IBI* SERIES WINNER

Student-Directed Discovery of the Plant Microbiome and Its Products

Endophyte Discovery, an IBI Prize–winning module, allows novice scientists to engage in meaningful discovery through inquiry-based research experiences.

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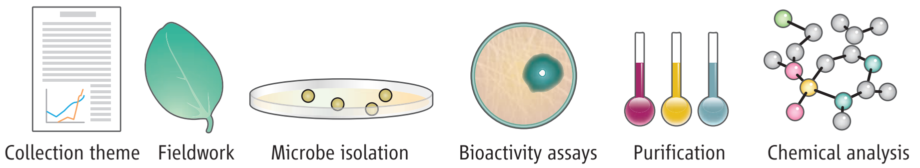
Macroscopic organisms, even when healthy, harbor diverse microorganisms that influence their development, physiology, and fitness. This microbial diversity is accompanied by chemical diversity, which provides a platform for integrative research training spanning the biological and physical sciences. Our programs focus on the development of scientific inquiry skills through the lens of isolating microorganisms associated with plants and characterizing their bioactive natural products.

Endophytes are microbes, such as fungi and bacteria, that inhabit healthy plant tissue without causing disease (*1*). As symbionts, they constitute part of a diverse and ecologically important plant microbiome. Endophyte communities differ in composition among biomes and host species (*2*). They exhibit a wide range of host specificity and often enhance plant health (*2*, *3*). The total number of estimated fungal species is in the millions, yet fewer than 100,000 have been characterized (*4*).

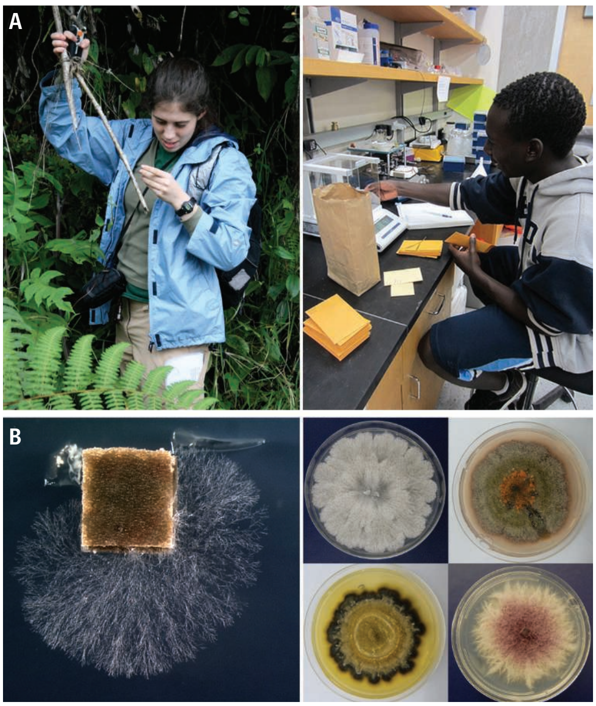
Novel endophytes and their natural products can be isolated on standard microbiological media from the plants that surround our campuses and communities. This accessible biological and chemical diversity provides novice scientists with the raw materials for inquiry-based research experiences that engender project ownership, interdisciplinary training, and the excitement of meaningful discovery.

We have used endophyte discovery as the foundation for a one-semester course at Yale University (*5*); a 10-week summer program at Diné College, the tribal college of the Navajo Nation in rural northeastern Arizona; and a semester-long course at Tucson High Magnet School, an urban high school in Arizona that serves a highly diverse student body. Our collective experience points to the substantial difference between a demonstration laboratory, where experimental outcomes are known, and an inquiry-based opportunity where discovery, exploration, and long-term buy-in are hallmarks of the experience.



Research objectives. [Adapted from (*5*)]

Our programs synthesize lectures, fieldwork, and laboratory experiences. As students isolate and characterize endophytic microbes, evaluate their bioactivity, and examine their chemical diversity, they gain new technical skills that translate into broader research abilities. They develop the capacity to ask scientific questions, test hypotheses, synthesize information, and step beyond the limits of current knowledge. They traverse multiple success points that build progressively in intellectual and technical complexity, which allows them to gain confidence and take intellectual ownership from the earliest stages of their work. Our goal is for these novice scientists to leave behind a fear of "wrong" answers and move on to envision possibilities, exercise their own quality control, develop collaborations, and think critically. Success in our programs is not wholly defined by students' achievement of research goals (listed below), which can yield novel organisms, new compounds, and novel bioactivities—but by the intellectual development that characterizes the most important and often elusive aspects of scientific training.



Fieldwork. (**A**) Students in the field and at the bench. (**B**) Endophytic fungus emerging from a tissue segment and four representative endophyte isolates.

