Behind the Scenes in Pharmacognosy: Fun with Aeroponics!

Last summer, the Journal of Natural Products *published "Thielavialides A–E, nor-spiro-azaphilones, and a bis-spiroazaphilone from* Thielavia *sp. PA0001, an endophytic fungus isolated from aeroponically grown* Physalis alkekengi," *work from the laboratory of ASP member Dr. Leslie Gunatilaka, Director and Professor, Southwest Center for Natural Products Research and Commercialization at the University of Arizona Cancer Center, Tucson, Arizona. Dr. Gunatilaka's laboratory pursues innovative techniques in understanding the relationship between plants and endophytic fungi in environmental stress. We are grateful to Dr. Gunatilaka for sharing his work in more detail, including the group's emphasis on local and international collaborations. Please read the article,* J. Nat. Prod., *2014, 77 (6), pp 1467-1472.doi: 10.1021/np500237h.*

By Dr. Amy Keller

How did you become interested in endophytic fungi and compounds therein?

My interests in natural products date back to my undergraduate research project on the investigation of medicinal plants of Sri Lanka. During my graduate studies with Dr. D. H. R. Barton and postdoctoral studies with Drs. Barton, Carl Djerassi, and David Kingston, I gained experience in studying natural product biosynthesis, isolation, identification, synthesis, and their evaluation for potential biological activity. When I moved to the University of Arizona, Tucson, Arizona, to take up my first independent position in the United States, I was fascinated to see how some plants without xerophytic adaptations survive in the harsh environment of the Sonoran desert. This observation led me to hypothesize that some microorganisms living in symbiotic association with these plants, such as endophytic and rhizosphere fungi, may produce small-molecule natural products capable of modulating stress (heat-shock) response in plants.

Thus, I became interested in plant-associated fungi. Our work on secondary metabolites of endophytic and rhizosphere fungi of Sonoran desert plants resulted in a number of natural products capable of modulating heat-shock response in a heat-shock induction assay routinely used in our laboratory. To our pleasant surprise, one of these compounds, monocillin I, was found to confer thermotolerance to the model plant, *Arabidopsis thaliana*, by interacting with the plant heat-shock protein, HSP100. Subsequently, we showed that co-culturing of *A. thaliana* seedlings with the fungus producing monocillin I resulted in survival up to 45° C for 75 minutes, whereas *A. thaliana* in the absence of the fungus did not survive at this high temperature. We have thus far investigated over 500 endophytic fungi and have isolated numerous natural products, some bearing unprecedented carbon skeletons or with potential anticancer, anti-HIV, and anti-tuberculosis activities.

From left to right: Mr. Brandon Freidhof, Mr. Felipe Hilario, Mr. Thiago Porto, Dr. Leslie Gunatilaka, Dr. Kithsiri Wijeratne, Ms. Patricia Espinosa-Artiles, Dr. Yaming Xu.

APRIL BAGLEY

Aeroponic chambers used for cultivation of plants under a controlled environment (in a greenhouse)

DANIEL BUNTING

Who in your laboratory carried out the research?

Although the work described in this paper was carried out by Dr. Kithsiri Wijeratne, Dr. Raphael Gruener, and Ms. Patricia Espinosa-Artiles, our project on investigation of endophytic fungi for bioactive and/or novel small-molecule natural products is a large collaborative effort between my group (Drs. Bharat Bashyal, Kamal Gunaherath, Angela Hoffman, Kithsiri Wijeratne, Jianguang Luo, Jair Mafezoli, Maria

