Dissecting microbial employment

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A targeted metagenomics approach profiles the methylotrophic microbes in lake sediments.

Microbial communities drive the Earth's biogeochemical pathways. Although broad functional characteristics of these diverse and genomically flexible communities can be identified by metagenomics, it is difficult to pinpoint which species are involved in particular metabolic processes. In this issue, Chistoserdova and colleagues[1] show that metagenomic analyses can be made more specific using DNA stable isotope probing (DNA-SIP). Focusing on methylotrophic organisms, the authors fed microbes from lake sediments one of five different isotopically labeled substrates, which were incorporated into the genome. Next, they separated each labeled DNA sample by isopycnic centrifugation before sequencing. In this way they constructed five 'functional' metagenomes that revealed each guild's specific contribution to the breakdown and assimilation of each substrate.

Metagenomics, or the sequencing of microbial metagenomes directly from the environment, has overcome one of microbiology's biggest headaches—the isolation and cultivation of microorganisms. Previously, culture-independent analysis with molecular markers, such as 16S rDNA, provided taxonomic assignments of uncultured organisms, but the functional capacities of these microbes had to be extrapolated from their nearest cultured relatives. To combine taxonomic assignment and functional analysis, researchers constructed early metagenomes using the 16S rDNA gene as an anchor or by looking for specific marker genes[2]. However, it was soon discovered that sequencing could proceed more rapidly if a sample of all of the genes was sequenced directly without any taxonomic anchor and the sequences were discriminated computationally. Using these techniques, the metabolic potential of microbes from diverse environments could be identified by direct comparisons of sequence abundances between metagenomes[3–7].

Direct sequencing provides a profile of the total microbial capabilities of the entire community[8]. To identify more subtle activities, one must manipulate and fractionate the microbial community before sequencing. For example, in a recent study marine microbes were exposed to varying levels of two common carbohydrates, dimethylsulfoniopropionate and vanillate, in the presence of the thymidine analog bromodeoxyuridine (BrdU)[9]. The BrdU-labeled DNA was purified, and the metagenomes of microbes actively growing on each substrate were constructed.

Chistoserdova and colleagues[1] have refined the specificity of the microbial community that is captured in a metagenome using DNA-SIP. Microbial communities from lake sediments were grown on isotopically labeled one-carbon substrates associated with methylotrophy, including methane, methanol, methylamine, formaldehyde and formate. Each substrate microcosm targeted microbes that actively utilized each carbon source. Total DNA was extracted, and the ^{13}C-labeled fraction was separated from the unlabeled fraction using isopycnic centrifugation. Next, the authors subjected the five fractions to whole-genome shotgun sequencing, enabling the recovery of five 'functional' metagenomes.

This approach simultaneously allowed metabolic reconstruction of substrate breakdown and near-complete genome sequencing of a few microbes responsible for the metabolism of a one-carbon substrate. The enrichment and fractionation enabled a detailed description of some of the microbes that relied on each substrate and identified the exact metabolic pathway that these organisms used. One potential problem, as noted by the authors, is the effect of cross-feeding: a microbe produces a by-product that is used by the next microbe in the metabolic pathway, thereby resulting in some contamination by other members of the community that are not directly utilizing the isotopically labeled compound but are included in the metagenome.

By combining enrichment, DNA labeling and sequence binning, the authors constructed nearly complete composite genomes of individual bacterial species involved in each metabolic pathway. For sequence binning the authors chose PhyloPythia, a compositional-based classifier that combines metagenomic sequences into higher-level generic clades[10]. Using this method they constructed a near-complete composite genome of *Methylotenera mobilis*. This organism is novel and numerically rare (0.4% of the population) but ecologically important within methane-utilizing environments.

M. mobilis dominated the methylamine microcosm metagenomes and displayed new pathways for energy generation and utilization of methylamine. Comparison of the composite *M. mobilis* genome with a previously sequenced genome of a close relative, *Methylobacillus flagellatus,* showed that the two organisms use different strategies in key energy-generating pathways. Secondary functions for well-classified genes were also identified. For example, the formaldehyde-activation proteins are known to convert formaldehyde into methylene-H_4MPT, but this study suggests that they could be a sensory component of a regulatory and/or signal transduction system. Thus, Chistoserdova and colleagues[1] were able to identify new functions within gene clusters without a genome of each cultured organism.

