Department of Biological Sciences Seminar Blog

Seminar Date: 3/23/18
Speaker: Dr. Nikki Shariat, Gettysburg College
Title: *"Probing* Salmonella *population diversity using CRISPRs"*

CRISPR-SeroSeq: A Developing Technique for *Salmonella* Subtyping

By: Emine F. Kahveci (Biology PhD Student)

Every semester graduate students in our department at Duquesne University nominate a speaker to visit the program. Dr. Nikki Shariat was chosen among six excellent candidates to meet with graduate students throughout the day and give our departmental seminar. Dr. Shariat received her BS degree from the UK before starting her career in the USA. She earned her Ph.D. from Vanderbilt University where she studied the role of small interfering RNAs in mice followed by a post-doctoral fellowship in a similar area at UCSF. At some point during this time, she attended a seminar about a new bacterial "immune" system called the CRISPR (Clustered regularly interspaced short palindromic repeats) system. Amazed by the work, she then started to work on CRISPR elements as a postdoctoral scholar at Penn State. She is currently a professor at Gettysburg College and studies the function, diversity, and evolution of CRISPRs to track food-borne pathogens. Her lab is currently developing a rapid and accurate CRISPR-based molecular method to identify and analyze *Salmonella* serotypes and strains in different environments.

Dr. Shariat has been working with *Salmonella,* which is the primary source of foodborne illness in the USA. *Salmonella* is associated with many food products including meat, tomatoes, peanuts, peppers, tuna fish, papaya, etc. *Salmonella* is a quite diverse species, and its

characterization is done by looking at cell surface antigens of different serotypes. These serotypes are called “serovars.” The over 2500 serovars of *Salmonella* can differ in host preference, pathogenesis, geographic region, and antibiotic resistance. For example, some serovars can only infect poultry, while some can infect both animals and humans. Dr. Shariat’s work focuses on two serovars of *Salmonella*. The first one is *S. enteritidis* causing infection in human, poultry and cattle. The other is *S. Kentucky* which demonstrates antibiotic resistance. Her goal is to utilize the existing CRISPR system to sequence and identify subtypes of these organisms.

CRISPRs are small DNA repeat sequences present in about half of all bacteria. CRISPRs have a potentially important role for bacteria by providing them an adaptive immune system against invading DNA of bacteriophages or plasmids. A CRISPR locus consists of CRISPR-associated (Cas) genes, a CRISPR array including spacers and direct repeats, and a leader sequence upstream of the array. The CRISPR loci function in three steps. First, acquisition or adaptation begins with recognition of a new invader and addition of new spacers to the 5’ end of the CRISPR array. Second, in expression or processing stage, the array is transcribed into a precursor CRISPR RNA (pre-crRNA) which is then cleaved by Cas proteins to be mature crRNAs. The last step is the interference when mature crRNAs form interference complexes with Cas proteins and this CRISPR-Cas complex can protect bacteria by degrading the foreign DNA (1).

Salmonella has two CRISPR loci, CRISPR1 and CRISPR2. When Dr. Shariat was a post-doc, she did loads of sequence analyses of CRISPR loci to generate “CRISPR profiles”. She found that direct repeats are the same within the two loci and across all *Salmonella*, but the spacers are heterogeneous (2). She has also found that there is no evidence of new spacers being added. Instead, there are missing or duplicated spacers within a serovar showing allelic polymorphism (2). These data actually

suggest that the CRISPR system may be an ancestral immune system that is not used any longer in these organisms. The reason that this system is maintained is a bit of a mystery.

The Shariat lab used the differences found in the spacers as a subtyping tool with four different serovars of *S. Enterica*, and has showed that spacer patterns are serovar specific (3). Her lab used this CRISPR subtyping in *S. Kentucky* as well, which does not typically cause human illness in the USA but is common in cattle and poultry. They used 40 human clinical isolates of *S. Kentucky* and identified two genetically distinct groups. They compared CRISPR typing results with the pulse-field gel electrophoresis (PFGE). They looked at the phenotypes by testing all isolates with 14 antibiotics and found that group I and group II exhibited distinct antibiotic resistances (4). In addition to the clinical isolates, they studied *S. Kentucky* isolates from domestic food animals for further investigation. They found all isolates belong to group I using CRISPR profile, so this result explains the possibility for transmission of group I *Salmonella* to humans from cattle and/or poultry. However, Dr. Shariat pointed out there are still questions of where group II *S. Kentucky* is coming from.

The next important study from the Shariat Lab is related to using serovar specific spacers to identify serovars in a mixed population. Her lab has developed a new approach called CRISPR-SeroSeq. After samples are collected, they are enriched for *Salmonella* growth. DNA is then isolated from the entire culture. A single PCR reaction is performed using primers binding to direct repeats. If there are two different serovars in a population, they can see this on a gel. Lastly, they extract spacers from the sequences and compare them to the CRISPR database that they have developed. They confirmed this CRISPR-SeroSeq approach by doing a pilot study with *S. Kentucky* and *S. Enteritidis* and could successfully differentiate the strains even at 1/10000 dilution.

As a further analysis, the Shariat Lab wanted to use CRISPR-SeroSeq to identify all serovars in a single population. They collected poultry samples from a selection of farms and processing plants in Pennsylvania. When they did CRISPR-SeroSeq analysis, they were very excited to see significant serovar diversity in a single sample. Dr. Shariat mentioned about the other sequencing approaches, such as 16S profiling and conventional methods. However, neither of them can identify all of the serovars present in a single sample, like CRISPR-SeroSeq does.

Her lab is currently working on a spacer-specific-probes for qPCR analysis to identify the antibiotic resistance of background isolates which they do not use for CRISPR-SeroSeq. Additionally, the Shariat Lab is trying to map diversity of *Salmonella* and investigate the risk on public health with their collaborators.

At the end of the seminar, I feel that I have learned a lot and am impressed by how important Dr. Shariat's work is to public health and understanding the basic science of the endogenous bacterial CRISPR system. Since the *Salmonella* population is quite diverse, they could use CRISPR-SeroSeq approach to identify different serovars not only in domestic food animals, but also in wildlife and our foods. During our meeting with her prior to seminar, she said that she spent her best three years in her life during her post-doc. We could see the enthusiasm and passion in her eyes. I am sure her academic success in science has inspired many students at the seminar.

References

1. Shariat, Nikki, and Edward G. Dudley. "CRISPRs: molecular signatures used for pathogen subtyping." *Applied and environmental microbiology* 80.2 (2014): 430-439.
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human clinical isolates of Salmonella enterica subsp. enterica serovar Enteritidis." *Food microbiology* 34.1 (2013): 164-173.

3. Shariat, Nikki, et al. "Characterization and evolution of Salmonella CRISPR-Cas systems." *Microbiology* 161.2 (2015): 374-386.
4. Vosik, Dorothy, et al. "CRISPR Typing and Antibiotic Resistance Correlates with Polyphyletic Distribution in Human Isolates of Salmonella Kentucky." *Foodborne pathogens and disease* (2018).