Exploring the Microbial Communities Within and Around Us

Next-generation sequencing with the MiSeq® System enables researchers to study the microbiota of humans, model organisms, and clinical environments.

Introduction

We are never really alone. Each of us is a host to flourishing populations of microorganisms arranged in communities referred to collectively as microbiota. Researchers are finding that regardless of whether they're located in the gut, skin, or airways, these communities possess great diversity that can change as we age, in response to certain diseases, changes in diet, or the ingestion of therapeutic drugs. While some pathogenic microorganisms can lead to disease or even death, many are essential to human health and well-being.

New microbial profiling approaches, such as 16S ribosomal RNA (rRNA) sequencing on the MiSeq System, have led to a greater understanding of our microbial communities and their interactions with us. Christopher Taylor, PhD, is part of the Louisiana State University Health Science Center (LSUHSC) Microbial Genomics Resource Group, an organization that supports microbial genomics with scientific expertise and research services. As an Associate Professor in the Department of Microbiology, Immunology, and Parasitology, Dr. Taylor uses rRNA and DNA sequencing approaches to investigate microbes of importance to human health.

iCommunity spoke with Dr. Taylor about his microbiome projects and how the MiSeq System has enabled his studies.

Q: How did you become involved in metagenomic studies?

Christopher Taylor (CT): I have a computer science and mathematics background and got involved with computational biology when I was in graduate school, where I was part of the US National Human Genome Research Institute's Encyclopedia of DNA Elements (ENCODE) project[1-3]. I began my career as a faculty member at the University of New Orleans by focusing on applying high-throughput DNA and RNA sequencing in biological studies. One of my early projects was in collaboration with Dr. Erik Flemington, a virologist at Tulane University. We became interested in the RNA sequence reads from human cancer cell lines that did not map back to the human genome. In many labs at the time, the typical workflow was to map as many sequencing reads as possible back to the host genome, and then discard the remaining 15–20% of the reads. We wanted to look more closely at the nonmapping reads to see if we could find any viral, bacterial, or other recognizable sequences.[4-6] This is still an active collaboration, and our most recent paper shows that there is a lot of microbial contamination in existing RNA sequencing data sets.[7]

Now that I'm at the LSUHSC School of Medicine, there's more of a health care focus to my work, and I've become immersed in research on microbial communities. My primary focus over the last 4 years has been using 16S rRNA sequencing to study the different microbial communities that populate model organisms, humans, and the environments in which they live.

Q: What microbiomes are you studying?

CT: We have various ongoing studies looking at gut, vaginal, airway, and environmental microbiota. In a recent collaborative research study with Drs. Michael Ferris and Duna Penn at Children's Hospital of New Orleans, we used sequencing to look at the gut microbiota of infants in the neonatal intensive care unit, particularly premature infants suffering from necrotizing enterocolitis.[8-9] Using 16S rRNA sequencing, we found that these infants have altered fecal microbiota characterized by a very low diversity in gut microbial communities, which might make them more susceptible to developing necrotizing enterocolitis.

Q: Have any of your studies looked at how diet impacts the gut microbiome?

CT: We've performed several studies where we've used sequencing to identify diet-associated variations in the gut microbiomes of mice.[10-11] In a recent collaborative study with Drs. Hans-Rudolf Berthoud, Annadora Bruce-Keller, Michael Salbaum, and David Welsh, we performed an antibiotic knockdown of the microbial gut community in a group of mice that had been on a standard mouse chow diet. By oral gavage, we then transplanted in the microbiota from mice that had been fed either a high-fat diet (HFD) or a standard mouse chow diet. Sequencing-based phylogenetic analysis using the MiSeq System confirmed the presence of a very distinctive difference in microbiota between the groups. The mice given HFD microbiota also showed

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significant and selective differences in laboratory measures of mouse behavior, such as fear conditioning, maze solving, and anxiety display. This data reinforced the link between gut dysbiosis and obesity-associated changes in neurocognitive behavior in mice.

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Q: What other microbiome studies have you performed that enabled you to see the impact that microbiomes have on disease?
CT: We have conducted several genitourinary tract studies in collaboration with Drs. David Martin and Michael Ferris at LSUHSC. In one study, we looked at a disease called bacterial vaginosis and the role of *Gardnerella vaginalis*, which was originally thought to be the etiologic agent for that disease. A recent paper had shown that there are 2 fundamental *G. vaginalis* genotypes, a biofilm-forming pathogenic variant that was found in all patients with BV, and a commensal variant that does not form biofilms.[12]

In our study, we obtained samples from 53 women and their male sexual partners and used 16S rRNA sequencing to investigate the 2 *G. vaginalis* genotypes and their impact on disease.[13] During that investigation, a graduate student in my lab, Murat Eren, developed a new method called oligotyping that allowed us to differentiate between various *G. vaginalis* strains based only on 16S sequencing data.

