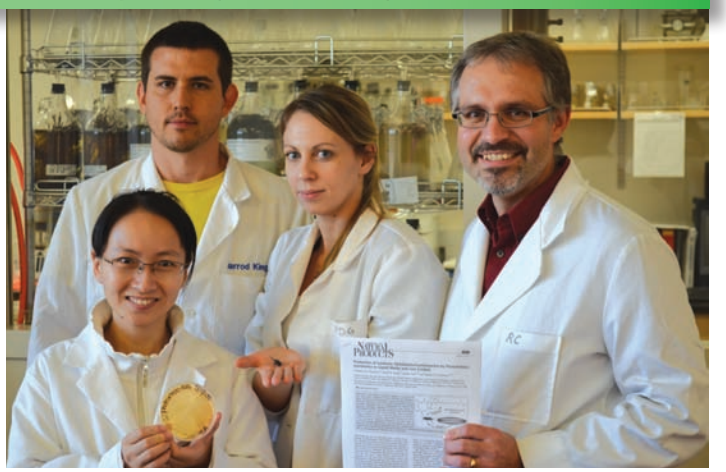
Behind the Scenes in Pharmacognosy: Jiminy Cricket!

By Dr. Amy Keller

This past October, the *Journal of Natural Products* published an article from ASP member Dr. Robert Cichewicz and his colleagues at the University of Oklahoma's Institute for Natural Products Applications and Research Technologies (INPART) in Norman, Oklahoma, entitled, "Production of Cytotoxic Glidobactins/Luminmycins by *Photorhabdus asymbiotica* in Liquid Media and Live Crickets". Please read the full article in the *Journal of Natural Products,* 2012, 75(11), 2007-2011. DOI: 10.1021/np300623x.



The University of Oklahoma Natural Products Applications and Research Technologies team members who carried out the cricket studies. From the left: Dr. Jianlan You (holding a plate containing *P. asymbiotica*), Mr. Jarrod King, Ms. Christine Theodore (holding a cricket), and Dr. Robert Cichewicz (holding a copy of the published cricket manuscript).

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1. How did you become interested in working with bacterial compounds, and how did you come to focus on their expression in live crickets?

Since 2005, our laboratory has focused on investigating secondary metabolites from microorganisms. While most of our work has been concerned with the drug development applications of fungal natural products, opportunities to work with bacteria have emerged from time to time. For example, we have worked with Dr. Felicia Qi at the University of Oklahoma Health Science Center to study hybrid polyketide-nonribosomal peptide metabolites produced by a bacterium that occupies the human oral cavity. More recently, we have engaged in new collaborative project with Dr. Brad Stevenson from the University of Oklahoma Department of Plant Biology and Microbiology to investigate bacteria from the microbiomes of mammals native to the southeastern United States as a source of new bioactive substances. During this time, a paper caught our attention describing *Photorhabdus asymbiotica* as an emerging bacterial pathogen that may infect humans via a nematode host (J. G. Gerrard, et al. Nematode symbiont for *Photorhabdus asymbiotica. Emerg Infect Dis.* doi.org/10.3201/eid1210.060464). Further supporting our interests in this bacterium was the tremendous insights provided in the work by Dr. Helge Bode about the natural product chemistry of *Photorhabdus* and *Xenorhabdus* species. In addition, these bacteria engage in a variety of curious symbiotic relationships with entomopathogenic nematodes. Despite reports of natural products from other taxonomically related isolates, *P. asymbiotica* had not been subjected to an investigation of its secondary metabolites.

Our initial results were disappointing. We knew the bacterium's genome contained nearly two dozen biosynthetic gene clusters (based on analysis with antiSMASH), yet culture extracts were devoid of detectable metabolites. Doctoral student, Ms. Christine Theodore, showed tremendous resolve testing a large number of media until she finally discovered conditions supporting the production of an assortment of the organism's cryptic metabolites. This led to a new question, "Why is the production of these metabolites under such strict control and how can we overcome this limitation in the lab?" Considering many possibilities, we arrived at the hypothesis that the production of these compounds may require very specific environmental cues such as those experienced when *P. asymbiotica* is released from its nematode host into an animal or insect. At the same time these events were unfolding, postdoctoral fellow Dr. Jianlan You, had been developing insect-based assay models of infectious disease for our group. It seemed reasonable that we could adapt those experiences to test whether an invertebrate host could provide an appropriate environment for the growth and production of *P. asymbiotica* metabolites. Both Jianlan and Christine worked together to establish a test system using crickets. We were very excited to see that the controlled inoculation of crickets with *P. asymbiotica* resulted in the accumulation of many of the bacterium's metabolites within the insects. Performing LC-MS on the crickets, we found that glidobactins/luminmycins metabolites were among the most prevalent of the microbial-derived compounds recovered from the organic extracts. Mr. Jarrod King, who operates our team's bioassay screening unit, was able to test these metabolites in cytotoxicity and proteasome-inhibition assays demonstrating that our compounds exhibited potent inhibitory effects similar to those for other members of the glidobactins/luminmycins family.

