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We have a community problem

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With the explosion of studies on microbial communities, from the human microbiome to characterizing microbes in the ocean, in soil, and on plants, it is clear that assemblages of microbes are an area of active research interest across the microbial sciences. This focus on communities, on one hand, seems quite natural as most microbes, perhaps outside of implant infections and a few extreme environments, live in a world where they co-exist with myriad other microbes. In contrast, since the development of Koch's postulates in 1890 (a nice summary of Koch's postulates and their history was published recently [1]), focusing on infectious diseases, as well as efforts by Beijerinck and Winogradsky to develop enrichment and isolation techniques, we have often viewed the microbial world through the lens of single species.

The microbial species that have received the lion's share of attention are often referred to as "model systems," maybe better called "model microbes." The best studied model microbe is *Escherichia coli*, as highlighted in a recent article in the Journal of Bacteriology (2). Other model organisms that have received a great deal of attention include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Caulobacter crescentus*, *Candida albicans*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*. More recently, steady progress in the development of molecular genetic techniques has enabled our ability to work with "non-traditional" model organisms such as various archaea, sulfate-reducing bacteria, as well as photosynthetic, electrogenic, intestinal, marine microbes, pathogenic fungi, parasites, and others. Thus, the foundation of the microbial knowledge base on which we can build is becoming both broader and deeper.

This singular focus on model microbes has helped us make stunning advances in understanding host–microbe interactions, fostered the discovery of antimicrobial agents, been the backbone of clinical microbiology, and allowed us to probe the depths of microbial biology. Indeed, a recently launched and ongoing collection of articles at JB titled "History of Microbial Model Systems" celebrates how a variety of microbial model organisms, typically studied in pure culture in the lab, have been key to enhancing our understanding of the microbial world. A theme running through these articles is that the models are studied via a variety of approaches to answer a spectrum of questions. A more holistic appreciation of microbial systems can be gained by tackling questions from different perspectives. Investigators from different disciplines—genetics, molecular biology, structural, biophysics, mathematics, ecology, evolution, etc.—will bring to their research very different viewpoints and tools. I would argue this is a good thing—when all this information is brought together, we cannot help but have a broad and deep appreciation and understanding of a microbe and its biology.

Within the pure-culture research framework of the past ~140 years, our picture can be quite comprehensive for a given microbe, but we are left with an important knowledge gap: how does the microbe relate to the others in its world? Here is where thinking about microbial communities is the critical next step. However, before I talk more about microbial communities, there is an important aside. I contend that before

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we can understand how microbes interact, we need to know (at least a little) about the biology of individual microbes in a community. That is, we must recognize the need for a strong foundation, built by the study of model microbes, for any hope of understanding microbial communities in all their beautiful complexity. Please keep in mind that as you mine your favorite metagenomic data set, most every assigned function (predicted or demonstrated) is derived from work done in a model microbe. Remember that the biology you infer from an amplicon sequence variant (ASV) has likely emerged from the study of a model microbe in pure culture. And every gene that is categorized as one with a "hypothetical function" means that a model microbe has not yet helped reveal the role of that gene product. So, it will never be "The Lab" or "The Real World," any progress in understanding microbial communities must be "The Lab" and "The Real World."

So how do we balance a world (and research framework) of microbial communities with the advantages of studying individual model microbes (Fig. 1)? I believe it is time to consider this critical question. Right now, we are in the wild, wild west. Are we studying microbial communities? Absolutely. Is there a rhyme or a reason for what communities are studied and why? Well, the answer to this question is less apparent.

I am most familiar with the studies of the microbial communities in the airways of persons with cystic fibrosis (CF; and thus, also have likely contributed to the current confusion!). That is, in the context of CF, there have been many investigations of interactions between two microbial species (*P. aeruginosa* and *S. aureus*, *P. aeruginosa* and *Streptococcus* spp., *P. aeruginosa* and fungi, including *C. albicans* and *Aspergillus fumigatus* [3–14]) and multi-microbe interactions (15–21)—I cite just a few such studies here. These communities have been studied on agar plates and in liquid culture, under aerobic and anaerobic conditions, and in lysogeny broth, tissue culture, and artificial sputum medium. Most model communities are supported by a reasonable rationale and have taught us important lessons. Unfortunately, it is sometimes difficult to compare the apple to the orange model system (or perhaps the orchard to the grove?).

