environmental microbiology reports

Environmental Microbiology Reports (2021) **13**(1), 27–30 doi:10.1111/1758-2229.12895

Crystal Ball

Getting to know our microbial friends by dropping into their neighbourhood

Edward Ruby *

Kewalo Marine Laboratory, Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, HI, 96813.

Imagine an interstellar explorer arriving in orbit around Earth, and deciding to examine the nature of its inhabitants. While much can be discerned just by measuring their general distribution and net activities at a distance, the explorer desires to get closer to the surface to better understand the diversity of organisms present and how their individual actions combine to produce global effects. Ideally, to walk among the inhabitants and discover whether the same kind of organism may have a different behaviour when its surroundings are different provides a deeper understanding of each inhabitants' predictable and, perhaps, unpredictable roles in the environment.

A similar situation is faced by microbial ecologists, who have been, until recently, stuck in their orbiting spacecraft, unable to land and wander among, or perceive interactions within, an environment's microbial community. But what if microbial ecologists could observe their subjects as easily as they can comprehend the citizens of a city during a walk around its crowded streets? How close are microbiologists to realizing the dream of such a walk, and what will they discover once they can? These questions are the motivation behind this essay.

At the end of the 19th century, two great traditions in microbiology were developing. One was pioneered by Robert Koch and Louis Pasteur (Blevins and Bronze, 2010), who devised methods to grow and study bacteria in pure culture, leading to a rapid advance in understanding disease mechanisms and launching the field of biotechnology. The other tradition was inspired by Sergei Winogradsky (Dworkin, 2012), who sought to comprehend the activities of microorganisms by examining their growth and chemistry within complex communities, such as those in soil or sediment, that we would now call environmental microbiomes. While it was assumed that these communities consisted of many species of bacteria and other microbes, only a fraction of these microorganisms could be grown and studied individually in culture. Even those that were cultured often failed to exhibit their key ecological activities when isolated from the whole microbiome. This latter observation drove microbiologists to find ways to examine microbial communities in their intact, native state. Such efforts initially were limited to measuring the net activity and products of the microbiome, and it was clear that any deeper understanding would require a knowledge of not only the membership of the community, but also each member's individual metabolic and ecological contributions as both a function of time (e.g. day/night, summer/winter) and its proximity to others (Cordero and Polz, 2014).

The development of ribosomal DNA (e.g. 16S) amplicon sequencing in the 1990s provided a means by which to catalogue the surprising number and abundance of both culturable and non-cultured species that are present in many natural environments. Similarly, during the last decade, metagenome-assembled genome (MAG) sequencing, coupled with annotation of the sample's total gene pool, provided a roadmap of the functional potential of the community as a whole, and even of its individual species. One resulting discovery was the surprising reproducibility of the species membership within microbial communities found in similar environments. While there were certainly 'tourists' that intermittently passed through the habitat, generally there was also an environmentally determined 'resident' microbiota that was predictably present.

In the case of the mammalian digestive tract, the literature became replete with evidence that the members in the resident gut microbiota were not only predictable, but their presence was also demonstrably beneficial to the host's normal development and health (Rook *et al.*, 2017). In spite of this increasing evidence, many studies continue to refer to these important microbial partners as 'commensal', a carry-over from an earlier time when the gut microbiota was believed to be of little consequence to

Received 7 October, 2020; accepted 8 October, 2020. *For correspondence, E-mail eruby@hawaii.edu; Tel. 808-539-7309; Fax 808-599-4817.

the host. The term commensal, whose meaning specifically denotes a microbe whose presence has no effect on its host (Veiga, 2016), continues to be misused, creating unnecessary confusion, especially when used to refer to a species whose beneficial nature is being demonstrated. In the future, editors and reviewers will need to be more vigilant in discouraging this inaccuracy because there are likely to be species, especially among the microbial tourists, whose presence has only a negligible effect on the host: reserving the term 'commensal' for the impact of these microorganisms, and refraining from applying it to clearly beneficial ones, will promote ecological clarity.

