PUBLISHED: 25 NOVEMBER 2016 | ARTICLE NUMBER: 16225 | DOI: 10.1038/NMICROBIOL.2016.225

ANTIMICROBIALS

Constraints on microbial warfare

Microorganisms produce antibiotics, which can exclude competitors, but bacteria typically only synthesize modest amounts of these compounds. New work suggests this may be an evolutionary strategy to balance the benefits of antimicrobial warfare against inadvertently providing help to resistant free-loaders.

Jeremy M. Chacón and William R. Harcombe

Many bacteria are stocked with an impressive arsenal of chemical weapons that can suppress competitors[1]. However, when bacteria are brought into the lab, they typically show surprising restraint, rarely producing significant levels of the antibiotics coded in their genomes[2]. In this issue, Gerardin and colleagues[3] report on the use of laboratory experiments and simulations to investigate the parameters that determine selection for antibiotic production. This work helps to explain microorganisms' restrained use of chemical warfare, and has the potential to inform a new method of antibiotic discovery.

Gerardin *et al.* quantified selection for antibiotic production by identifying the relevant costs and benefits. They employed a system that involves three strains of *Escherichia coli*: one that produces an antibiotic protein called a colicin, one that is sensitive to the colicin, and one that is resistant but does not produce any of the antibiotic. When grown on an agar plate, a colony that releases colicins can suppress sensitive competitors in the vicinity. The authors chemically induced different levels of colicin production and used image analysis to measure the effects on growth when strains are mixed at varying densities. They showed that producing more antibiotic led to a bigger susceptible-free zone around the producing colony, allowing the producers to access more resources and grow to a higher density. However, the benefit of increasing production plateaus once the zone of inhibition exceeds the area from which a colony can effectively access resources. Interestingly, the relevant cost of producing antibiotics arises indirectly through interactions with resistant genotypes. As antibiotic production increases, it becomes more likely that a non-producing, resistant colony will be in a zone free of susceptible competitors. Sharing the benefits of competitor-suppression reduces the relative advantage of antibiotic producers over resistant non-producers. Thus, intermediate levels of production maximize the trade-off between helping oneself while not helping resistant free-loaders in the population (Fig. 1).

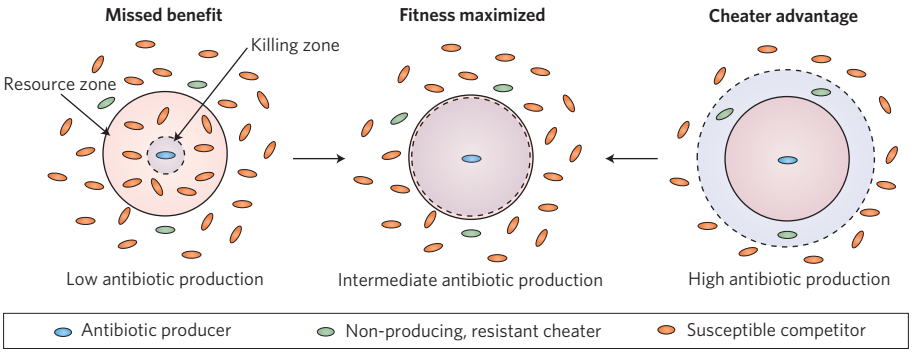

Figure 1 | Trade-offs for antibiotic production. Low levels of antibiotic production allow sensitive strains to compete with producers for local resources in a spatially structured environment (left). In contrast, overproduction of antibiotic provides no additional nutrients for producers, and instead clears out sensitive stains, allowing resistant cheaters to proliferate (right). Selection for moderate antibiotic production may maximize available nutrients for producers while reducing unintended benefits for cheaters (middle).

The careful analysis of costs and benefits helps identify the key parameters for selecting high antibiotic production. Specifically, the analysis demonstrates that selection is driven by the density of both sensitive competitors and producers. Increasing the density of sensitive competitors increases the benefit of producing antibiotics. Conversely, reducing the density of producers decreases the likelihood that resistant cells can benefit from the production of antibiotic by other colonies (Fig. 1, middle panel). This work builds on a rich history of research into the evolution of antibiotic production. The importance of localizing interactions and controlling the density of competitors and producers has been previously recognized[4–7]. However, the current work integrates these parameters to generate a foundation for quantitatively predicting how evolution will shape antibiotic production. The results presented here therefore help to explain why previous attempts to select for antibiotic production in the lab have met with mixed results[8,9]. For example, Le Gac and Doebeli[8] evolved colicin-producing *E. coli* on agar plates in the presence of sensitive competitors and found that the level of antibiotic produced decreased rather than increased. In light of the current results, it is likely that this outcome arose because producers were grown at high density and resistant mutants were not separated from the producers between transfers. The close proximity of mutants to producers thus favoured mutants that evolved to produce less antibiotic. The current work suggests that the optimal approach is to grow separated colonies of producers amidst a dense lawn of susceptible bacteria. The producers should then be diluted and redistributed with each transfer so that colonies arise from single cells and zones of inhibition rarely overlap.

Excitingly, understanding the evolutionary forces shaping antibiotic production may synergistically enhance other methods for discovery of novel antibiotics. First, knowing the factors that select for antibiotic production can inform what environments are mined for bacteria that secrete novel drugs[10]. Additionally,

experimental selection can be combined with synthetic approaches. Synthetic biologists often attempt to engineer high levels of antibiotic production[2]. These engineering approaches could be integrated with bouts of selection under ecological conditions that favour increased production. Following this, natural selection, rather than painstaking researcher screens, could be used to identify the optimally productive colonies across multiple phases of growth. This sort of integrative approach is likely to be particularly useful for coaxing antibiotics from genetically recalcitrant species.

There are still important questions that need to be addressed. For one, while Gerardin *et al.* provide a pathway to select for increased antibiotic production, they have not yet carried out this experimental evolution, and there may be genetic or metabolic constraints that block the evolution of high production even in the face of strong selection. Further, it remains to be seen whether experimental evolution can effectively activate latent antibiotic production, or simply modulate the levels of compounds already produced. Activation of biosynthesis remains a major obstacle in compound discovery. Finally, the authors suggest that the scale over which bacteria compete plays a fundamental role in the evolution of toxin production in natural systems. This highlights the importance of further research into the scale over which microorganisms interact in complex spatially structured environments. ❐

Jeremy M. Chacón and William R. Harcombe are at the Biotechnology Institute and the Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota 55108, USA.
e-mail: harcombe@umn.edu

References

1. Johnston, C. W. *et al. Nat. Chem. Biol.* **12,** 233–239 (2016).
2. Rutledge, P. J. & Challis, G. L. *Nat. Rev. Microbiol.* **13,** 509–523 (2015).
3. Gerardin, Y., Springer, M. & Kishony, R. *Nat. Microbiol.* **1,** 16175 (2016).
4. Chao, L. & Levin, B. R. *Proc. Natl Acad. Sci. USA* **78,** 6324–6328 (1981).
5. Frank, S. A. *Evol. Ecol.* **8,** 369–386 (1994).
6. Kerr, B., Riley, M. A., Feldman, M. W. & Bohannan, B. J. M. *Nature* **418,** 171–174 (2002).
7. Greig, D. & Travisano, M. *Evolution* **62,** 521–527 (2008).
8. Le Gac, M. & Doebeli, M. *Evolution* **64,** 522–533 (2010).
9. Charusanti, P. *et al. PLoS ONE* **7,** e33727 (2012).
10. Smanski, M. J., Schlatter, D. C. & Kinkel, L. L. *J. Ind. Microbiol. Biotechnol.* **43,** 115–128 (2016).