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What will it take to understand the ecology of symbiotic microorganisms?

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Summary

Many microbial taxa associated with healthy animals have both within-host and free-living populations, but little is known about the magnitude, persistence and dispersal of their populations in the external environment. Advances, particularly in our understanding of the population dynamics of free-living populations and microbial cycling between the external environment and hosts, can be achieved by the creative use of current technologies. In particular, there are opportunities to adapt methods, such as capture-mark-release techniques widely used in animal ecology, to the study of symbiotic microorganisms. Future technological advances are, however, required to monitor the location, proliferation and metabolic status at the scale of the single cell, especially under natural conditions. These advances will enable us to achieve precise quantification of host impacts on both micro-habitat suitability for microbial proliferation and microbial dispersal in the external environment. The resultant understanding of the fate of microorganisms shed from animal hosts is essential for the development of environmentally-safe and reliable microbial therapies for humans and animals of economic and environmental importance. Achieving an understanding of the ecology of symbiotic microorganisms is a key challenge for the discipline of environmental microbiology in the coming years.

Keywords

free-living populations; microbial ecology; microbiome; population dynamics; symbiosis

I have been invited to “predict where the field of environmental microbiology will go in the next 20 years”. What a wonderful opportunity to reflect on the current status and future opportunities for our discipline. But recent history suggests that this endeavor may be hazardous. If the last 20 years is a valid predictor of the next 20 years, technological advance will be the chief agent of change; and, disconcertingly, any predictions of the most important future technological advances will most likely be wrong. After all, no “realistic” predictions 20 years ago would have anticipated the advances in sequencing technologies, nanoscale fabrication, microscopical tools and bioinformatics that have led to today’s standard methods to investigate the composition and function of complex microbial communities in natural environments. They would also have failed to foresee our increasing capacity to

monitor and direct microbial evolution, both as a tool in fundamental research and for biotechnological and biomedical applications.

How can we avoid foolish predictions? Probably the best protection is to avoid tightly-defined prescriptions for how the future of our discipline will unfold. Instead, we can ask some simple questions, in the hope that exploring these questions may help us navigate the uncharted territory that lies ahead. Specifically, for today's first-order problems in environmental microbiology:

- To what extent can the problems be solved by creative use of current methodologies?
- What key innovations would speed our progress to solutions?

This article concerns the first-order problem posed by the ecology of symbiotic microorganisms. I will focus specifically on microorganisms that are associated with animals, and that are benign or beneficial to their hosts. I do not include pathogens, even though pathogens are treated as one type of symbiotic microorganism by the original definition of symbiosis as “any association between different species”, and advances in our understanding of beneficial microbes may promote research on pathogens. I will, first, address why the ecology of symbiotic microorganisms is a first-order problem; and then consider how the discipline of environmental microbiology can meet this challenge.

The problem posed by the ecology of symbiotic microorganisms is a consequence of one of the great successes in modern microbiology: microbiome science. The last 20 years have witnessed a transformation in the perceptions of most biologists from the belief that microorganisms associated with healthy animals including humans were trivial and not worthy of study, to the realization that these microorganisms are key players shaping the health, wellbeing and vigor of their hosts (Douglas, 2018). To date, microbiome science has focused principally on the host, to identify the microbial taxa within a host and how the presence and composition of the microbial community influences host traits, including health and fitness (Byrd et al., 2018; Douglas, 2015; McFall-Ngai et al., 2013; Robinson et al., 2010; Schmidt et al., 2018). This offers exciting opportunities for microbial therapy, i.e. to augment or otherwise manipulate the microbiome to promote health and even cure disease (Vazquez-Baeza et al., 2018). For example, microbial therapies are already in clinical practice for patients with intractable *C. difficile* infections (van Nood et al., 2013), they are being applied to reduce methane production by cattle (Yanez-Ruiz et al., 2015), they are under consideration to promote honey bee health (Raymann and Moran, 2018), and they are being proposed for protection of coral reefs (van Oppen et al., 2015). These fast-moving scientific developments create a first-order need - and a first-order opportunity for environmental microbiology. The design of safe and effective microbial therapies will require an understanding of the fate of administered microorganisms. Taking the human microbiome as an example, we know in general terms that microorganisms within the GI tract and on the skin are shed in large numbers back to the external environment, for example in feces, exhaled air and shed skin particles (Browne et al., 2017; Byrd et al., 2018; Kelley and Gilbert, 2013; Meadow et al., 2015). What is the size and location of the free-living populations of these microorganisms? Do shed microbial cells proliferate in the

external environment? For how long can the shed cells (and their descendants) persist? What proportion of these cells cycle back into hosts, and over what timescales? There is, of course, no single set of answers to these questions. Aerobic and aerotolerant taxa are, generally, predicted to sustain larger and more persistent free-living populations than most obligate anaerobes, especially taxa with no capacity to form spores or other resistant stages (Browne et al., 2017; Tetz and Tetz, 2017). But, to a large extent, we lack quantitative data for the population dynamics of the many microbial taxa that inhabit humans and other animals.