A second insight driven by amplicon and MAG sequencing has been that two strains of the same species might have significantly different genome content and, thus, may have distinct ecological roles. This realization established the importance of understanding microbial diversity at the population level and encouraged efforts to discover the functional distinctions differentiating strains (Bongrand and Ruby, 2019; Zeevi *et al*., 2019). Strain-specific functionality has now been documented in a number of natural environments, from the marine bacterioplankton to terrestrial hot springs (e.g. Preheim *et al*., 2011; Olm *et al*., 2020). Similarly, strain specificity has proved to be a particularly informative concept when examining symbiotic associations between bacteria and their hosts (Mandel *et al*., 2009; Murfin *et al*., 2015; Bongrand *et al*., 2016). For example, the genetic specialization of closely related strains of *Escherichia coli* to pathogenic, mutualistic or environmental lifestyles is a well-known example of the impact of strain-level differences on biological outcome.

While much was learned by developing and applying various molecular analyses to bulk samples like seawater and soil, to actually visualize whether two bacterial cells are close enough to each other to interact metabolically required the development of fluorescence-microscopy staining approaches that target a cell's species-specific DNA sequence (i.e. fluorescence *in situ* hybridization; FISH). By imaging natural samples under the microscope, one could link a cell's identity with its location (e.g. Schimak *et al*., 2016) and even possible function (e.g. Tropini *et al*., 2017) within the community. The targeting of 16S rRNA by FISH at present precludes this approach from easily differentiating between strains; however, the growing interest in visualizing specific strains in their natural environments is likely to drive further refinement of this technique in the coming years.

More recently, the development of sensitive mRNA-specific fluorescent probes (i.e. hybridization chain reaction-FISH) has allowed the layering of species (and even strain)-specific gene-expression patterns onto FISH images (Choi *et al*., 2016; Grieb *et al*., 2020). These studies have begun to allow a cell-by-cell analysis of gene-expression levels among a clonal population of bacteria, revealing how individual a cell's response can be, even to a seemingly identical environment. Such observations have already begun to have a significant effect on how microbial evolution and population microbiology is modelled.

These and other imaging approaches are just now being applied by microbial ecologists and physiologists to investigate bacterial and archeal interactions in both abiotic and host-specific associations (Rosenthal *et al*., 2013; Nikolakakis *et al*., 2015). In the near future, we can expect that such imaging will reveal the importance of strain-specific microhabitats at different locations, either within an environmental sample or along a tissue's surface (McGlynn *et al*., 2018; Donaldson *et al*., 2020; Essock-Burns *et al*., 2020), or at the same location during different times in a host's life history (Thaiss *et al*., 2016; Taft *et al*., 2018). Determining the role of host factors in driving differences in species- and strain-level responses at a tissue's surface has become an area of increasing interest and has given rise to the term microbial 'biogeography' (Donaldson *et al*., 2016).

It is clear that microbial ecologists and population microbiologists now find themselves at an exciting point in their quest to roam within the microbial world and to understand it at the micron and femtolitre scales that are relevant to its inhabitants. So, what does the future hold? What molecular, technological and computational advances should we both promote the development of, and prepare to apply? The advances that need to be developed and/or combined include (i) those that are currently in use, but whose resolution is being increased, and (ii) those that are not yet available, but are on the horizon. A number of these emerging technologies have been well described in recent reviews (e.g. Grandin *et al*., 2018; Hatzenpichler *et al*., 2020; Kaster and Sobol, 2020). In addition, we can expect that model systems will be used to develop and test such technologies in a controlled manner before they can be deployed to complex environmental samples. These experimentally approachable models will include both constructed (e.g. microfluidic devices, gut-on-a-chip) and natural (e.g. binary symbioses) examples (Lambert *et al*., 2017; Bosch *et al*., 2019; Ashammakhi *et al*., 2020) and will become increasingly important as they are enlisted to address questions like the following:

i. *What is the distribution of different strains within an environment, and how do their metabolic and ecological potentials differ?* Traditionally, ecological theory predicts that diversity within populations is likely to be a destabilizing factor, leading to competition between closely related strains. When the strains share a symbiotic niche, even as mutualists, their competition is

expected to negatively affect the host (Frank, 1996). However, as analyses of natural gut-tract symbioses are revealing, diverse strains not only coexist stably, but appear to be adaptive for all partners (Ellegaard *et al*., 2020); future work will need to apply strain-specific imaging technology to determine whether different strains are separated into distinct microhabitats along a tissue and/or are functionally specialized, thereby minimizing the opportunity for disruptive competition (Ansorge *et al*., 2019).

