Research Statement - Morgan Carter

Overview:

My research applies molecular, genetic, and computational tools to understand the partnerships between endofungal bacteria (EFB) and their hosts, within the plant microbial community. For each research theme I propose, I focus on the initial steps that will lead to the development of genome-wide and niche-wide resources and hypotheses to drive future functional studies in the rapidly emerging field of facultative EFB. My research can be tailored for a wide range of funding opportunities due to the relevance of bacterial-fungal interactions to both agriculture and human health. I have outlined funding sources for each project and will apply for an NSF CAREER award.

I am excited about the potential to collaborate at UNC-Charlotte: within the Host-Microbe Interactions Group, other faculty associated with the Biotechnology Resource Center, and beyond. My research requires access to high performance computing and long-read and short-read sequencing, so I expect to work closely with the BRC to achieve my research goals, especially with continuing work on RB-TnSeq (Theme 2 below) and other high throughput technique development. The inclusion of this position as part of a cluster hire is very appealing as I work well in team environments where complementary research interests and skills can build into the most creative projects and problem-solving.

Research experience:

My previous research topics ranged from fungal toxin production as an undergraduate, to plant resistance engineering and bacterial effector proteins as a graduate student, to the genetics of bacterial-fungal interactions as a postdoc. **The diversity of topics and systems I have studied is indicative of my ability to make progress quickly and effectively in non-model systems by making connections and adapting techniques.** From my training, I have a strong skill set in biochemical and microbial techniques, including protein methods, cloning, in planta assays, microbial genetics, and nucleic acid extraction from various organisms, as well as computational analysis such as GWAS, RNA-seq, and genome assembly.

My research experience began at North Carolina State University, where I investigated fungal polyketide synthase gene clusters (Noar et al., 2019). At Cornell University, I discovered an unintuitive confounding effect in an assay used in a previously published study to screen fungal effector genes by bacterial delivery into barley leaves (Carter et al., 2018). Next, I identified barley lines that have a cell death response to the *Pseudomonas* effector AvrPphB, then mapped and functionally characterized the underlying resistance gene (Carter et al., 2019). This work provided new opportunities for engineering novel disease resistance traits in cereals. Finally, I combined my expertise in bacterial and fungal genetics for my USDA NIFA predoctoral fellowship project. I characterized the first effector, Btl19-13, from an EFB (**Theme 1**), finding it to be a secreted effector that impacts the host transcriptome and stress tolerance (Carter et al., 2020). Now, as a postdoctoral researcher, I study the interaction between the endophytic fungus *Pestalotiopsis* sp. 9143 and a bacterial symbiont, *Luteibacter* sp. 9143 (**Theme 2**) as well as plant pathogenic *Fusarium* spp. (**Theme 3**).

Background information:

Endofungal bacteria (EFB; also referred to as endohyphal bacteria, EHB) were first discovered in arbuscular mycorrhizal fungi in the 1970s and have since been found in diverse fungi across ecological niches including endophytes, plant and human pathogens, and soil saprophytes (Araldi-Brondolo et al., 2017). Ranging from transient to obligate, bacterial-fungal relationships are understudied compared to other eukaryotic-bacterial partnerships. **Yet, EFB cause diverse phenotypes within fungal hosts, such as altering plant pathogenicity, reproduction, and nutrition source use, making them an untapped resource for fungal control and manipulation** (Araldi-Brondolo et al., 2017). Studies of EFB can reveal potential targets for modulating pathogenicity and metabolite production. Furthermore, how bacteria can invade and inhabit fungi is critical knowledge for engineering EFB or investigating natural partnerships.