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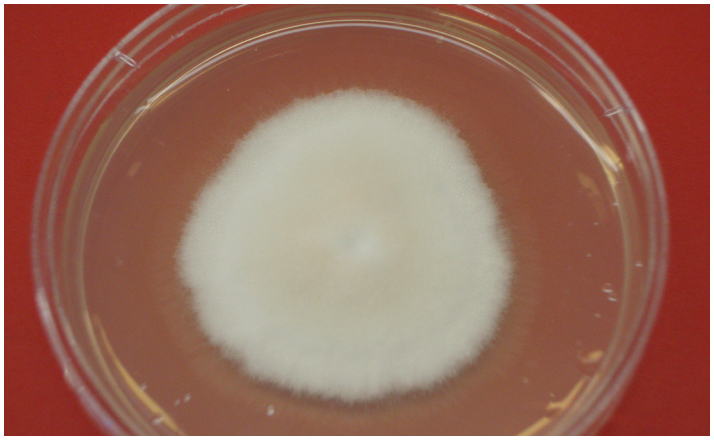
Oliveira, Priyani Paranagama, Jacqueline Takahashi, Yaming Xu and Jixun Zhan, and Ms. Patricia Espinosa-Artiles and Manping Liu), and the groups led by Drs. Nafees Ahmad, Elizabeth Arnold, Eli Chapman, Istvan Molnar and Donna Zhang (all from University of Arizona), Stan Faeth (Arizona State University, Tempe, Arizona), Scott Franzblau (University of Illinois at Chicago, Chicago, Illinois), and Luke Whitesell (Whitehead Institute, Cambridge, Massachusetts).

Could you provide a brief explanation of the work and results in your own words?

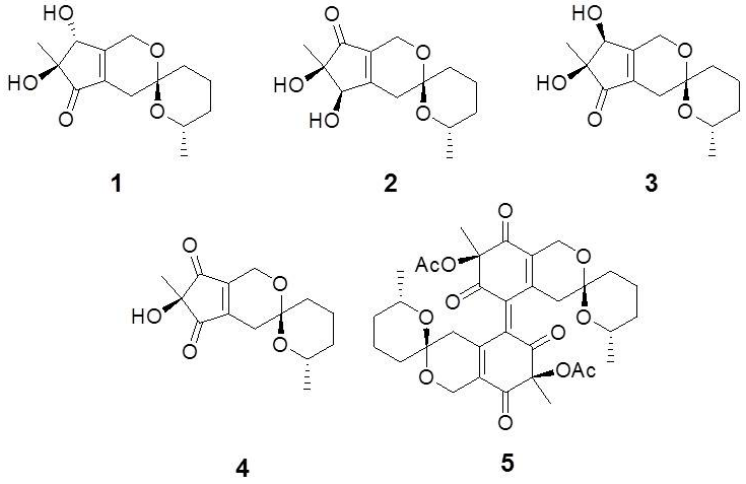
We have recently developed an innovative soil-free aeroponic technique for the cultivation of plants under environmentally-controlled conditions. This facilitates the efficient production and structural diversification of their bioactive metabolites. As high-lighted by Dr. David Newman in his series on "Hot Topics in Pharmacognosy," published in the 50th Anniversary Issue of the *ASP Newsletter* (Vol. 50, Issue 4, pp 18-19), successful application of this technology to *Withania somnifera* (ashwagandha) resulted in efficient production of the commercially available, but expensive, plant natural product withaferin A (current Sigma-Aldrich price for 5.0 mg is $352).

Prior to our work reported in our publication, we and others have investigated endophytic fungal strains inhabiting plants and mosses found in their natural environments. We thought that it would be interesting to see if plants grown under environmentally controlled conditions would also harbor endophytic fungi, and if so, would these produce metabolites with biological activity? To this end, we sampled live tissues of several medicinal and related plants, including *Withania somnifera* and several *Physalis* sp., and cultivated them using our environmentally controlled aeroponic technique for their endophytic fungi. We were



A petri dish with the endophytic fungus, *Thielavia* sp. PA0001

PATRICIA ESPINOSA-ARTILES



Chemical structures of thielavialides A (1) – E (5)

intrigued by the fact that in contrast to plants growing under natural conditions that harbor numerous endophytic fungi, these plants cultivated under a controlled environment were found to host only a few culturable endophytic fungi. This observation may support the hypothesis that plants harbor symbiotic endophytic fungi for ecological interactions with their environment; under controlled environmental conditions this symbiotic association may not be essential for their survival.