ii. *Are two bacterial cells exchanging metabolites, and in what directions?* This goal will be complicated not only by the micron-level scale of the measurements, but also by an inconvenient truth of flux measurements: in steady state, desirable metabolites are usually taken up by one microbe as quickly as they are produced by another; in addition, the more rapidly a metabolite is exchanged and catabolized, the more difficult it is to follow by its chemical identity (e.g. by mass spectrometry). A major advantage of nanoscale secondary ion mass spectrometry (nanoSIMS) is that it allows the tracking of a molecule's exchange between cells (Dekas *et al*., 2019), even when it is quickly modified by the receiving cell's metabolism; however, except in a few special circumstances, arranging for a cell to have only one of its exchanged products be stable-isotope labelled has been problematic. Nevertheless, recent developments are working around this limitation by directly coupling nanoSIMS with other substrate-specific fluorescence or electron-microscopy visualization assays like immunocytochemistry (Loussert-Fonta *et al*., 2020).

iii. *When and where is a strain expressing specific genes and proteins? What is the role of host cell activities in strain-specific selection along a tissue (DePas et al*., *2016; Solis et al*., *2020)?* Once we can measure metabolomic and gene-expression parameters at the single bacterial-cell level, significant phenotypic heterogeneity can be expected to emerge, even within clonal populations (Salek *et al*., 2019). The resulting distributions of phenotype levels will be due not only to stochastic forces, but also to biological factors like adaptive bet-hedging (Carey *et al*., 2018); as a consequence, we can anticipate the need for significant advances in computational and modelling tools (e.g. Nikolic *et al*., 2017; He *et al*., 2019; Labarthe *et al*., 2019) to allow a more robust differentiation of signal from the noise in all of the newly developed population and imaging analyses.

At present, the answers to these and other challenges faced by explorers of the microbial world may feel a long way off. However, looking back at the speed with which currently commonplace technologies like DNA sequencing, gene-expression analysis and quantitative fluorescence imaging have emerged and improved over the past 10 years, it is not unlikely that the approaches and analyses envisioned here will become available in the coming decade. Now is the time for us to prepare our spacecraft and ourselves for landing.

References

Ansorge, R., Romano, S., Sayavedra, L., Porras, M.A.G., Kupczok, A., Tegetmeyer, H.E., *et al*. (2019) Functional diversity enables multiple symbiont strains to coexist in deep-sea mussels. *Nat Microbiol* **4**: 2487–2497.

Ashammakhi, N., Nasiri, R., Barros, N.R., Tebon, P., Thakor, J., Goudie, M., *et al*. (2020) Gut-on-a-chip: current progress and future opportunities. *Biomaterials* **255**: 120196.

Blevins, S.M., and Bronze, M.S. (2010) Robert Koch and the 'golden age' of bacteriology. *Int J Infect Dis* **14**: e744–e751.

Bongrand, C., Koch, E.J., Moriano-Gutierrez, S., Cordero, O. X., McFall-Ngai, M., Polz, M.F., and Ruby, E.G. (2016) A genomic comparison of 13 symbiotic *Vibrio fischeri* isolates from the perspective of their host source and colonization behavior. *ISME J* **10**: 2907–2917.

Bongrand, C., and Ruby, E.G. (2019) The impact of *Vibrio fischeri* strain variation on host colonization. *Curr Opin Microbiol* **50**: 15–19.

Bosch, T.C.G., Guillemin, K., and McFall-Ngai, M. (2019) Evolutionary "experiments" in symbiosis: the study of model animals provides insights into the mechanisms underlying the diversity of host-microbe interactions. *Bioessays* **41**: e1800256.

Carey, J.N., Mettert, E.L., Roggiani, M., Myers, K.S., Kiley, P.J., and Goulian, M. (2018) Regulated stochasticity in a bacterial signaling network permits tolerance to a rapid environmental change. *Cell* **173**: 196–207.

Choi, H.M., Calvert, C.R., Husain, N., Huss, D., Barsi, J.C., Deverman, B.E., *et al*. (2016) Mapping a multiplexed zoo of mRNA expression. *Development* **143**: 3632–3637.

Cordero, O.X., and Polz, M.F. (2014) Explaining microbial genomic diversity in light of evolutionary ecology. *Nat Rev Microbiol* **12**: 263–273.