Proposed research program:

The research that I propose conducting on EFB at UNC-Charlotte focuses on the following three themes with indicated focus study systems and detailed initial directions:

1. **Effector biology and type III secretion systems:** *Mycetohabitans* spp. & *Rhizopus microsporus*
2. **Adaptation to an endofungal lifestyle:** *Luteibacter sp.* 9143 & *Pestalotiopsis* sp. 9143

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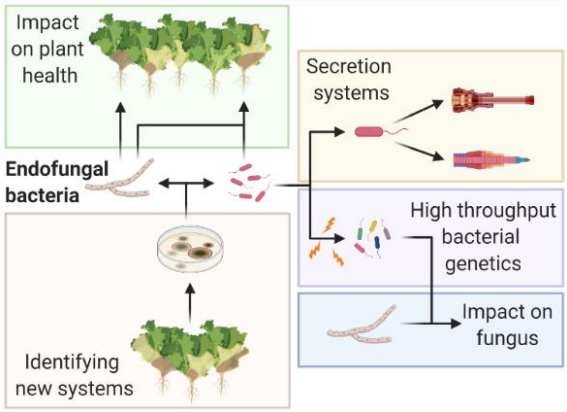
3. **Impact of endofungal bacteria on plant health:** Novel bacteria & *Fusarium* spp.



<u>Theme 1: Effector biology and type III secretion systems (T3SSs)</u>

T3SSs and their effectors are well-studied in plant- and animal-associated bacteria, where they are critical for protein exchange in symbiotic interactions and inform disease control strategies, but are not well studied in EFB. I will use my background in effector protein biology and partnership between *Mycetohabitans* spp. and *Rhizopus microsporus*, a plant and human pathogen, to probe EFB T3-secreted effectors.

***A. Investigate the mechanism of Btl effector proteins*:** Building on my graduate work on Btl19-13, I will spearhead a collaboration with the Bogdanove Lab at Cornell University to identify interactors with Btl proteins (yeast 2-hybrid and ChIP-seq) and assess diversity of *btl* gene presence and role across EFB; I have sequenced and analyzed genomes of *Mycetohabitans* spp. to survey *btl* gene content (*in prep*). **Future work will determine *btl* gene presence, role, and protein function across symbionts.**

B. Characterize type III secreted effector repertoires: Btl proteins are the only known T3SS-compatible *Mycetohabitans* proteins, but they are not required for invasion (Carter et al., 2020), necessitating additional study to identify the effectors critical for infection. Effectors in *Mycetohabitans endofungorum* strain B13 will be identified using computational signal prediction and a strain I created that overexpresses the T3SS master regulator, *hrpB*. **Future work will focus on characterization and identification of core *Mycetohabitans* effector genes** shared with related EFB, like *Burkholderia* sp. 9120 (Baltrus et al., 2017).

<u>Strategy for funding:</u> (A) NIH R01 (B) NSF MCB or IOS

<u>Theme 2: Adaptation to an endofungal lifestyle</u>

In contrast to *Mycetohabitans* spp., many facultative EFB lack a T3SS or T2SS, but do have a T6SS. Beyond secretion systems, genes such as metabolic transporters may play a role in adaptation to an endofungal lifestyle and need to be identified in a high throughput manner to accelerate characterization. I will identify genes critical for an endofungal lifestyle using the *Luteibacter* sp. 9143 and *Pestalotiopsis* sp. 9143 system, a model for the most transient EHB relationships that seem common in tree endophytes from Dikarya but have not been investigated from a molecular standpoint.

A. Determine the role(s) of type VI secretion systems in facultative EFB: T6SSs are often involved in inter-bacterial antagonism, but may be tied to metal scavenging, pathogenicity, and motility (Jurenas and Journet, 2020). I created T6SS mutants of the facultative EFB *Luteibacter* sp. 9143 (Baltrus et al., 2017) to determine why the T6SS is upregulated when co-cultured with its host, *Pestalotiopsis* sp. 9143 (Shaffer et al., 2021). I propose to identify the role of the T6SS through competition assays, quantitative re-association assays, motility assays, etc. Notably, experimental characterizations like these make excellent undergraduate research projects. **Future work will determine, compare, and contrast the role of the T6SS in other EFB from the same ecological niche, broadening our understanding of roles for T6SSs.**

***B. Identify fitness contribution of genes for invading and inhabiting fungi*:** Beyond secretion systems, genes such as metabolic transporters may play a role in adaptation to an endofungal lifestyle and need to be identified in a high throughput manner to accelerate characterization. Using my experience in bacterial genetic manipulation and genome-wide computational tools, I have developed a random barcode transposon mutagenesis (RB-TnSeq) (Wetmore et al., 2015) library of *Luteibacter* sp. 9143. This library will be used for high-throughput evaluation of gene fitness contributions under various conditions, including nutrient stress and fungal reassociation. **Future work can follow up on genes identified as important for fungal association and other conditions, or on applying this pipeline to other EFB to identify conserved mechanisms.**

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Strategy for funding: NSF Understanding the Rules of Life: Microbiome Interactions and Mechanisms program - an expansion of my withdrawn NSF PRFB that was recommended for funding.