I propose that we, as a field, need to consider consolidating around "model communities" that will serve the same function as "model microbes." That is, investigators start with the same microbes, medium, and growth conditions. One can compare those findings to a published system (i.e., a benchmark) to make sure they can indeed replicate

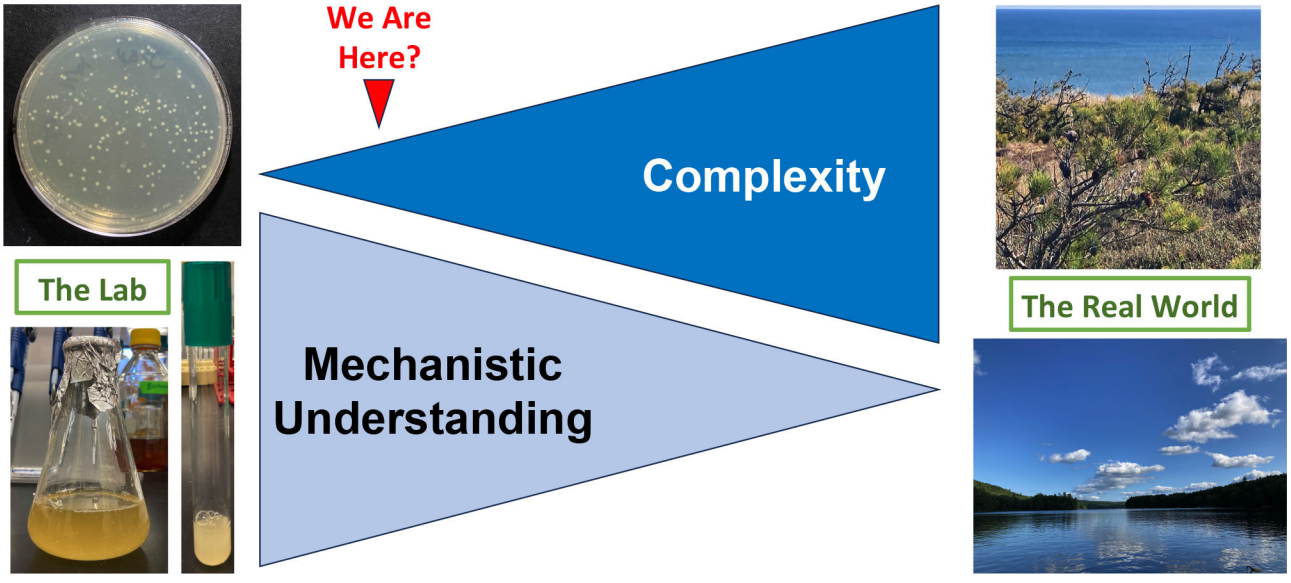

FIG 1 From "The Lab" to "The Real World." The diagram depicts the dynamic between working in single-species lab model organisms versus studying natural environments. Specifically, how does one balance the ability to address detailed (causal) mechanism in less complex lab models ("The Lab") versus the challenge of moving beyond correlative inferences in complex natural environments ("The Real World"). "We Are Here?" indicates that the use of model microbial communities in the lab could be a small step toward approaching the complexity of natural environments while being able to bring to bear the full kit of study tools available in a lab setting.

central findings, and if not, dig into why (the answers could turn out to be very informative!). Once model community systems are developed and carefully described, the same sort of rigorous, cross-disciplinary approaches that are used to study model microbes in isolation can be applied. I predict such an approach will yield analogous major advances in our understanding of microbial communities.

What should a model microbial community look like? I do not believe there is a specific answer to this question—at least I will not venture one. Instead, I will offer some thoughts about features, or perhaps requirements, that one should consider in a widely adopted model community.

