-regulate their antibiotic or bacteriocin attacks dependent on stress responses that detect either nutrient competition or direct cell damage. *Streptomyces* species have several established examples of this, with antibiotic production known to be up-regulated by starvation (11), envelope damage (12), and, potentially, DNA damage (13). Ruling out a role of other cues and even nonadaptive effects, like the influence of pH shifts on antibiotic efficacy, will be important (14). Nevertheless, the induction of inhibition seen by Abrudan et al. (4) appears to have its roots in the sensing of competition via both nutrient limitation and cell damage.

There are interesting parallels in bacteria that use Type VI secretion systems (T6SS), molecular spears that directly deploy toxins onto or into neighboring cells (15). Amazingly, the pathogen *Pseudomonas aeruginosa* will respond to an incoming T6SS attack in kind (16). Not only can cells detect a Type VI attack from another, but they mount a counterattack by assembling their own poisoned spear at the site of the attack. In another surprising twist, once a cell is lysed by T6SS, the lysate can induce T6SS activity in addition to other antibacterial compounds in neighboring kin (17). This suggests that cells not only sense competition directly but also indirectly via damage done to neighboring cells. Lastly, it has been seen that T6SS attacks induce *soxS*, whose expression is associated with reactive oxygen stress (18). This observation ties in with the more general observation that clinical antibiotics induce reactive oxygen stress (19) and suggests that responses to oxidative stress may be a common currency allowing a cell to detect and respond to diverse damage from competing genotypes. Consistent with this idea, recent work has shown that bacteria respond to ecological competition and cell damage by forming dense groups in the form of surface-attached biofilms (20). Like antibiotic production, biofilm formation is a key candidate competition sensing response. However, rather than being a weapon like antibiotics, biofilms may represent a circling of wagons, which allows strains to both defend and, from a safe position, initiate counterattacks.

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In addition to up-regulation of attacks, there is also the suggestion of evolved defense strategies in the data from Abrudan et al. (4). Strains that made antibiotics when alone were commonly suppressed when grown next to another strain. This is likely due to the inhibition of biosynthetic pathways, general metabolic suppression, or antibiotic degradation. Indeed, antibiotic degradation is a well-known defensive strategy in *Streptomyces* and other species via enzymes like beta-lactamases that destroy the structure and function of competitors' antibiotics (21). However, an intriguing alternative is that a suppressed strain is actually playing an active role in its own toxin reduction. We have all seen spectacular examples of conflict in nature documentaries, like the ferocious fighting of polar bears in the Arctic. However, the reality is that many of these conflicts end quickly, and it is common for an organism to back down rather than face the escalation of deadly conflict. A large body of theory in both economics and biology backs up this underlying intuition that conflict avoidance can be as effective a strategy as attack itself (22). Upon sensing a competitor, therefore, some strains may cooperatively reduce their own antibiotic production in a manner reminiscent of a Cold War détente. Such a possibility may seem, and may well be, far-fetched. However, it underlines how little we understand the evolutionary logic behind bacterial regulatory networks. Ecological competition is a major piece of the puzzle of how and why bacteria respond to their environment, but we are only now scratching the surface.

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