Studying Microorganisms along the Mississippi River

Michael Sadowsky, PhD, leverages the speed and multiplexing ability of the HiSeq® and MiSeq® Systems to identify microbes along the river.

Introduction

Formed by glaciers millions of years ago, the Mississippi River is the longest river in North America. It flows 2350 miles from its source at Lake Itasca in the northwest corner of Minnesota through the center of the continental United States to the Gulf of Mexico[1]. Most of the wildlife along the river has been documented. Its ecosystem is home to a diverse array of fish and wildlife, and the Mississippi Flyway above it is the migration corridor for 40% of North America's waterfowl and shorebirds. Yet studies of microbial life in the Mississippi River have been limited.

Wildlife and microbial inhabitants of the river are affected by human activity along the river. An average of 175 million tons of freight is shipped each year on the Upper Mississippi[2]. Next to the river, 66% of the nearly 1,200,000 acres in the Upper Mississippi River floodplain are now used for crop and pastureland[3]. In fact, the Mississippi is the only inland river to be designated by the U.S. Congress as a nationally significant ecosystem and a nationally significant navigation system, and part of it is the only liquid national park in the United States.

Michael Sadowsky, PhD, became curious about the impact all this activity has on microorganisms. Dr. Sadowsky is a professor in the Department of Soil, Water, and Climate and Director of the Biotechnology Institute at the University of Minnesota (UMN) where he teaches microbial ecology and researches interactions between microorganisms and their environment. He also leads the Minnesota Mississippi Metagenome Project (M3P), an effort to identify and characterize microbes at the headwaters of the Mississippi and to evaluate the human impact on the microbial community downstream.

iCommunity spoke with Dr. Sadowsky to learn how he's using next-generation sequencing (NGS) in the M3P to study microbes in the river.

Michael Sadowsky, PhD, is a professor in the Department of Soil, Water, and Climate, and Director of the Biotechnology Institute at the University of Minnesota.

Q: What is the goal of the M3P?

Michael Sadowsky (MS): The goal of M3P is to understand what the microbiota looks like in relationship to land use, industrial and agricultural inputs, and other anthropogenic changes to the Mississippi River. The Mississippi River starts as a very small stream from Lake Itasca that you can almost step over. We leveraged our close proximity to these pristine headwaters, with all microbial comparisons made in relation to this location. We sampled the river's northern most point until it leaves the state. We follow the changes in microbiota at many locations along the river that are upstream and downstream of sewage treatment facilities, centers of human activity, and where other rivers form their confluence with it in Minnesota. We identified the microbiota using genome sequencing technologies and correlate those to chemical and physical parameters that we determine in the river.

Q: Why is cataloging bacteria living in the Mississippi River important?

MS: The microorganisms in the Mississippi River impact its function because many of them are involved in degradation and the transformation of materials and pollutants that humans add to the river. Microorganisms also contribute to the biogeochemical cycling of elements that are present in the river. Nobody, up until this time, had a good idea of how many microbial species were present in the river. We're cataloging which microorganisms are present and trying to understand their functions. In doing so, we can develop strategies, or guidelines, to remediate potential problems in the river. That's our goal.

Q: Is M3P a longitudinal study?
MS: The M3P study is being conducted over multiple years. At some sites it's performed at multiple times over multiple years, and at multiple depths. We hope to continue this particular study all the way down to the Gulf of Mexico. We'll be requesting funding from the National Science Foundation to do that.

> "NGS is the only way we can effectively understand the taxonomic distribution of microbes without the need to grow them."

Q: How many different bacteria have you identified?
MS: We have identified 4000–6000 unique species present in the Mississippi River. Some microorganisms are location-specific. Because the river is a moving body of water, microorganisms found upstream eventually make their way downstream and mix as the river empties into the Gulf of Mexico. For example, there might be a unique set of organisms present in the Minnesota River or the St. Croix River, but after they're mixed with the Mississippi River they move down together to the next site where another set of inputs occurs.

Q: How do you collect samples in a flowing river?
MS: Sampling a river is an interesting problem because it's a moving waterway. Typically when we use genome sequencing, we go to an environment, take multiple or replicate samples, and extract DNA. In the Mississippi River, as you turnaround to pick up the bottle, that bit of water that you just sampled is now gone. So, we conduct multiple types of sampling on the river. We collect volumes ranging from 40 down to about 2.5 liters. The water is filtered through different pour sizes, from 0.45 to 0.2 microns, to capture different sizes of microorganisms. Once those microorganisms are captured in the filters, we extract DNA using various extraction technologies and sequence using NGS.