Each of our programs is tailored to its specific target audience, but all are organized around six research objectives (see the first figure). Instructors are encouraged to tailor a course based on their area of expertise and the physical and personnel resources available at their home institution.

We use a parallel-project structure based on individualized but similar activities to simplify mentoring by the instructor and to facilitate peer mentoring among class members (*6*). Note that students need not complete all of the research objectives outlined below to gain a meaningful scientific experience: Adaptations of our courses at Diné College and Tucson High focus on objec-

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tives 1 to 4, for example, with students still gaining significantly in cognitive and technical skills through multidisciplinary, inquiry-based activities. The goals for our programs are as follows:

1. *Development of a plant collection theme.* Students first identify target plants to survey for endophytes. This preparation encourages them to consider diverse aspects of plant biology ranging from taxonomy to ethnobotany (Yale), or to frame ecological research questions with instructor support (Diné College and Tucson High). Allowing students to define and convey their collection themes provides a low-stakes opportunity to participate in empirical design.

2. *Fieldwork.* After a basic introduction to botany, students identify and collect plants for endophyte isolation [see the second figure (A)]. Our collection trips range from half-day excursions to botanical gardens, reserves, and state and national parks to multiweek expeditions to South American rainforests. Field experiences help students observe biology directly, appeal to students with diverse learning styles, and promote teamwork. We have found that fieldwork is transformative for students no matter what their future career choices might be.

3. *Endophyte isolation and characterization.* Endophytes are isolated by surface-sterilizing fresh plant tissue and plating small fragments on nutrient media [see the second figure (B)]. Students characterize the diverse microbes that emerge from the interior of symptomless tissue using traditional microbiology, microscopy, and molecular sequence analysis. They upload DNA sequence data to GenBank, thus contributing directly to the scientific knowledge base, and then use their data to construct phylogenetic trees that reveal the relations of newly discovered endophytes to known relatives. A hallmark of our programs is that individual student observations can be compiled to test larger research questions. We have published three research papers to date detailing novel observations resulting from the efforts of an entire class.

4. *Bioactivity assays.* Endophytes have a demonstrated, but underexplored, potential to make novel chemical products important for human sustainability (*7*). Students select and culture a subset of their endophytes on a larger scale for use in standard bioassays, testing culture filtrates and extracts for enzymatic activities relevant to industry, agriculture, and medicine (e.g., cellulase, ligninase, and protease activity or activity against human and plant pathogens). Each student also identifies an assay in conjunction with a collaborating faculty member and tests the library of extracts compiled by the entire class for activity. These diverse assays (e.g., anti-inflammatories, inhibition of β-amyloid oligomeric assembly, and effects on zebrafish development) allow students to exercise creative imagination and generate collaborations with peers and faculty. Positive results provide an impetus for further research projects beyond the course.

5. *Fractionation and purification.* Positive assays are followed by bioactivity-guided fractionation to isolate the active natural products with thin layer–, column-, and/or high-performance liquid chromatography.

6. *Chemical analysis.* Characterization of active natural products by analytical methods (e.g., mass spectroscopy, nuclear magnetic resonance analysis, and small-molecule x-ray crystallography) usually occurs after the course by alumni who continue their research projects. These analyses are technically challenging, but most students are remarkably motivated to provide a chemical explanation for their biological observations.

Assessment through the Classroom Undergraduate Research Experience survey (CURE) (*8*) indicates that alumni of the Yale program consistently self-report a readiness for more demanding research, greater self-confidence, and a better understanding of the research process. The vast majority (>80%) continue their research in subsequent semesters. Upon graduation, they matriculate into Ph.D. programs at a rate about three times that of other Yale graduates with a science degree. Diné College students highlight this experience in their applications to 4-year institutions and graduate school, and high school students use their experience as an entry point for science fairs, independent projects, and research after matriculating into college.

These discovery-based experiences centered on the biodiversity and chemical richness of endophytes have inspired a diversity of students with substantially different levels of scientific preparedness. Although execution of such an inquiry-based course requires a willingness to accept a level of uncertainty regarding research outcomes, with that uncertainty comes the potential for every student to make original and meaningful observations about the natural world.

About the authors

Authors (left to right). **Carol Bascom-Slack** is a lecturer at Yale University and is a National Academy of Sciences Education Fellow. In addition to the course described here, she teaches a research-based biology course for freshmen and a microbiology course. **A. Elizabeth Arnold** (Betsy) is an Associate Professor in the School of Plant Sciences at The University of Arizona. Her research focuses on the diversity, ecology, evolution, systematics, and applications of endophytic fungi. **Scott A. Strobel** is the Henry Ford II Professor of Molecular Biophysics and Biochemistry at Yale and is a Howard Hughes Medical Institute Professor. His research explores application of endophytic fungi to production of alternative fuels and bioremediation.

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