1. **Know your community**. As highlighted by my colleague John LiPuma (personal communication), before one can study a community in the lab, one must understand as much as possible about the community in the real world. It is, frankly, a near impossibility to achieve this goal on its face because one would need to have a deep understanding of community structure and function, know who is there, how much of each (absolute and relative abundance), where they are, and what are they doing. Additionally, we would need a complete understanding of the chemical environment, from oxygen availability to pH to the concentration of micronutrients. Unfortunately, a key conundrum is one almost always does not have such information—that is why we are studying the communities! And we must keep in mind that model development will be an iterative process—more information gained from studying the community is used to better refine the model. However, there are some possible strategies to employ. For example, we can start by picking systems for which there are already robust data sets—a well-studied lake (Lake Mendota, for example [22]) or marine environment, a hot spring in Yellowstone (23, 24), a simple community in acid mine drainage (25), a plant microbial community, or infection site. For example, my lab's choice of an airway community in CF has built off literally hundreds of 16S rRNA gene amplicon and metagenomic sequences (26–30). The oral cavity is another excellent example of a community that has been studied deeply—arguably the earliest studied microbiome (31–33). I am concerned where we stand now is a shallow understanding of many, many communities (i.e., via the easy road of DNA sequencing) rather than diving deep into a limited set of communities.
2. **A functional output for the community**. A functional output of any type provides two key advantages. First, in building the model, one can associate community composition/structure to functional outputs to measure how much variance in the function is explained by one (or more) model communities for a given system. So, the model community is linked to a real-world output we care about. Second, once the community is built, these functional outputs can help us understand the role of specific community members (or their gene products) or microbe–microbe interactions in how the community works. I think, at the end of the day, "function" can take many forms. For our recently developed CF airway infection model, we used patient outcomes as the functional output in designing the community (15, 34). This patient outcome function is, however, not particularly useful *in vitro*, where cellular viability and antibiotic tolerance can serve as alternative outputs. In principle, functional outputs could include metabolite production, substrate consumption, spatial structure, impact on the host, enhanced crop yield and/or resilience, and more. It is important to note that for any functional output chosen, we must recognize that because so many variables likely impact measures of community function, such outputs are likely (hopelessly) confounded even with the most rigorous analyses.
3. **Benchmarking the community**. How can we tell whether our lab model most accurately reflects the real world? Using benchmarks, such as the functional outputs mentioned above, could be useful means to assess the relevance of

an *in vitro* model to the real-world environment. Alternatively, recent studies by Whiteley and colleagues have used an "accuracy score" framework (35, 36) with transcriptional data to benchmark CF-relevant models for *P. aeruginosa*. For communities, such an accuracy score framework should be expanded for the multiple microbes and likely move beyond just using transcriptional data.

4. **Balance complexity with feasibility**. One goal of a model is to try to reflect "reality." As illustrated in Figure 1, there will be a balance to strike between complexity and experimental tractability. One approach is to consider how to simplify model systems. For example, if there are three organisms with similar physiologies, it may be possible to include one as a stand-in for all three. The ability to assess community function could help determine if reducing complexity in this way is possible while minimizing the loss of information. The balance between complexity and feasibility will, in part, be influenced by the specific scientific question one seeks to answer.
5. **The community over time**. One feature of a model community would be the ability to incorporate a temporal aspect, which could take several forms. As examples, perturbations caused by exposures to antimicrobial agents (important in the clinical setting), shifts in the chemical environment (i.e., how does community vary with respect to evolving geochemistry or the dynamic aspects of an ecosystem; for example, many Yellowstone hot springs are never the same 2 days in a row), or physical perturbations (perhaps caused by grazing or changes in flow). Overall, such perturbations would allow one to address questions such as community stability, a well-studied question in ecological circles. For example, are there feedback loops that contribute to the community's resilience to perturbation? In many ways, lab microbial model systems are the perfect playground for ecologists, because few other systems can be perturbed reproducibly in so many ways and over such short time spans. A related question could include evolution of the community, both in a stable and in a changing environment.
6. **Reproducibility**. The ability to use constructed communities in a lab setting allows one a degree of reproducibility that is not easily attained in "the wild." That is, the ability to store communities (or their members) in the freezer allows for repeatable studies across time and between labs. Similarly, a frozen stock of a pre-mixed community could be outgrown with some consistency and repeatability.
7. **The model does not need to be perfect**. For me, an ideal model system would include at least one genetically tractable organism, but such a feature is not essential for all investigators and depends on the questions the model is designed to ask. I would say, however, that the more features that are useful to the largest number of investigators would increase the likelihood that a given model is likely to be broadly adopted. As mentioned above, model development will be an iterative process (this was the case for model microbes, too), so one may need to dive in to model building at the outset with incomplete information!