Theme 3: Impact of endofungal bacteria on fungal pathogenicity

Plant-associated *Fusarium* spp. have been reported to have EFB, but surveys of the most agronomically important *Fusarium* spp. are lacking (Dean et al., 2012; Obasa et al., 2019; Shaffer et al., 2017). In addition to continuing my current work, I propose to collaborate with other field pathologists across the state to screen for novel EFB in fungal pathogens important to North Carolina agriculture and determine whether they impact fungal pathogenicity.

A. Impact of endofungal bacteria on plant health: In collaboration with Dr. Barry Pryor (University of Arizona) and Dr. Gary Bergstrom (Cornell University), I aim to assess EFB presence, diversity, and impact in their field isolates of *Fusarium oxysporum* f.sp. *lactucae* (Fol) and *Fusarium graminearum* (Fg), pathogens of lettuce and grains, respectively. Identifying EFB model systems for studying pathogenicity control in relevant fungi is a priority for funding and application of EFB studies. **Amenable partnerships will be used in future work to investigate pathogenicity mechanisms or other fungal behavior.**

Strategy for funding: Current USDA AFRI EWD Postdoctoral fellowship; Future USDA AFRI FAS Program on Pests and Beneficial Species in Agricultural Production System, submitted as a New Investigator Seed Grant.

Strategies for Inclusion and Equity through Mentoring and Advocating:

Beyond my formal research plan, I wanted to comment on my commitment to diversity, inclusion, and equity within my laboratory. Mentored research experience is critical to empower graduate and undergraduate students to be independent scientists and competitive for graduate programs, fellowships, and careers. As such, **I am excited about being an engaged mentor and supporting a diverse group of trainees at UNC-Charlotte - I will be intentional in the accessibility of my group and the community that I foster.** I believe in an open, cooperative environment and one concrete example of this is my use of an electronic lab notebook, so resources can be shared, catalogued, and edited collaboratively so no one is left out. I look forward to working with undergraduate students through summer programs as well as during the academic year for course credit and/or hourly wage. I will strive to properly compensate undergraduates, especially to improve recruitment and retention among students of color and first-generation students who view pay as the largest barrier to gaining research experience (Jensen et al., 2021). I believe everyone does their best work when they feel comfortable taking the time they need for life outside of the lab, so I encourage work-life balance and activities outside of the lab, whether that be family care, career exploration, hobbies, or skill development.

Beyond mentorship, I am an advocate for my mentees and enable exploration of independent projects, nominate them for awards, and facilitate their professional development. I have informally mentored many graduate students through submitting applications for USDA predoctoral fellowships and NSF GRFPs, and I have facilitated numerous workshops for career exploration for trainees at the school and society levels. I am careful to know the career goals of students I work with and tailor their experiences to those goals, while suggesting relevant activities and opportunities out of the lab. In this way, I have connected mentees to summer positions and new jobs.

I believe strongly in giving credit to trainees of any stage through authorship and in increasing trainee networks through collaborator meetings, conference attendance, and interaction with visiting scientists; three undergraduates I have worked with are authors on papers I have published. I am proud to have mentored students from a range of backgrounds, including first-generation college students, people of color, and gender minorities. I recognize that many of my students will need other mentors and communities that share aspects of their identity that I do not share, who can provide mentorship and comradery grounded in their experiences. Thus, I will encourage and facilitate, through financial support, their engagement in communities and conferences focused on relevant minoritized groups like SACNAS, MANRRS, and ABRCMS.

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