Story by Courtney Swyden

The Baron of Bugs

Keeping waterborne pathogens at bay

While a student at The University of Arizona, Dr. George Di Giovanni planned to attend medical school. But a slight shift in his college career led him to the occasional title of the "baron of bugs" today.

As the primary developer of the most comprehensive *E. coli* culture collection and Bacterial Source Tracking (BST) library in the state, Di Giovanni, professor of environmental microbiology at the Texas AgriLife Research and Extension Center at El Paso, did not exactly get his start in detecting water pathogens.

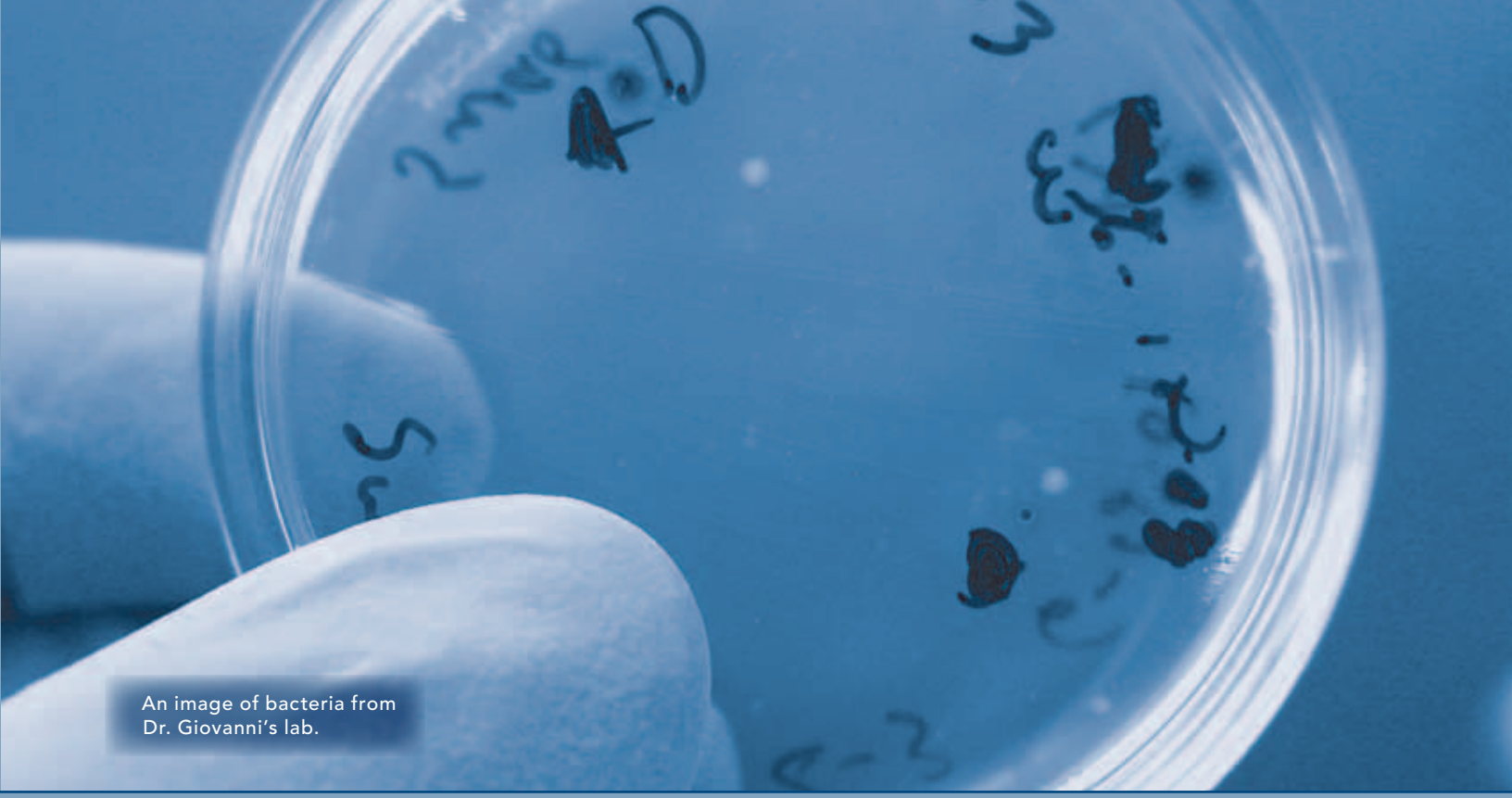
"I consider myself an environmental microbiologist, and there are a lot of sub-disciplines in that area," said Di Giovanni, whose interest in detecting and characterizing microorganisms in the environment eventually shifted from soil to water. "The whole overriding theme was the analysis of organisms from environmental settings, and that's always been an interest to me."

As a child, Di Giovanni participated in science fairs, focusing his projects on microorganisms. "I had a microscope and was fascinated by the delicate structure of bread mold, algae, and protozoa," he said.

Majoring in microbiology and immunology at The University of Arizona, he began working in an environmental microbiology laboratory during his sophomore year, studying the use of bacteria to remediate pesticide-contaminated soil. During this time he gained experience and a strong interest in molecular biology techniques applied to soil microorganisms and environmental samples.

His doctoral research at The University of Arizona focused on gene transfer and bioremediation of pesticide-contaminated soil, where he applied both culture and molecular-based analyses to microorganisms in the environment. His postdoctoral position with the U.S. Environmental Protection Agency took him to Oregon, where he worked as a National Research Council associate and continued research in environmental microbiology.