GOING FORWARD

How would microbiologists begin the process of coalescing around a set of model systems? One pathway is to work with funding agencies. For example, the National Science Foundation (NSF) Long-Term Ecological Research Program (LTER) funds work at specific sites, allowing investigators from a variety of disciplines to focus their efforts on one locale, rather than having individual researchers use their particular (narrowly focused) tools to study their specific, unique research site. Similarly, NSF Engineering Research Centers program helped launch, for example, the Center for Biofilm Engineering—this was a strategic investment in growing a field. A call by the NSF, DOE, or NIH for proposals that focus on model community development, with a requirement for trans-disciplinary engagement, coupled with an outreach and/or visiting scientist program to help "spread the word," could be an effective strategy.

A more "bottom-up" approach might leverage a group of scientists already thinking deeply about building microbial model communities. For example, a virtual workshop I hosted at the Dartmouth Cystic Fibrosis Center (DartCF) brought together 35+ investigators working on CF to discuss what are some key aspects of building a model of airway infection relevant to CF (37). Two ideas came out of this workshop. First, "the right model for the right question," that is, we need to think deeply about the kind of questions we would like to answer as we develop model microbial communities. Second, the clear need for more than one model system, because of the broad set of questions we would like to answer about microbial communities found across diverse environments. Maybe a happy middle ground is ~10 communities that we can rally around.

What do we stand to gain by developing a set of broadly adopted microbial community model systems? To perhaps overwork an analogy used above, as a field, we can compare apples to apples, oranges to oranges, and mangos to mangos for individual communities, and if we are lucky, make generalizations that apply to most/all fruit, and if we are really lucky, uncover generalizable "rules of life." As depicted in Figure 1, these model communities may allow us to make the first baby steps on the road to understanding mechanisms that drive the structure/function of natural systems. We still have a long way to go to come close to any model communities approaching natural complexity, but these first steps are analogous to those taken by the pioneers using microbial model organisms to reveal the wonder of the biology of individual microbes.

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The genesis of this idea came from my time as a course faculty and co-director at the Microbial Diversity Course at the Marine Biological Laboratory. Roberto Kolter, in a lecture in 2017, talked about the value of synthetic microbial communities (indeed, his work on synthetic plant root communities inspired my work in CF-relevant models). The ideas lingered, grew, and evolved over the >700 hours of lecture and seminars I sat in at Microbial Diversity over seven summers as part of this course. Aspects of this article also grew out of a lecture with my Co-director Rachel Whitaker summing up a "community week" block at the course. Finally in 2023, Roberto also gave a talk at Microbial Diversity on what can (and can't) we learn from synthetic microbial communities. All of this went in the mix with many discussions with colleagues and the 2021 "Model systems to study the chronic, polymicrobial infections in cystic fibrosis" workshop and accompanying article that grew out of the workshop.