Of those investigated, only the leaf tissue of *Physalis alkekengi* (bladder cherry) contained a culturable endophytic fungal strain, identified as a *Thielavia* sp. Investigation of the ethyl acetate extract derived from a culture of this fungus resulted in the isolation and characterization of five new metabolites consisting of four nor-spiro-azaphilones and a bis-spiro-azaphilone together with the known spiro-azaphilone, pestafolide. Although none of the compounds encountered showed any activity in our cell and mechanism-based anticancer bioassays, pestafolide has been reported to have mild antifungal activity. Co-occurrence of these metabolites in this endophytic fungus led us to postulate biosynthetic pathways to all new metabolites starting from pestafolide.

You successfully isolate compounds from endophytic fungi in a plant grown aeroponically. Do you think this method of growth had any influence on the compounds you discovered?

There is evidence that the prevalence of endophytic fungi in a given plant species may be influenced by the environment (ecological factors) in which it grows. We believe that under environmentally controlled greenhouse conditions used for growing plants aeroponically, it may not be essential for plants to harbor many endophytic fungi which may produce metabolites required for their survival in their native environ-

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ments. Although the compounds we discovered in *Thielavia* sp. PA0001 had no activity in biological assays available to us, it is possible that they serve some useful functional role in the host plant.

What is a favorite nonscientific activity of your lab?
We receive many foreign visiting scholars who bring cultural diversity and new ideas to the group. When they leave, all of us get together with food and drinks to bid farewell to them. In addition, we organize an annual Halloween lunch party with international cuisine. We invite interested family members and university administrative staff.

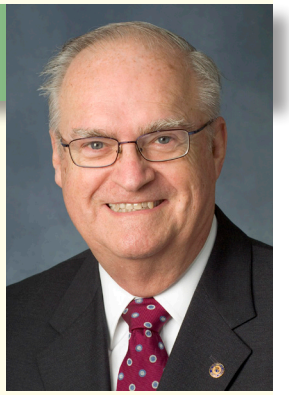
What is your laboratory's motto or slogan?
Our laboratory's motto is "work diligently with colleagues and other scientists from academia and industry worldwide involved in areas related to natural products discovery and development in a mutually beneficial manner," as everything we do with isolation and medicinal chemistry of natural products is focused on our intended common goal of discovery and development of new and effective therapeutic agents for cancer, neurological disorders, and HIV.

What is your greatest extravagance in the laboratory?
We provide our group with whatever resources they need to work efficiently to keep up with our above "motto." The greatest extravagance in our lab is the recent acquisition of a 400 MHz NMR spectrometer which has accelerated our work and collaboration with scientists from developing countries. ■

Physalis alkekengi plant growing under aeroponic conditions

DR. RAPHAEL GRUENER

Former ASP President Mitscher Dies



by Dr. Thomas Prisinzano

Former ASP President and Professor Emeritus, Dr. Lester A. Mitscher, died May 8, 2015, at the age of 83. The research of Dr. Mitscher, former Department of Medicinal Chemistry Chair and University Distinguished Professor at the University of Kansas, ranged from the development and popularization of the use of circular dichroism for structural elucidation to the isolation of novel entities using bioassay-guided fractionation. He specialized in the search for new antimicrobial agents and was a key player in the development of both tetracycline- and quinolone-based agents.

Throughout his highly decorated career, he earned numerous awards and honors, including the Research Achievement Award in Natural Products Chemistry from the American Pharmaceutical Association in 1980, the Ernest H. Volwiler Award for Research Achievement from the American Association of Colleges of Pharmacy in 1985, the Higuchi-Simons Award in Biomedical Sciences at Kansas University in 1986, the Bristol-Myers Squibb Edward E. Smissman Award in Medicinal Chemistry from the American Chemical Society in 1989, fellow of the American Association for the Advancement of Science in 1995, the Division of Medicinal Chemistry Award from the American Chemical Society in 2000, and the Norman R. Farnsworth Research Achievement Award from the American Society for Pharmacognosy in 2007. ■

Editor's Note: *In light of Dr. Mitscher's immense contributions to ASP and natural products as a whole, Dr. Prisinzano will contribute a comprehensive obituary for our Fall issue.*