Q: How is NGS used in the project?
MS: NGS allows us to determine the taxonomic identities of organisms that are present in the river without the requirement that we grow those organisms. Many of these microorganisms can't be grown, or have not been successfully grown in the past. NGS is the only way we can effectively understand the taxonomic distribution of microbes without the need to grow them.

Q: What did you need from NGS to conduct the project?
MS: We needed NGS to be rapid and cost effective. Because of the large sampling requirement—replicated samples, at multiple sites, and over different depths at multiple sites—cost became a real issue. We were looking for a platform that could multiplex samples and generate a large amount of sequence data rapidly.

I've done a lot of genome sequencing, mostly single microorganisms over the years. The last large project I did began with sequencing 48 microorganisms simultaneously on Illumina platforms and ramped up to sequencing 200 at a time. I was very familiar with Illumina technology and its advantages relative to other platforms.

The HiSeq and MiSeq systems have the throughput that we needed to do a project of this magnitude. The ability to use multiplexed primers and obtain hundreds of millions of sequences in a short time period enabled us to initiate this project.

Over the years we've transitioned to Illumina chemistries that allow us to change what primers we use to identify the microorganisms. As the length increases, our ability to speciate the organisms increases. We're transitioning into primer sets that allow us to look at archaea and fungi as well.

Q: What attributes do you use to assess a good quality sequencing run?
MS: We make stringent comparisons of our sequences to make sure that we don't have any chimeras present and that the read lengths are good. Most important is that we have balanced sequence production throughout the run. Because we multiplex our samples using barcodes, it's important that we obtain even distribution across all barcodes. Downstream statistical analyses require relatively similar size pools for analysis.

> "The HiSeq and MiSeq platforms have the throughput that we needed to do a project of this magnitude."

Q: Have the results so far surprised you?
MS: This project had two separate goals. The first was structural-based, focusing on what microorganisms were living in the river. We expected to find microbial diversity, but I didn't expect it to be as great as what we found. Typically, soils are thought to be one of the most diverse sites on the planet, containing specie numbers approaching 10,000 per gram of soil. I knew that there were soil inputs to the river, but I thought it would be a less complex ecosystem than it turned out to be. I was also surprised at the large impact human activity has on the river. For example, agricultural, urban, and forested land use impacts the types of microorganisms present.

The second goal was determining the functions of the organisms found in the river. Classical microbiological methods rely on enrichment technology. With our ability to clone

genes and check functionality directly in other organisms, we observed that the degree of resistance to antibiotics and heavy metals was a lot less than we thought it would be.

> "Our ability to use multiplexed primers and get hundreds of millions of sequences in a short time period really allowed us to initiate this project."

Q: How are humans impacting biodiversity along the river?
MS: We're doing it in subtle and less subtle ways. One of the more subtle impacts involves land runoff. As the river passes through urban areas, runoff from impervious surfaces like cement and blacktop have a big impact, carrying point and non-point sources of pollution into the river. Feces from animals, and nitrogen and phosphorus from fertilizers have a direct impact on which microorganisms are present in the river. This is reflected in seasonal trends, which also impact the types and relative numbers of microbes present. As temperature changes, nitrogen and phosphorus increase, resulting in algal blooms that occur periodically throughout the river.

Some impacts are even more subtle. For example, runoff from forested areas adds a lot of nutrients to the river such as nitrogen and phosphorus. These naturally occurring nutrients have an impact on microbial life.

One of the largest human impacts is from the sewage treatment facilities that are located all along the Mississippi River from Minnesota to the Gulf of Mexico.

Q: What impact do you expect the project to have on water quality?
MS: It's a little early to say. Initially it was more of a survey, but we're doing more focused analyses now. I'm interested in studying the seasonal changes within the river. We hope the results will impact how managers control the flow of materials into the waterway. We also hope it will enable farmers to make more informed decisions. For example, choosing not to farm right up to the water's edge where fertilizers can enter the river, or to control erosion problems that could add microorganisms to the river.

References

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