As the questions posed in the previous paragraph illustrate, there is a strong scientific imperative to expand the scope of microbiome science from a host-centric perspective to investigate the total biology of the symbiotic microorganisms: both the within-host populations and free-living populations, and the patterns of microbial transfer between the host and the external environment. The key area of ignorance is the free-living populations of symbiotic microorganisms.

Some information on the abundance and distribution of the free-living populations may come from on-going large-scale surveys of the microbiology of natural environments, e.g. soils, oceans, as well as buildings and other habitats dominated by humans (Delgado-Baquerizo et al., 2018; National Academies of Sciences, 2017; Thompson et al., 2017). However, we cannot rely on these data alone because the taxonomic information is relatively coarse, and would fail to discriminate between many symbiotic microorganisms and closely related taxa with an exclusively free-living existence.

I believe that our understanding of the ecology of symbiotic microorganisms will be promoted by focused, experimental approaches. As indicated in previous paragraphs, a central area of current ignorance is the fate of symbiotic microorganisms released from hosts into the external environment. Some aspects of this problem are addressed by applied microbial ecologists, for example in the use of 16S sequence data to track fecal contamination among humans and across landscapes (Griffin et al., 2017; Knights et al., 2011; Shaffer and Lozupone, 2018). Looking ahead, we have the opportunity to address this topic from the perspective of individual cells, rather than DNA sequences, by applying methods widely used by animal ecologists, especially capture-mark-recapture techniques (CMR, also known as “mark-release-recapture”) (Amstrup et al., 2005; Henderson and Southwood, 2016). In CMR, marked individuals from a natural population are released back into the environment and the population is resampled at a later date. Depending on the detailed design of the resampling, CMR can be used to determine survivorship of the released organisms, population size (the proportion of marked individuals in the recaptured sample) and dispersal (the spatiotemporal distribution of marked individuals that are recaptured). CMR of symbiotic microorganisms is different from animal CMR in two linked respects: that the marked microbial cells may proliferate extensively over the timescale of the experiment and that, in many systems, unmarked conspecifics with equivalent symbiotic traits may be difficult to identify reliably. Nevertheless, we can use CMR to investigate the fate of symbiotic microorganisms in the external environment.

Multiple variants of a CMR experimental design for symbiotic microorganisms can readily be envisaged. The marked microorganisms may be administered to the environment in a diversity of ways. For some experiments, it will be important to know precisely how many, where and when the marked microbial cells are released. For other experimental designs, it may be more valuable to use microorganisms shed from colonized host(s), thereby ensuring that the microorganisms of interest are definitively derived from a within-host source. (The hosts would need to be removed directly after the microorganisms are shed, to ensure a point source of release.) CMR experiments can be done under tightly defined laboratory conditions, in semi-natural enclosures or mesocosms, and in the field, with post-release sampling designed to determine both the timescales of changes in abundance (increase or decline) and dispersal from the point of release. There is also the opportunity to investigate how the presence and traits of hosts may influence the size and spatial distribution of the free-living populations of the symbiotic microorganisms, particularly through experiments that include manipulation of hosts in the environs of released microorganisms (presence/absence and abundance of the hosts, different host species and developmental stages etc). I will return to the value of host manipulation experiments later in the article.