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REFERENCES

1. Ross LN, Woodward JF. 2016. Koch's postulates: an interventionist perspective. Stud Hist Philos Biol Biomed Sci 59:35–46. https://doi.org/10.1016/j.shpsc.2016.06.001
2. Ruiz N, Silhavy TJ. 2022. How *Escherichia coli* became the flagship bacterium of molecular biology. J Bacteriol 204:e0023022. https://doi.org/10.1128/jb.00230-22
3. Orazi G, Jean-Pierre F, O'Toole GA. 2020. *Pseudomonas aeruginosa* PA14 enhances the efficacy of norfloxacin against *Staphylococcus aureus* Newman biofilms. J Bacteriol 202:e00159-20. https://doi.org/10.1128/JB.00159-20
4. Orazi G, O'Toole GA. 2017. *Pseudomonas aeruginosa* alters *Staphylococcus aureus* sensitivity to vancomycin in a biofilm model of cystic fibrosis infection. mBio 8:e00873-17. https://doi.org/10.1128/mBio.00873-17
5. Price CE, Brown DG, Limoli DH, Phelan VV, O'Toole GA. 2020. Exogenous aginate protects *Staphylococcus aureus* from killing by *Pseudomonas aeruginosa*. J Bacteriol 202:e00559-19. https://doi.org/10.1128/JB.00559-19
6. Price KE, Naimie AA, Griffin EF, Bay C, O'Toole GA. 2016. Tobramycin-treated *Pseudomonas aeruginosa* PA14 enhances *Streptococcus constellatus* 7155 biofilm formation in a cystic fibrosis model system. J Bacteriol 198:237–247. https://doi.org/10.1128/JB.00705-15
7. Limoli DH, Warren EA, Yarrington KD, Donegan NP, Cheung AL, O'Toole GA. 2019. Interspecies interactions induce exploratory motility in *Pseudomonas aeruginosa*. Elife 8:e47365. https://doi.org/10.7554/eLife.47365
8. Li K, Gifford AH, Hampton TH, O'Toole GA. 2020. Availability of zinc impacts interactions between *Streptococcus sanguinis* and *Pseudomonas aeruginosa* in coculture. J Bacteriol 202:e00618-19. https://doi.org/10.1128/JB.00618-19
9. Baty JJ, Huffines JT, Stoner SN, Scoffield JA. 2022. A commensal streptococcus dysregulates the *Pseudomonas aeruginosa* nitrosative stress response. Front Cell Infect Microbiol 12:817336. https://doi.org/10.3389/fcimb.2022.817336
10. Baty JJ, Stoner SN, McDaniel MS, Huffines JT, Edmonds SE, Evans NJ, Novak L, Scoffield JA. 2023. An oral commensal attenuates *Pseudomonas aeruginosa*-induced airway inflammation and modulates nitrite flux in respiratory epithelium. Microbiol Spectr 11:e0219823. https://doi.org/10.1128/spectrum.02198-23
11. Scoffield JA, Duan D, Zhu F, Wu H. 2017. A commensal streptococcus hijacks a *Pseudomonas aeruginosa* exopolysaccharide to promote biofilm formation. PLoS Pathog 13:e1006300. https://doi.org/10.1371/journal.ppat.1006300
12. Hogan DA, Kolter R. 2002. *Pseudomonas-Candida* interactions: an ecological role for virulence factors. Science 296:2229–2232. https://doi.org/10.1126/science.1070784
13. Morales DK, Hogan DA. 2010. *Candida albicans* interactions with bacteria in the context of human health and disease. PLoS Pathog 6:e1000886. https://doi.org/10.1371/journal.ppat.1000886
14. Zhao J, Yu W. 2018. Interaction between *Pseudomonas aeruginosa* and *Aspergillus fumigatus* in cystic fibrosis. PeerJ 6:e5931. https://doi.org/10.7717/peerj.5931
15. Jean-Pierre F, Hampton TH, Schultz D, Hogan DA, Groleau M-C, Déziel E, O'Toole GA. 2023. Community composition shapes microbial-specific phenotypes in a cystic fibrosis polymicrobial model system. Elife 12:e81604. https://doi.org/10.7554/eLife.81604
16. O'Brien S, Fothergill JL. 2017. The role of multispecies social interactions in shaping *Pseudomonas aeruginosa* pathogenicity in the cystic fibrosis lung. FEMS Microbiol Lett 364:fnx128. https://doi.org/10.1093/femsle/fnx128
17. Vandeplassche E, Tavernier S, Coenye T, Crabbé A. 2019. Influence of the lung microbiome on antibiotic susceptibility of cystic fibrosis pathogens. Eur Respir Rev 28:190041. https://doi.org/10.1183/16000617.0041-2019
18. Flynn JM, Cameron LC, Wiggen TD, Dunitz JM, Harcombe WR, Hunter RC. 2020. Disruption of cross-feeding inhibits pathogen growth in the sputa of patients with cystic fibrosis. mSphere 5:e00343-20. https://doi.org/10.1128/mSphere.00343-20
19. O'Brien TJ, Hassan MM, Harrison F, Welch M. 2021. An *in vitro* model for the cultivation of polymicrobial biofilms under continuous-flow conditions. F1000Res 10:801. https://doi.org/10.12688/f1000research.55140.1
20. O'Brien TJ, Welch M. 2019. A continuous-flow model for *in vitro* cultivation of mixed microbial populations associated with cystic fibrosis airway infections. Front Microbiol 10:2713. https://doi.org/10.3389/fmicb.2019.02713
21. Hendricks MR, Lashua LP, Fischer DK, Flitter BA, Eichinger KM, Durbin JE, Sarkar SN, Coyne CB, Empey KM, Bomberger JM. 2016. Respiratory syncytial virus infection enhances *Pseudomonas aeruginosa* biofilm growth through dysregulation of nutritional immunity. Proc Natl Acad Sci U S A 113:1642–1647. https://doi.org/10.1073/pnas.1516979113
22. Brock TD. 1985. A Eutrophic Lake. In . Springer-Verlag, New York, NY.
23. Petryshyn VA, Junkins EN, Stamps BW, Bailey JV, Stevenson BS, Spear JR, Corsetti FA. 2021. Builders, tenants, and squatters: the origins of genetic material in modern stromatolites. Geobiology 19:261–277. https://doi.org/10.1111/gbi.12429
24. Spear JR, Walker JJ, McCollom TM, Pace NR. 2005. Hydrogen and bioenergetics in the yellowstone geothermal ecosystem. Proc Natl Acad Sci U S A 102:2555–2560. https://doi.org/10.1073/pnas.0409574102
25. Baker BJ, Banfield JF. 2003. Microbial communities in acid mine drainage. FEMS Microbiol Ecol 44:139–152. https://doi.org/10.1016/S0168-6496(03)00028-X
26. O'Toole GA. 2018. Cystic fibrosis airway microbiome: overturning the old, opening the way for the new. J Bacteriol 200:e00561-17. https://doi.org/10.1128/JB.00561-17
27. Jean-Pierre F, Vyas A, Hampton TH, Henson MA, O'Toole GA. 2021. One versus many: polymicrobial communities and the cystic fibrosis airway. mBio 12:e00006-21. https://doi.org/10.1128/mBio.00006-21
28. Cuthbertson L, Walker AW, Oliver AE, Rogers GB, Rivett DW, Hampton TH, Ashare A, Elborn JS, De Soyza A, Carroll MP, Hoffman LR, Lanyon C, Moskowitz SM, O'Toole GA, Parkhill J, Planet PJ, Teneback CC, Tunney MM, Zuckerman JB, Bruce KD, van der Gast CJ. 2020. Lung function and microbiota diversity in cystic fibrosis. Microbiome 8:45. https://doi.org/10.1186/s40168-020-00810-3
29. Ghosh A, Saha S. 2022. Meta-analysis of sputum microbiome studies identifies airway disease-specific taxonomic and functional signatures. J Med Microbiol 72. https://doi.org/10.1099/jmm.0.001617
30. Pienkowska K, Pust M-M, Gessner M, Gaedcke S, Thavarasa A, Rosenboom I, Morán Losada P, Minso R, Arnold C, Hedtfeld S, Dorda M, Wiehlmann L, Mainz JG, Klockgether J, Tümmler B. 2023. The cystic fibrosis upper and lower airway metagenome. Microbiol Spectr 11:e0363322. https://doi.org/10.1128/spectrum.03633-22
31. Borisy GG, Valm AM. 2021. Spatial scale in analysis of the dental plaque microbiome. Periodontol 2000 86:97–112. https://doi.org/10.1111/prd.12364
32. Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. 2016. Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci U S A 113:E791–800. https://doi.org/10.1073/pnas.1522149113
33. Kolenbrander PE, London J. 1993. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 175:3247–3252. https://doi.org/10.1128/jb.175.11.3247-3252.1993