2. Who in your laboratory carried out the research?

Similar to all projects in our lab, this work was a collaborative effort among several individuals within our research group. Although we are affiliated with the Department of Chemistry and Biochemistry, we pride ourselves on maintaining a diverse group of students, postdoctoral fellows, and research associates with formal academic training in chemistry, biochemistry, molecular biology, microbiology, and pharmaceutics. Christine is a biochemistry Ph.D. candidate who is training in the area of natural products chemistry. Both Christine and I worked together to design the experiments. She also performed all of the metabolite purification, structure determination analysis, and analytical chemical studies. Dr. Jianlan You is a microbiologist who has been develop-

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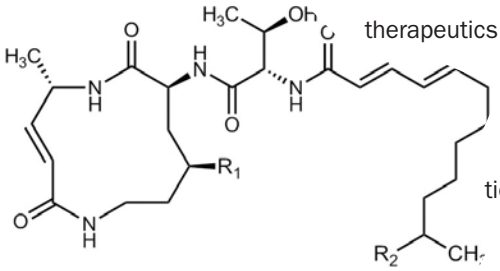
ing new assays for our research progran as well as providing a wealth of expertis concerning the molecular biology of boti bacteria and fungi. She collaborated witl Christine to establish the cricket-basec experiments. Mr. Jarrod King is the leac biologist responsible for all of the antibacterial, antifungal, and cancer cell cytotoxicity screening operations in our group. He is also a key player in our fungal and bacterial isolate procurement efforts. This bottom-up integration of scientists with diverse backgrounds is one of our fundamental strengths and it was certainly an important factor in this project.



glidobactin A R_1 = OH, R_2 = H
luminmycin A R_1 = H, R_2 = H
luminmycin D R_1 = H, R_2 = CH_3

Structures of the glidobactins/ luminmycins obtained from crickets infected with *P. asymbiotica.*

therapeutics to treat a myriad of human diseases. This is our group's primary research focus. We are also rapidly expanding our efforts in this area with the establishment of the new Institute for Natural Products Applications and Research Technologies at the University of Oklahoma. We are in the process of hiring and sustaining six natural products research groups who will work on a collaborative basis toward the development of new lead molecules for several disease indications. We are using the same ecological principals utilized in this study to further enhance the bioactive compound discovery process and help provide an efficient path toward preclinical drug development.

3. Could you provide a brief explanation of the work and results in your own words? In what way are the data in your paper new?

One of the important themes contained in this paper is the incredible level of acute sensitivity that many microorganisms exhibit when it comes to biochemical responses to the environment. While this is by no means a new observation, it is certainly one that has created an enormous range of challenges and possibilities for the field of microbial natural products. Although molecular-based approaches to controlling secondary metabolite production have provided important opportunities for uncovering the products of silent biosynthetic pathways, they also inadvertently perpetuate a fundamental scientific dilemma: How do the microorganisms interpret their local environments and why are they capable of making such an amazing array of secondary metabolites? The combination of modern genomics technologies and systems biology have helped reveal many new insights concerning these problems, but they alone are insufficient to understand how microbial systems (populations of cells) function as the basic operational units within an ecological context. When one considers the extent to which many bacteria and fungi exhibit a profound mutual reliance on other microbes, plants, and animals, it becomes apparent that understanding the role of natural products within an ecological context is an immense challenge. This is why theory from the field of ecology coupled with classic descriptive biological sciences will continue to play important roles in addressing the "big" questions about natural products (*e.g.*, Why are natural products made at all?). Our paper represents a very modest, but potentially useful contribution to moving us incrementally closer to understanding why secondary metabolites have evolved into one of nature's most fascinating forms of organic chemistry.

4. What impact does this research have on natural product science and health research in general?

Using ecological insights to manipulate microorganism into revealing their secondary metabolites is the best approach to effectively mining nature for new bioactive compounds. Although the multitude of roles that natural products play in our world is fascinating, a more pressing problem is the need for new small-molecule

5. What is a favorite nonscientific activity of your lab?

Outside of weekly group research meetings, we do not have any compulsorily nonscientific lab activities. The group is highly diverse and our members work incredibly hard based on their passion for their research. Individuals or small groups of lab members engage in a variety of activities including sports (*e.g.*, soccer, olympic weightlifting, running, etc.), reading, drawing, coin collecting, and life with family. Our group's greatest strength, its diversity of individual backgrounds and ideas, is optimally maintained by having each member engage in their own unique pastimes.

6. What is your lab's motto?

Our lab's motto, "Transforming the chemistry of nature into products that improve lives," has evolved into the guiding principal for the Institute for Natural Products Applications and Research Technologies. This motto is a constant reminder of why we are here and what our goals are. Everything we do is focused on the development of new therapeutic agents ranging from direct natural products discovery to efforts aimed at enhancing the hunt for new bioactive compounds. Our recent paper highlights the latter as a means for improving the approaches we use to find new hit molecules.

7. What is your greatest extravagance in the lab?

We really do not have any "extravagances" in our lab. Every tool, instrument, and machine was purchased with state or federal funds and we have a responsibility to the taxpayers of the United States and state of Oklahoma to ensure their money is put to the best responsible use. With that said, our group and institute are appropriately outfitted to take on a range of research challenges including analytical studies (LC-MS), compound purification and structure determination (HPLCs, various evaporators, and spectroscopy tools), and biological testing (BSL2 facilities with hoods, incubators, plate readers, and other molecular biology equipment). Having this equipment is also a key component of student training with individuals who have worked in our group having hands on exposure with all of our instruments. ■