CMR experiments for symbiotic microorganisms pose multiple technical issues, particularly relating to how to “mark” and how to “recapture” the symbiotic microorganisms released into the external environment. Many microorganisms can be marked genetically, for example with unique sequence barcodes, genes coding fluorescent proteins or other readily distinguished products (Burns et al., 2017; Goodman et al., 2009; Mark Welch et al., 2016; Wu et al., 2015). These methods have been used with success to monitor the spatiotemporal distribution of various symbiotic microorganisms within hosts, indicating their feasibility for study of free-living populations. For laboratory studies, these methodologies can be applied directly, with sequencing and microscopical methods to quantify the spatiotemporal changes in free-living populations. Field experiments using genetically-engineered microorganisms are necessarily constrained by risks of genetic contamination by the marked microorganisms. Current and improved technologies for suicide constructs to protect against the persistence of marker sequences transferred to other microorganisms and time-dependent suicide constructs to eliminate the marked cells from the environment at the end of the experiment will facilitate field experimentation (Chan et al., 2016; Stirling et al., 2017; Wright et al., 2013). In principle, there are other solutions, with many opportunities for innovation. We can turn to animal ecologists for inspiration, if not necessarily for detailed solutions. The movement of many animals is now monitored in real time using radio-telemetry and PIT (passive integrated transponder) tags (Gibbons and Andrews, 2004; Smyth and Nebel, 2013). Just imagine if we could modify our favorite microorganisms to emit a signal that was proportional to abundance (or activity) and could be detected with the spatial resolution of a single cell. The monitoring could be conducted by a scanner that continuously tracks the laboratory arena or mesocosm, or a drone operating over natural landscapes. Perhaps this system could be multiplexed to communicate to the sensor not only abundance and distribution, but also metabolic status, proximity to other microorganisms and history of cycling between the external environment and hosts of individual cells and their descendants – and, ideally, for multiple microbial taxa, simultaneously.

As mentioned above, experiments that include manipulation of hosts, especially host exclusion, will be very informative to our understanding of the ecology of symbiotic microorganisms. For many systems, the free-living populations of symbiotic microorganisms are apparently restricted to the immediate environs of their hosts (Granados-Cifuentes et al., 2015; Lee and Ruby, 1994; van Elsas et al., 2011). These observations are commonly interpreted as evidence that the symbiotic microorganisms are short-lived in the external environment, raising the possibility that their free-living populations are sink populations maintained by microbial cells shed from hosts, i.e. the persistence of the free-living populations is dependent on cycling between hosts and the external environment with population amplification in hosts (Adair and Douglas, 2017). These dynamics may be typical for many symbiotic microorganisms. There are, however, two alternative interpretations for the co-occurrence of hosts and free-living symbiont populations. For systems where the microbial taxon is present in all members of a host population, perfect host-symbiont co-occurrence can be explained as restrictions on hosts to environments where free-living populations of their microbial symbionts are abundant. We would expect elimination of hosts to have little or no effect on free-living populations of these microbial taxa. Alternatively, animals can modify the external environment, with substantial effects on the microbial communities (Bonaglia et al., 2017; de Menezes et al., 2018; Eldridge et al., 2015; Fall et al., 2007; Laverock et al., 2010), including promotion of free-living populations of symbiotic microorganisms (O'Rorke et al., 2017; Silliman and Newell, 2003; Wong et al., 2015). Such interactions are examples of ecological engineering by animals (Hastings et al., 2007; Jones et al., 1994), and can be identified by the promotion of free-living populations of symbiotic microorganisms under conditions that exclude access to the host.

These various approaches have great potential to advance our understanding of the ecology of symbiotic microorganisms. We also have the opportunity to achieve better integration between short-term ecological experiments and analysis of the genetics/genomic population structure of symbiotic microorganisms (Lapierre et al., 2016; Martiny et al., 2006). Do different types of interactions with hosts have predictable effects on the genetic structure of free-living populations of symbiotic microorganisms? Where microbial dispersal is promoted by carriage in mobile hosts, the host may reduce spatial stratification of the microbial populations; but local adaptation, either to the host or the environs of a host, may increase genetic subdivision of free-living populations. Interactions with hosts may additionally increase genetic diversity, as suggested by evidence that residence in animal guts can promote genetic transfer among microorganisms (Reuter et al., 2007; Smillie et al., 2011). The ecology of some microbial taxa that have large, persistent free-living populations and low incidence of capture by hosts may, however, be largely independent of variation in host ecology – even for microbial taxa that promote host vigor and fitness.

So, what will it take for us to understand the ecology of symbiotic microorganisms? The short answer is smart experimental designs with creative application of methodologies already in place, together with swift uptake of appropriate novel technologies that will assuredly emerge in the coming years. This is all within our reach. I have the strongest sense that the community of environmental microbiology researchers and funding agencies are aware of the potential of this research agenda for enhanced understanding of fundamental

processes and as underpinning research for effective microbial therapies. There are many challenges, but we are up to the task.

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