Dekas, A.E., Parada, A.E., Mayali, X., Fuhrman, J.A., Wollard, J., Weber, P.K., and Pett-Ridge, J. (2019) Characterizing chemoautotrophy and heterotrophy in marine Archaea and bacteria with single-cell multi-isotope NanoSIP. *Front Microbiol* **10**: 2682. https://www.frontiersin.org/articles/10.3389/fmicb.2019.02682/full.

DePas, W.H., Starwalt-Lee, R., Van Sambeek, L., Ravindra Kumar, S., Gradinaru, V., and Newman, D.K. (2016) Exposing the three-dimensional biogeography and metabolic states of pathogens in cystic fibrosis sputum via hydrogel embedding, clearing, and rRNA labeling. *mBio* **7**: e00796–e00716.

Donaldson, G.P., Chou, W.C., Manson, A.L., Rogov, P., Abeel, T., Bochicchio, J., *et al*. (2020) Spatially distinct physiology of *Bacteroides fragilis* within the proximal colon of gnotobiotic mice. *Nat Microbiol* **5**: 746–756.

Donaldson, G.P., Lee, S.M., and Mazmanian, S.K. (2016) Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* **14**: 20–32.

Dworkin, M. (2012) Sergei Winogradsky: a founder of modern microbiology and the first microbial ecologist. *FEMS Microbiol Rev* **36**: 364–379.

Ellegaard, K.M., Suenami, S., Miyazaki, R., and Engel, P. (2020) Vast differences in strain-level diversity in the gut microbiota of two closely related honey bee species. *Curr Biol* **30**: 2520–2531.

Essock-Burns, T., Bongrand, C., Goldman, W.E., Ruby, E.G., and McFall-Ngai, M.J. (2020) Interactions of symbiotic partners drive the development of a complex biogeography in the squid-vibrio symbiosis. *mBio* **11**: e00853–e00820.

Frank, S.A. (1996) Host-symbiont conflict over the mixing of symbiotic lineages. *Proc Biol Sci* **263**: 339–344.

Grandin, H.M., Guillaume-Gentil, O., Zambelli, T., Mayer, M., Houghtaling, J., Palivan, C.G., *et al*. (2018) Bioinspired, nanoscale approaches in contemporary bioanalytics. *Biointerphases* **13**: 040801.

Grieb, A., Bowers, R.M., Oggerin, M., Goudeau, D., Lee, J., Malmstrom, R.R., *et al*. (2020) A pipeline for targeted metagenomics of environmental bacteria. *Microbiome* **8**: 21.

Hatzenpichler, R., Krukenberg, V., Spietz, R.L., and Jay, Z.J. (2020) Next-generation physiology approaches to study microbiome function at single cell level. *Nat Rev Microbiol* **18**: 241–256.

He, X., Chadwick, G., Kempes, C., Shi, Y., McGlynn, S., Orphan, V., and Meile, C. (2019) Microbial interactions in the anaerobic oxidation of methane: model simulations constrained by process rates and activity patterns. *Environ Microbiol* **21**: 631–647.

Kaster, A.K., and Sobol, M.S. (2020) Microbial single-cell omics: the crux of the matter. *Appl Microbiol Biotechnol* **104**: 8209–8220.

Labarthe, S., Polizzi, B., Phan, T., Goudon, T., Ribot, M., and Laroche, B. (2019) A mathematical model to investigate the key drivers of the biogeography of the colon microbiota. *J Theor Biol* **462**: 552–581.

Lambert, B.S., Raina, J.B., Fernandez, V.I., Rinke, C., Siboni, N., Rubino, F., *et al*. (2017) A microfluidics-based *in situ* chemotaxis assay to study the behaviour of aquatic microbial communities. *Nat Microbiol* **2**: 1344–1349.

Loussert-Fonta, C., Toullec, G., Paraecattil, A.A., Jeangros, Q., Krueger, T., Escrig, S., and Meibom, A. (2020) Correlation of fluorescence microscopy, electron microscopy, and nanoSIMS stable isotope imaging on a single tissue section. *Commun Biol* **3**: 362.

Mandel, M.J., Wollenberg, M.S., Stabb, E.V., Visick, K.L., and Ruby, E.G. (2009) A single regulatory gene is sufficient to alter bacterial host range. *Nature* **458**: 215–218.