Dr. Martin had observed a phenomenon in the clinic where women diagnosed with bacterial vaginosis would finish a course of antibiotics, and be cured, but would then return to the clinic several weeks later with the same symptoms. Our idea was that the bacteria were harbored by the male sexual partner and transmitted back to the woman during sexual activity following the completion of antibiotic treatment. When we looked at the bacterial 16S oligotyping profiles of *G. vaginalis* from the women and men in the study, we found that we could determine which participants were sexual partners, without a priori knowledge and with a high degree of certainty. The ability to look this deeply into strain level variation using only 16S rRNA sequencing was unprecedented and has since been extended with a method called Minimum Entropy Decomposition by Murat and his colleagues and applied to several interesting microbial community studies.[14]

Q: What is the value of 16S sequencing?
CT: 16S sequencing enables us to identify bacterial species present in a community and to separate similar strains using techniques like oligotyping. We're currently working on an airway study in collaboration with Dr. David Welsh where we're using 16S sequencing data to look at relatively subtle differences between microbiota sampled from the oral cavity with microbiota from tissue samples taken further down the airway with different sampling brushes. Compared to gut microbiota, the bacterial burden of airway microbiota is a lot lower. The further down the airway you go, the fewer bacteria you will find. We want to determine how these microbiota transit between these different environments and what this might tell us about the composition of microbiota living deeper in the airways.

Q: What types of environmental microbiome studies are you performing?
CT: We are planning a 16S sequencing study using the MiSeq System to analyze the metagenomic landscape of the Intensive Care Units (ICUs) in the new University Medical Center that is scheduled to open in mid-2015. We're very interested in looking at environmental samples from the new trauma ICU and medical ICU before patients are moved in and as these ICUs are put into service. Based on a trial run in another operating medical facility using qPCR to quantify 16S ribosomal RNA, we found the largest microbial counts came from the floor and from the lever on the hand sanitizer. In the new medical center, we're proposing to sample patient rooms and common areas where health care workers will be walking and using equipment, such as computer terminals, hand sanitizers, and scanners for tracking drug administration.

We also want to look at how antibiotic-resistant organisms end up entering and moving around in these new ICUs. One of our collaborators on the study is proposing a simultaneous culture-based investigation to look for particular antibiotic-resistant organisms. We're hoping to associate our 16S sequencing data with antibiotic-resistant and nonresistant versions of the cultured microorganisms and better understand how to control and prevent the spread of antibiotic-resistant microorganisms.

Q: What types of sequencing approaches did you use before obtaining a MiSeq System?
CT: In some of my initial research, we used genome tiling microarrays, but by the mid-2000s they had become obsolete with the advent of high-throughput sequencing. When I arrived at LSU, the School of Medicine had a pyrosequencing system that was being used to obtain long read lengths for 16S rRNA studies. This pyrosequencing system was older technology and was fraught with problems due to the large number of complex steps required for sample preparation. On many occasions, we struggled with this technology and had to perform multiple repeated sequencing runs attempting to resolve questionable results. It was also a long process, when you consider the time required for sample preparation, sequencing, and the weeks-long, intensive computation that was required to de-noise and prepare the data for analysis.

Q: How has the MiSeq System improved your workflow?
CT: We obtained a MiSeq System in August 2013, and the difference was like night and day. The cartridge-based MiSeq System eliminated many sources of variation where things could go wrong in sequencing preparation. Our sequencing throughput has been reduced from weeks to days. Due to the consistent and high-quality data generated by the MiSeq System, we were able to move forward with several of our projects. We didn't have to go back and repeat the same sequencing run over and over, trying to get usable data.

For our first MiSeq sequencing run, we used samples from the mouse gut repopulation study I mentioned earlier. When we ran these samples on the MiSeq System, the results were some of the best data we had ever seen. The beta diversity plot groupings obtained for the HFD mice versus the control diet mice were incredibly distinct and provided a better separation than we could have expected. We were

even able to identify a mislabeled sample from the study due to the extremely high fidelity of the diet groupings.

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Q: What do you see for the future of metagenomics?

CT: One thing I envision is the prescriptive use of probiotics. Currently, there is a lot of pseudoscience in the supplement industry, promoting probiotics that are just different strains of *Lactobacillus*. The market for these products is based on the idea that *Lactobacillus* is generally helpful as a way to improve or maintain human health. Sequencing approaches will enable us to acquire a much more nuanced and specific understanding of what a healthy gut microbial community is and what kinds of treatments are possible, and the benefits of certain probiotics. Similar to the promise of personalized medicine, I think that in the future, we'll be able to determine a person's gut microbiota and the kinds of probiotics that could be most beneficial for them. That is something I see on the long-term horizon for microbiome research.

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 Pub. No. 1270-2015-002 Current as of 14 May 2015

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