Dr. George Di Giovanni developed the *E. coli* culture collection and Bacterial Source Tracking library for Texas.



An image of bacteria from Dr. Giovanni's lab.

"At that point, things shifted a little bit, and I was actually looking at biotechnology risk assessment of transgenic plants," he said. Di Giovanni became interested in microorganisms in the root zone of these transgenic, or genetically engineered, plants and their impact on microbial communities.

"At the end of my post doctorate, I decided to continue with molecular environmental microbiology research, but shifted from the detection and characterization of soil microorganisms to the detection of waterborne pathogens," he said.

The expertise gained while working with soil-based environmental samples was transferrable to the water environment, and in 1997 he became an environmental scientist for the American Water Works Service Company in Belleville, Ill. At the largest privately owned water and wastewater utility company in the United States, Di Giovanni's work focused on waterborne pathogens.

"The key thing there was detection and characterization of organisms in the environment, except instead of working with soil now I was working with water," he said. "Since it was a drinking water company, the focus was detecting pathogens in source water used for drinking water production, as well as finished drinking water itself."

Though he believed great advancements in basic and applied waterborne pathogen research were being made, he foresaw limited research opportunities at the water company. At this time, Di Giovanni said, the water industry and regulatory communities were beginning to take a serious interest in molecular methods for waterborne pathogens.

"With the exciting research opportunities on the horizon, I felt an academic environment would be a better fit for my interests," Di Giovanni said. In 2001, he joined Texas AgriLife Research where he continued in this line of research. His focus has been on the detection of waterborne pathogens—specifically *Cryptosporidium* and *Giardia*—and source tracking to identify and control human and animal sources of surface water pollution.

In 2003 Di Giovanni and his team of researchers began the first BST studies in the state with support from the Texas State Soil and Water Conservation Board and the Texas Commission on Environmental Quality (TCEQ) in the Lake Waco and Lake Belton watersheds. Their BST research focused on *E. coli*, and the team developed a comprehensive *E. coli*-based library.

"The thing about bacterial source tracking and the way it is applied here in Texas and in many other states is that we are not specifically looking at pathogens," Di Giovanni said. "What we are looking at are indicators of fecal pollution because that fecal pollution can contain pathogenic organisms."

The library was developed by isolating and sampling known fecal sources, including wildlife, domestic animals, livestock, and wastewater samples of human origin. Di Giovanni's team isolated *E. coli* bacteria from the samples and typed them using DNA fingerprinting methods to create a library of *E. coli*.

The team then isolated *E. coli* from water samples and compared them with the known source sample library to obtain source ⇨

identifications. The libraries from the Lake Waco watershed and the Lake Belton watershed were combined for cross-validation to match the correct sources and for use in future studies.

"At that point we had the basis of the state library because those two projects were fairly large," Di Giovanni said. The library has been refined, and continuing cross-validations will allow for further improvements. Cross-validations take his team to various watersheds to obtain *E. coli* from known source samples and to attempt identification of those isolates using the current library. Currently the library includes 1,173 *E. coli* isolates from 1,045 different fecal source samples selected from more than 5,000 *E. coli* isolates.

"To test the identification accuracy of the library, we treat the *E. coli* library isolates as unknowns, and then test them against the remaining library and look at the rates of correct identification. The accuracy is okay at this point (87 percent), but we could do better," Di Giovanni said.

This intensive library will save researchers time and money while performing studies in smaller watersheds. The library will assist in identifying water isolates from other watersheds. Di Giovanni said that *E. coli* collected from other watersheds will be incorporated into the continually developing state library.

Di Giovanni and his team received the 2007 Texas Environmental Excellence Award, the state's highest environmental honor, presented by TCEQ, for their extensive BST research and development of the statewide library. The library was recognized as saving millions of dollars on future pollution source tracking projects as well as supporting the development of effective pollution control strategies to ensure safe drinking water.

Currently, Di Giovanni's lab is leading an international project on *Cryptosporidium* with funding from the Water Research Foundation in hopes to transfer this technology to water testing labs. The collaboration includes laboratories in Scotland, England, Wales, Australia, Canada, South Africa, and the United States.

"We are getting the water industry prepared to move into the 21st century," Di Giovanni said. "Unfortunately it is pretty scary; they have been behind a long time."

The United States requires testing of *Cryptosporidium* from drinking water sources, and the current project involves meeting these mandates by using microscopy-based testing. However when a test sample is recovered and viewed under a microscope, *Cryptosporidium* can be identified but the particular species or genotype cannot be identified.

Di Giovanni's lab and cooperators are solving this problem by taking the microscope slides and removing the oocyst—the stage of *Cryptosporidium* found in water—and using molecular techniques to identify the species and genotype. This allows for a more accurate human-health risk assessment and identification of potential sources impacting a watershed. For example, Di Giovanni explained, if all of the *Cryptosporidium* found in a particular source water were animal-associated and not pathogenic to humans, it would contain very little risk to humans.

"It is currently an exciting time in waterborne pathogen research since the water industry and regulatory communities are beginning to embrace and adopt molecular methods," Di Giovanni said. "It is very gratifying to contribute to this revolution in water quality testing and the protection of public health."

Dr. Karina Barrella, post-doctoral research associate at the Texas AgriLife Research and Extension Center at El Paso, works on the E. coli library.