GENOMICS

Microbes right on target

Researchers use a targeted metagenomic approach to functionally characterize complex microbial communities.

Ever wonder what lives in your backyard?

Mila Chistoserdova at the University of Washington decided to find out just that. For many years, her team has studied the microbes that live beneath the surface in the sediment of Lake Washington located next to Seattle. "We do not need to travel to Italy, some hot spring or a volcano," says Chistoserdova. "We bet we would find something novel in our own backyard, and we did."

Typically, to characterize such complex bacterial communities, researchers shotgun sequence together the genomes of the resident microbes. However, although such metagenomics studies determine the identity of these microbes, they often do not uncover information about their lifestyles.

Chistoserdova's group now introduced a targeted-metagenomics approach, focusing on so-called methylotrophs that feed on single-carbon (C_1) compounds. By looking at the sequences of these microbes, they hoped to discover which organisms metabolize C_1 compounds and which pathways these bacteria use to do so. This is exciting because methylotrophs serve as a methane sink, potentially combating the greenhouse effect.

To enrich for these microbes, Chistoserdova's team took advantage of a technique pioneered by Colin Murrell of the University of Warwick, called stable-isotope probing. They incubated lake sediment samples with five different ^{13}C-labeled food sources. Then, they isolated the ^{13}C-labeled genomic DNA by extraction and subsequent density gradient ultracentrifugation.

With the genomic DNA of the microbes in hand, Chistoserdova's group turned to the US Department of Energy Joint Genome Institute (JGI) in Walnut Creek, California, USA to sequence them. ^{13}C-enriched DNA libraries were generated for each of the five microcosms and sequenced on the Applied Biosystems Prism 3730.

The coring device used for collecting undisturbed samples of the sediment in Lake Washington. Image courtesy of Mila Chistoserdova.

It is no easy task, however, to put together the genomes of dozens of microbes. Taking a look at existing metagenomic datasets, the JGI team noted that there were quite a few low-quality reads that undoubtedly put a wrench in the works. This issue is especially important when trying to put together entire microbial genomes to ensure accurate gene annotation. Therefore, the JGI team first ran all reads through Lucy, a program that recognizes and removes poor-quality sequences.

To assemble the microbial genomes, the JGI team tested commercially available genome assemblers and settled on the one from the company Paracel. "There is no assembler specifically designed for metagenomic projects," says Alla Lapidus, head of microbial assembly and finishing at JGI. Researchers must see what works best for their own datasets.

Isidore Rigoutsos' team at IBM phylogentically classified the sequences using PhyloPhythia. The JGI team then deposited the data into the integrated microbial genomes with microbiome samples (IMG/M) system, a web-based resource that contains data analysis and visualization tools designed for even the smallest labs to analyze and visualize metagenomic datasets. These include training-set selection for phylogenetic classification and gene-annotation programs. "IMG/M has democratized the analysis of metagenomes," says Alex Copeland, head of quality control and assurance at JGI.

How well did the enrichment work? "For a community as complex as this one," says Natalia Ivanova, head of metagenomics at JGI, "it works wonders." The sequence coverage of individual contigs was at least 1.6-fold, a depth remarkable for such a large and complex metagenomics project.

Taking a look at the results, Chistoserdova's team identified many genes known to be involved in C_1 metabolism to be overrepresented in the metagenomes, providing confidence in their approach.

Notably, they identified several closely related *Methylotenera mobilis* strains that incorporated four out of five C_1 compounds tested. A closer look at the genomic sequences of these microbes revealed differences in key metabolic enzymes, providing insight into how these microbes diverged metabolically. The group also discovered several uncultivated bacteria. A methyltransferase that is likely part of one of these genomes could be the key to deciphering how these organisms use these carbon sources.

However, the JGI team could mostly or fully assemble only a few microbial genomes. Christoserdova anticipates that as more reference sequences become available and as metagenomes are sequenced at even greater depth, more such genomes could be solved: "If you have more sequence coverage, you should have more complete genomes."

Chistoserdova's team is now delving into the mechanisms of the C_1 metabolic pathways discovered by performing targeted metatranscriptomics and metaproteomics. Such studies can explain how bacteria reduce the amounts of these environmentally damaging C_1 compounds. Stay tuned.

Michelle Pflumm

RESEARCH PAPERS

Kalyuzhnaya, M.G. *et al.* High-resolution metagenomics targets specific functional types in complex microbial communities. *Nat. Biotechnol.* **26**, 1029–1034 (2008).