Substrate-specific comparison of composite genomes showed that the methylamine-oxidizing capability of *M. mobilis* was an acquired trait, because these genes were not identified in all metagenomes. This observation

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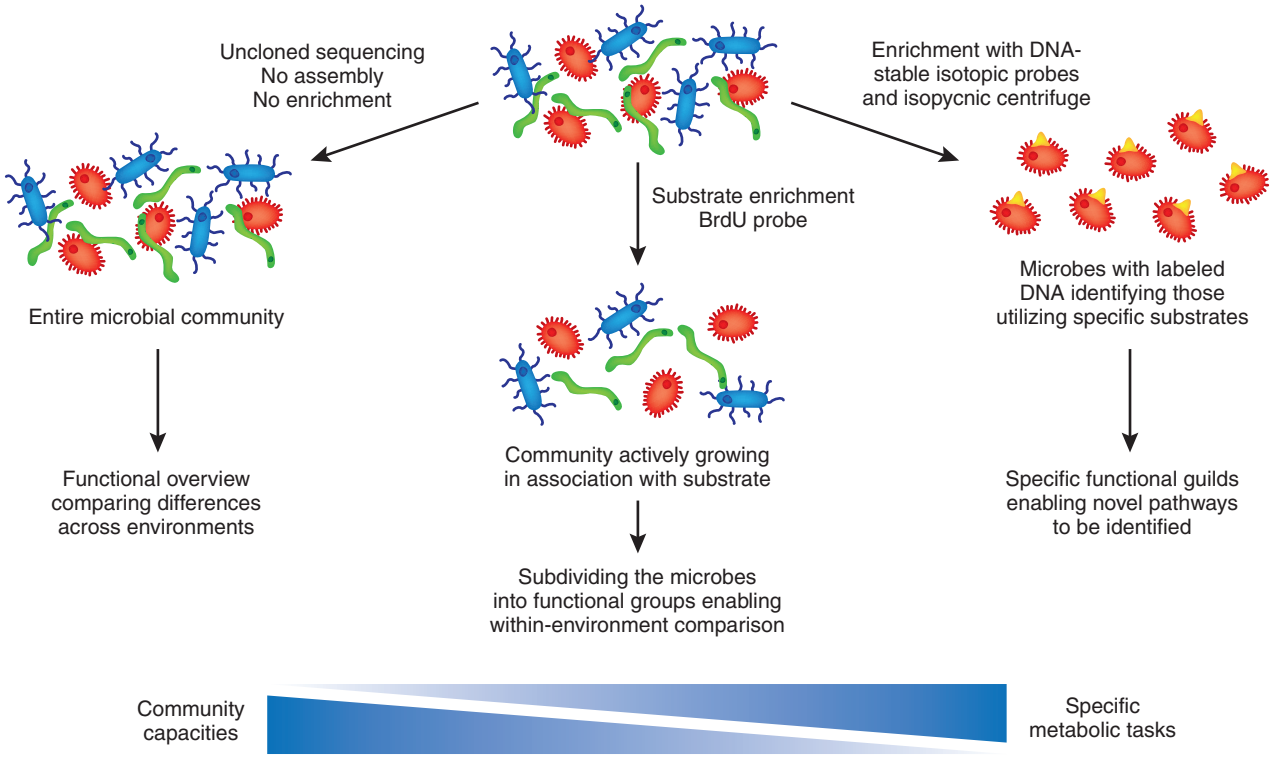

Figure 1 Different methods for analyzing microbial communities provide different levels of selectivity and functional description. These tools enable researchers to describe microbes from community capacities to individual metabolic tasks.

once again demonstrates that microbes incorporate new DNA when exposed to different nutrient sources and that these new acquisitions allow them to use multiple chemicals. *M. mobilis* displayed remarkable flexibility: for example, it used the formaldehyde-activation proteins to convert methylamine when grown on that substrate and incorporated the gene cluster RuBisCo in the presence of methanol. The movement and incorporation of DNA appears to be a fundamental strategy of microbial adaptation, and phage may hold the key to DNA transmission. As with all microbial metagenomes[11], Chistoserdova and colleagues[1] identified high coverage of bacteriophage; other studies have shown that viromes encode extensive metabolic capabilities[7]. This finding corroborates the notion that phage may act as a store of potentially useful DNA that enhances microbial growth and activity.

The new high-resolution technology described by Chistoserdova and colleagues[1] enables novel microbial functions to be identified without the need for culturing. By combining different metagenomic techniques, microbial function can now be described from the community level (by whole-community sequencing), to the individual level (by extracting active species via BrdU labeling[9]), to the level of single metabolic pathways (by DNA-SIP) (**Fig. 1**)[1]. These metagenomic tools will provide deeper insights into the productivity of microbial communities and the specification of individual metabolic tasks.

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Agrobacterium-mediated DNA transfer, and then some

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In addition to its plasmid DNA, *Agrobacterium tumefaciens* can transfer its chromosomal DNA to plant genomes.

The emergence of today's agricultural biotechnology industry stems largely from the discovery, ~30 years ago, that the pathogenic soil bacterium *Agrobacterium tumefaciens* can integrate so-called transfer DNA (T-DNA) on the tumor-inducing (Ti) plasmid into the genomes of most crops. In the late 1990s, scientists found that the transferred sequences can include regions of the Ti plasmid, or T-DNA 'binary vectors', outside the T-DNA borders[1]. In this issue, Ülker *et al.*[2] show that the plant genome may also incorporate bacterial chromosomal DNA.

In the past decade, *Agrobacterium*-mediated transformation has been used to generate hun-

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