environmental microbiology reports

Environmental Microbiology Reports (2015) **7**(1), 26–28 doi:10.1111/1758-2229.12248

Microbes don't play solitaire: how cooperation trumps isolation in the microbial world

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Primitive cells did not carry a stable organismal genealogical trace. Primitive cellular evolution is basically communal. The high level of novelty required to evolve cell designs is a product of communal invention, of the universal HGT field, not intralineage variation. It is the community as a whole, the ecosystem, which evolves.

– Woese (2002)

Symbiosis, defined by DeBary as 'the living together of unlike organisms', can increase the collective problem solving power of biological systems. The best-known and most charismatic examples of symbiosis usually involve microorganisms and eukaryotic cells that exchange nutritional, energetic or protective services. In these cases, the members of the symbiosis are clearly unlike each other; the host is a eukaryote, the symbiont a bacterium. But it is becoming increasingly clear that microbes form metabolically interdependent associations at the population and community levels. These interdependencies have the potential to direct the evolution of microbial lineages and likely play integral roles in shaping community assembly and ecosystem function. Here, we shine a spotlight on converging lines of evidence consistent with this view and consider future innovation at the interface of microbial ecology and synthetic biology that builds on cooperative design.

Lessons from symbiosis

Microbial communities are interesting in part because they are dynamic and complex in space, time and organization. This complexity makes knowing which organisms are interacting challenging, unless the community is simple enough that the interactions can be somehow observed experimentally or inferred from genomics. It is instructive therefore to look at patterns that emerge from simple microbial communities. In terms of species diversity and clarity of metabolic interactions, the least complex microbial communities are probably those found living inside eukaryotic cells such as the nutritional symbiotic communities found in sap-feeding insects (McCutcheon and Moran, 2010). In these cases, the associations consist of only a few bacterial species, are stable over time, involve no known bacteriophages and the metabolic interdependencies are unambiguous. Thus, these endosymbiotic communities can provide a window into the evolution of wild microbial communities, albeit a window that is clouded by the idiosyncrasies of intracellular life.

What organizing principles emerge from the study of endosymbiotic microbial communities? The most relevant lesson for our argument here relates to the rate at which bacterial genomes can lose genetic information. Genome reduction can occur extremely rapidly after the establishment of symbiosis (Clayton *et al.*, 2012), which gives rise to obligate forms of metabolic interdependence. While it is tempting to think that transplantation from nutritionally depauperate surroundings to a more replete intracellular milieu is the primary driver for gene loss, very similar patterns of genome reduction have been observed in *Salmonella* grown with severe population bottlenecks in the lab (Nilsson *et al.*, 2005). It is in part the population genetic environment, not only the intracellular environment, that dictates the rapidity of gene loss. Thus, while forces such as horizontal gene transfer seem to drive adaptive changes in microorganisms through gene acquisition (Ochman *et al.*, 2000), genome reduction can drive cooperation and interdependencies in communities through gene loss.

How might fluctuations in population structure shape the evolution of wild microbial communities? Microbes in the sea or in soil are typically thought to have very large effective population sizes. This makes sense because microbes are small and numerous in these environments, and these environments are expansive. However, population sizes can vary widely in temporally and spatially variable environments. In fact, metabolic interactions themselves are likely to influence the effective population size of the participating organisms – if two microbes

form a temporarily beneficial symbiosis leading to the formation of a microenvironment, this can immediately and dramatically reduce their effective population sizes. If the interaction persists for several generations, and if genome reduction is rapid, then gene loss that is masked or compensated by the transient symbiosis can 'lock-in' the relationship (Ellers *et al*., 2012; Morris *et al*., 2012). We argue that these sorts of compensatory gene loss events, whether driven by chance (Moran, 2002) or selection (Morris *et al*., 2012), occur with such frequency and rapidity to make the concept of a free-living microbe obsolete.

Lessons from plurality and single-cell genomics

Wild microbial genomes assembled using plurality and single-cell genomic methods tend to reinforce a metabolic blueprint tuned for interdependence. Indeed, evidence for widespread genomic streamlining in marine microbes is consistent with a general process of reductive evolution resulting in nutrient and energy dependencies that mandate a cooperative mode of existence.

But what about the subset of microbial strains that can be isolated and grown as clones in laboratory settings? The short answer is that just because a microorganism can be grown alone doesn't mean it lives alone or could survive alone in the wild. Even *Escherichia coli*, the ultimate model bacterium requires precursor molecules from several pathways [such as vitamin B_{12} (Lawrence and Roth, 1995)] that must be sourced externally.

And what of the remaining invisible and uncultured majority? Approximately half of the 60 major branch points in Bacterial and Archaeal domains of life are represented by candidate divisions with no known cultivated representatives, the so-called microbial dark matter (Rinke *et al*., 2013). Plurality and single-cell genomic sequencing and process-oriented studies suggest that bacteria affiliated with numerous candidate divisions participate in cooperative or syntrophic growth modes. For example, a single-cell genomic study of fermentative OP9 bacteria from hot spring sediments suggests that these microbes are dependent on exogenous vitamins sourced from surrounding community members (Dodsworth *et al*., 2013). Such public good dynamics appear to be a recurring organizing principle in structuring microbial community interaction networks (Morris *et al*., 2012) and could help explain why most environmental microorganisms, including candidate divisions, resist clonal isolation.

Future developments: from genomes to biofactories

Of course, readers of *Environmental Microbiology Reports* can appreciate the fundamental advances in molecular genetics, physiology and biochemistry gleaned from the application of toothpicks and logic on clonal isolates. But we argue here that our modern understanding of metabolism has become too insulated from real world microbial interactions. Over the past 25 years, we have come to recognize that microorganisms represent the invisible majority of living things on Earth. This uncultured majority represents a virtually limitless reservoir of genetic information and metabolic innovation that has evolved through 3.5 billion years of *interaction and cooperation*.

The concept of community metabolism has recently emerged as a topic of interest in synthetic biology. At its core, synthetic biology is built upon 'BioBricks,' or standardized parts composed of DNA that conform to an assembly standard. Synthetic biologists engineer biological systems in cellular 'chasses', including *E. coli*, by combining BioBricks into integrated devices (e.g. reactions, pathways and structures). Engineered microbial communities expand upon the BioBricks concept to include cellular organisms as information processing units implementing a distributed genetic algorithm. These BioFactories perform complex tasks more effectively than single cells (Shou *et al*., 2007; Kerner *et al*., 2012), complement enzyme complexes mediating biomass conversion (Arai *et al*., 2007) and resist environmental perturbation (Burmølle *et al*., 2006), suggesting a beneficial role for ecological design principles in engineering new materials and industrial processes from naturally engineered genetic parts.

In summary, we argue that metabolic symbiosis was, is and will be the default state for cellular life and suggest that holistic understanding of ecosystem function requires a deeper understanding of microbial interactions. We should apply this new understanding towards building the next generation biotechnologies that operate in sync with the natural world.

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