McGlynn, S.E., Chadwick, G.L., O'Neill, A., Mackey, M., Thor, A., Deerinck, T.J., *et al*. (2018) Subgroup characteristics of marine methane-oxidizing ANME-2 archaea and their syntrophic partners as revealed by integrated multimodal analytical microscopy. *Appl Environ Microbiol* **84**: e00399-18.

Murfin, K.E., Lee, M.M., Klassen, J.L., McDonald, B.R., Larget, B., Forst, S., *et al*. (2015) *Xenorhabdus bovienii* strain diversity impacts coevolution and symbiotic maintenance with *Steinernema* spp. nematode hosts. *mBio* **6**: e00076.

Nikolakakis, K., Lehnert, E., McFall-Ngai, M.J., and Ruby, E. G. (2015) Use of hybridization chain reaction-fluorescent *in situ* hybridization to track gene expression by both partners during initiation of symbiosis. *Appl Environ Microbiol* **81**: 4728–4735.

Nikolic, N., Schreiber, F., Dal Co, A., Kiviet, D.J., Bergmiller, T., Littmann, S., *et al*. (2017) Cell-to-cell variation and specialization in sugar metabolism in clonal bacterial populations. *PLoS Genet* **13**: e1007122.

Olm, M.R., Crits-Christoph, A., Diamond, S., Lavy, A., Matheus Carnevali, P.B., and Banfield, J.F. (2020) Consistent metagenome-derived metrics verify and delineate bacterial species boundaries. *mSystems* **5**: e00731-19.

Preheim, S.P., Timberlake, S., and Polz, M.F. (2011) Merging taxonomy with ecological population prediction in a case study of *Vibrionaceae*. *Appl Environ Microbiol* **77**: 7195–7206.

Rook, G., Backhed, F., Levin, B.R., McFall-Ngai, M.J., and McLean, A.R. (2017) Evolution, human-microbe interactions, and life history plasticity. *Lancet* **390**: 521–530.

Rosenthal, A.Z., Zhang, X., Lucey, K.S., Ottesen, E.A., Trivedi, V., Choi, H.M., *et al*. (2013) Localizing transcripts to single cells suggests an important role of uncultured deltaproteobacteria in the termite gut hydrogen economy. *Proc Natl Acad Sci U S A* **110**: 16163–16168.

Salek, M.M., Carrara, F., Fernandez, V., Guasto, J.S., and Stocker, R. (2019) Bacterial chemotaxis in a microfluidic T-maze reveals strong phenotypic heterogeneity in chemotactic sensitivity. *Nat Commun* **10**: 1877.

Schimak, M.P., Kleiner, M., Wetzel, S., Liebeke, M., Dubilier, N., and Fuchs, B.M. (2016) MiL-FISH: multilabeled oligonucleotides for fluorescence *in situ* hybridization improve visualization of bacterial cells. *Appl Environ Microbiol* **82**: 62–70.

Solis, A.G., Klapholz, M., Zhao, J., and Levy, M. (2020) The bidirectional nature of microbiome- epithelial cell interactions. *Curr Opin Microbiol* **56**: 45–51.

Taft, D.H., Liu, J., Maldonado-Gomez, M.X., Akre, S., Huda, M.N., Ahmad, S.M., *et al*. (2018) Bifidobacterial dominance of the gut in early life and acquisition of antimicrobial resistance. *mSphere* **3**: e00441-18.

Thaiss, C.A., Levy, M., Korem, T., Dohnalova, L., Shapiro, H., Jaitin, D.A., *et al*. (2016) Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **167**: 1495–1510.

Tropini, C., Earle, K.A., Huang, K.C., and Sonnenburg, J.L. (2017) The gut microbiome: connecting spatial organization to function. *Cell Host Microbe* **21**: 433–442.

Veiga, J.P. (2016) Commensalism, amensalism and, synnecrosis. In *Encyclopedia of Evolutionary Biology*. Kliman, R.M. (ed). Amsterdam, Netherlands: Academic Press, pp. 322–328.

Zeevi, D., Korem, T., Godneva, A., Bar, N., Kurilshikov, A., Lotan-Pompan, M., *et al*. (2019) Structural variation in the gut microbiome associates with host health. *Nature* **568**: 43–48.