Biofilms

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Eating a way out of antibiotics

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Understanding the physiological effects of antibiotics on bacterial cells is important for informing antibiotic use. Bacterial communities treated with antibiotics in a microfluidic device maintain glucose consumption at the community periphery, protecting interior cells from the effects of antibiotics.

Antibiotics are a cornerstone of modern medicine, but their widespread use and misuse have contributed to a resistance crisis that will cause an estimated 10 million global deaths per year by 2050[1]. Understanding how bacteria respond to antibiotics can inform new treatment strategies that make better use of our dwindling antibiotic arsenal. Many studies have investigated the physiological response of bacterial cells to antibiotics in planktonic, or suspension, culture. However, bacteria in the wild often form structured two- and three-dimensional communities, including biofilms. In this issue of *Nature Chemical Biology*, Zhang et al.[2] leverage microfluidics, fluorescence microscopy, and chemical and proteomic analyses to quantify glucose consumption and bacterial physiology in communities treated with antibiotics. Spatial depletion of glucose and decreased membrane potential at the community interior protected cells from the effects of antibiotics (Fig. 1). These results indicate that bacterial communities display distinct metabolic and physiological attributes that enable them to respond to and mitigate the effects of antibiotics.

Although the primary binding targets of commonly used classes of antibiotics have been characterized, further studies have highlighted the role of metabolism in dictating bacterial susceptibility to antibiotics[3]. For example, metabolically active bacteria are highly susceptible to antibiotics, and metabolically dormant bacteria give rise to persister cells that can withstand antibiotics and facilitate the evolution of resistance[4]. In addition, technologies such as microfluidics and high-resolution microscopy now enable spatiotemporally resolved studies of the physiological responses of single bacterial cells and communities to perturbations. These technologies have led to a nuanced understanding, beyond the engagement of primary binding targets, of how antibiotics induce phenotypic changes that are associated with their action[5].

Further work is needed to study how these discoveries translate to real-world contexts of bacterial infection, but there is an obvious elephant in the room. In many cases, bacterial infections are driven by bacterial communities or biofilms – structured three-dimensional communities with extracellular matrices. The effects of antibiotics on bacterial communities are different from their effects on single cells in suspension, owing to factors such as reduced cellular growth rates, limited availability of metabolites and other molecules, changes in gene expression, and cell-to-cell signaling[6]. Better understanding of the interplay between spatial organization, cellular metabolism and bacterial physiology in the context of antibiotics is a timely goal, with relevant clinical implications.

Zhang et al.[2] used a microfluidic platform to treat largely two-dimensional *Escherichia coli* communities with tetracycline in the presence of glucose. The microfluidic platform builds on a previous approach by this group showing that metabolic co-dependence between the peripheral and interior cells of a biofilm results in

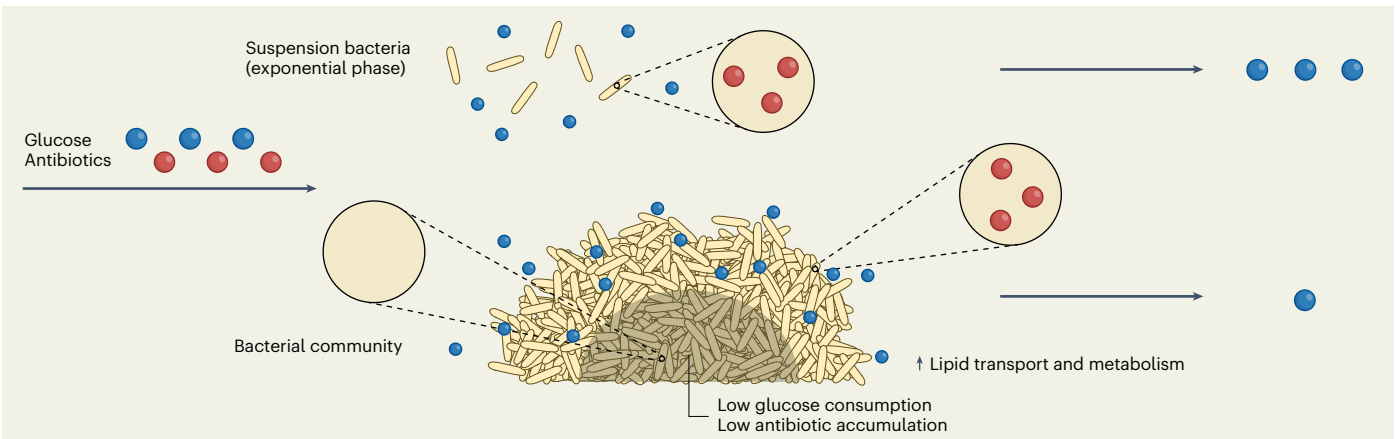

Fig. 1 | Nutrient consumption, cellular physiology and antibiotic accumulation in bacterial communities treated with antibiotics. Semi-two-dimensional bacterial communities grown in a microfluidic chamber and subjected to flow of medium containing glucose and antibiotics exhibit spatial gradients in nutrient consumption, cellular physiology and antibiotic accumulation, unlike their suspension counterparts. Peripheral cells maintain a high rate of glucose consumption and high membrane potentials, enabling these cells to uptake antibiotics and metabolically insulate interior cells, which display low glucose consumption.