34. Hampton TH, Thomas D, van der Gast C, O'Toole GA, Stanton BA. 2021. Mild cystic fibrosis lung disease is associated with bacterial community stability. Microbiol Spectr 9:e0002921. https://doi.org/10.1128/spectrum.00029-21
35. Lewin GR, Kapur A, Cornforth DM, Duncan RP, Diggle FL, Moustafa DA, Harrison SA, Skaar EP, Chazin WJ, Goldberg JB, Bomberger JM, Whiteley M. 2023. Application of a quantitative framework to improve the accuracy of a bacterial infection model. Proc Natl Acad Sci U S A 120:e2221542120. https://doi.org/10.1073/pnas.2221542120
36. Lewin GR, Stocke KS, Lamont RJ, Whiteley M. 2022. A quantitative framework reveals traditional laboratory growth is a highly accurate model of human oral infection. Proc Natl Acad Sci U S A 119:e2116637119. https://doi.org/10.1073/pnas.2116637119
37. O'Toole GA, Crabbé A, Kümmerli R, LiPuma JJ, Bomberger JM, Davies JC, Limoli D, Phelan VV, Bliska JB, DePas WH, et al. 2021. Model systems to study the chronic, polymicrobial infections in cystic fibrosis: current approaches and exploring future directions. mBio 12:e0176321. https://doi.org/10.1128/mBio.01763-21