oscillations in the biofilm growth rate[7] but has modified design features[8]. Here, the authors[2] cultivated circular biofilms of over 500 µm in diameter and found that the amount of spent glucose did not significantly change after tetracycline treatment, as would be expected for cultured cells in suspension (Fig. 1). Detailed fluorescence microscopy studies using the fluorescent glucose analog 2-NBDG indicated that glucose was depleted at the interior of the community, a finding supported by high-performance liquid chromatography–mass spectrometry (HPLC–MS) measurements of glucose-6-phosphate in interior and peripheral cells. Similar results were observed after treatment with different antibiotics, including kanamycin, ceftazidime, ciprofloxacin, trimethoprim, chloramphenicol and rifampicin. By using the fluorescent sensor ViBac2 to measure cellular membrane potential together with fluorescence microscopy, LC–MS and HPLC experiments to measure the accumulation of kanamycin, ceftazidime and ciprofloxacin, Zhang et al.[2] found that membrane potential was high in peripheral cells but low in interior cells; this pattern tracked the availability of glucose and was similar to the measured patterns of antibiotic accumulation (Fig. 1). Thus, the authors[2] hypothesized that sustained glucose consumption by peripheral cells enables the cells to maintain membrane potential and antibiotic uptake, metabolically insulating interior cells from the effects of antibiotics by depleting glucose in the interior. This working model was further supported by experiments that staggered the flow of medium containing glucose and tetracycline, and experiments involving perturbations in membrane potential.

Bacterial communities consume glucose at similar rates before and after antibiotic treatment. One may ask where the glucose goes after antibiotic treatment, given that bacterial growth is halted. To address this question, Zhang et al.[2] profiled the proteomes of tetracycline-treated peripheral and interior cells, and found that, across both subpopulations, proteins involved in lipid transport and metabolism were upregulated compared with tetracycline-treated suspension cells. Increases in lipopolysaccharide synthesis were further supported by ^{13}C-tracing experiments, and impairing lipid synthesis by knocking out the *fabH* gene (which encodes β-ketoacyl-ACP synthase III, a protein that regulates fatty acid and membrane phospholipid production) resulted in communities that could not maintain glucose consumption. These findings suggest that bacterial communities may produce more membrane material when cells are unable to replicate, a suggestion reminiscent of the generation of L-forms and the blebbing of outer membrane vesicles – both of which represent additional stress responses against antibiotics[9].

Not all antibiotics induce similar bacterial community responses, and not all bacterial communities are well-represented by the model system in this study. Future work is needed to investigate three-dimensional bacterial assemblies, to translate the findings of this study to the development of antibiotic adjuvants that can help treat bacterial infections in the clinic, and to expand on potential implications for antibiotic resistance. Previous studies have demonstrated that modulating bacterial cell metabolism with chemical stimuli can potentiate antibiotic activity and classified antibiotics as weakly or strongly dependent on bacterial metabolism[3,10]. This body of work might help to translate insights pertaining to the interplay between bacterial metabolism, spatial organization and antibiotic action.

Overall, the study by Zhang et al.[2] represents an interdisciplinary approach that applies comprehensive chemical biology methods to discover insights that may be generally relevant to antibiotic action in bacterial communities. These techniques and findings should help to advance our understanding of the multi-faceted effects of antibiotics on spatially structured assemblies of bacteria, broadly informing the next generation of antibiotic treatment strategies to combat bacterial infections.

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References

1. The Review on Antimicrobial Resistance. *Tackling a Crisis for the Health and Wealth of Nations*; https://go.nature.com/4d2fYme (2014).
2. Zhang, Y. et al. *Nat. Chem. Biol.* https://doi.org/10.1038/s41589-024-01708-z (2024).
3. Stokes, J. M. et al. *Cell Metab.* **30**, 251–259 (2019).
4. Levin-Reisman, I. et al. *Science* **355**, 826–830 (2017).
5. Wong, F. et al. *Nat. Commun.* **12**, 2321 (2021).
6. Hall, C. W. & Mah, T.-F. *FEMS Microbiol. Rev.* **41**, 276–301 (2017).
7. Liu, J. et al. *Nature* **523**, 550–554 (2015).
8. Zhang, Y. et al. *Small Sci.* **10**, 2200047 (2022).
9. Schwechheimer, C. & Kuehn, M. J. *Nat. Rev. Microbiol.* **13**, 605–619 (2015).
10. Zheng, E. J. et al. *Nat. Commun.* **13**, 2525 (2022).

Competing interests

The author declares no competing interests.