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Volatiles Produced by *Serratia marcescens* and Their Inhibitory Effects on *Rhizopus stolonifer* and *Neurospora crassa*

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Derreck Adam Carter-House

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Dissertation Committee:

Dr. Jason E. Stajich, Chairperson
Dr. Katherine Borkovich
Dr. James Borneman

The Dissertation of Derreck Adam Carter-House is approved:

Committee Chairperson

University of California, Riverside

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I am grateful to my advisor, without whose help, I would not have been here.

To all my incredible teachers I have been blessed with, from Ms. Rupp in third grade who took the extra time to make me feel special to Mrs. DePriest in high school who showed me that science could be a career.

To my family for your patience amidst the questions I had growing up regarding how things worked or why or "do mosquitoes poop and pee?"

To Benson. It's because of you that I am here today. Thank you for everything you do.

ABSTRACT OF THE DISSERTATION

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Derreck Adam Carter-House

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Dr. Jason E. Stajich, Chairperson

Abstract: Bacteria and fungi have shared similar niches for millions and possibly billions of years. Researchers often study the antagonism between microbes to produce novel, effective antimicrobials, but stop short of the large compounds secreted into the nearby environment. Here I show that bacteria produce anitfungals that can volatilize and inhibit fungal growth from a distance. These are even produced at high enough levels to inhibit some of the fastest growing fungi like *Neurospora* and *Rhizopus*. Further, I explore how the fungi sense, react, and protect themselves from bacteria in their vicinity through their transcriptional response to pure volatiles. This contribution to the growing field of bacterial-fungal interactions highlights the importance of volatiles for long distance interactions between microbes.

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Chapter 1

Introduction: Bacterial-Fungal Volatile Interactions

1.1 Abstract

Fungi are important, beneficial, eukaryotic organisms that can live as saprophytes, degrading organic matter and as symbionts associating with hosts in mutualistic or pathogenic lifestyles. Fungi that are pathogenic to their hosts can devastate plants and animals in agricultural and environmental contexts and cause severe disease in humans. It is important to understand these fungi and their lifestyles to develop necessary tools to manage and treat disease in agricultural, veterinary and clinical settings. While there are many ways to treat fungal infections, the most powerful technique is prevention. Volatile compounds emitted by microbes and plants have been shown to be an important tool for battling fungi and could provide insight into how these microbes are interacting and fighting in their natural

environments. Fungi can have a negative impact on animals and plants that we are only beginning to understand. However, with our increased understanding of the threat that fungi can pose, we can prepare for the future, and potentially use applications of fungal-bacterial volatile interactions to do it.

1.2 Fungal Impact on Animal Health and Agriculture

Climate fluctuations as well as trade and transportation, have opened up new environments for fungi. That, coupled with their ability to survive long periods in adverse conditions, high virulence and opportunistic pathogenicity has helped to create world-wide epidemics affecting plants and animals [Fisher et al., 2012]. Wildlife, especially endangered species, have felt the impacts of fungi over the last two decades. In fact, fungi are one of the top five leading causes of endangered species extinction [SMITH et al., 2006]. The list of devastation continues to grow each year and includes a variety of mammals (including humans), amphibians and insects. Some examples of the impact of fungi on animals includes the rise of White Nose Syndrome (WNS). WNS is caused by a fungus that can grow at cave temperatures and disrupts the hibernation of bats. This disease, which has decreased the bat population in the eastern US by 75%, is due to the fungal pathogen, *Geomyces destructans* [Lorch et al., 2011]. This decrease in bat population has ecological side effects including reduced insect control, plant pollination, and seed dissemination [Blehert et al., 2009]. *Batrachochytrium dendrobatidis* has affected amphibian populations all over the world. This fungi has a broad host range of over 350 different species [Fisher et al., 2009]. Interestingly though, the signs and symptoms for chytridiomycosis are minimal despite the fact that this

disease has wiped out entire species of frog and drastically reduced the populations of many others. *Nosema bombi* has been driving down the bee population reducing their numbers by up to 96% [Cameron et al., 2011]. Even coral are affected by fungi, *Aspergillus* has nearly eradicated seafan sites off the coast of Florida [Kim and Harvell, 2004]. For humans, the outlook is not any better as resistance mechanisms for these pathogens are being developed in both medicine and agriculture through the use of antifungals [Fisher et al., 2018].

Fungal diseases in humans range in severity from dermatological irritations like vaginal Candidiasis and *Trichophyton, Microsporum,* and *Epidermophyton* infections known as ringworm, that cause disease in about 25% of the world's population [Havlickova et al., 2008], to *Aspergillosis, Cryptococcus* and *Coccidioides* causing hundreds of thousands of deaths each year. To further complicate matters, fungal infections are difficult to treat. Unlike bacteria, fungi are eukaryotes and as such they share many similarities with their plant or animal host, such as multicellularity as well as many proteins and biosynthetic pathways. This characteristic makes it difficult to target in medicine, as the drug should target the fungi, not human organelles or proteins. Finding these fungi specific targets requires phylogenetic analysis or domain analysis can help to identify minor differences in proteins between a host and the fungal pathogen [Bencurova et al., 2018]. Of course, all of this research and hard work is futile if the fungi can development resistance to novel drugs, adding to the complexity of treating infections [Carter-House, 2019].

Fungi can be deadly, killing more people than malaria [Ballou, 2017], and causing approximately 1.5 million deaths worldwide every year [Almeida et al., 2019]. Approximately 97,000 Americans die from hospital-related fungal infections each year alone; ninety

percent of these often deadly infections are caused by just three common fungi, *Candida albicans* and *Aspergillus fumigatus* [Stein et al.,] and *Cryptococcus* [Ballou, 2017]. In addition, 1 billion people suffer worldwide from fungal infections [Almeida et al., 2019]. It is predicted that fungi with continue to adapt to rising temperatures which poses an increased risk to humans [Casadevall, 2018]. In a horrifying example, early diverging fungi can cause infection as seen when *Mucor sp.* contaminated baby formula led to Mucormycosis in a newborn [Vallabhaneni et al., 2015]. Many fungal infections are opportunistic, these infections are typically connected to a weak or suppressed immune system. However, some fungal diseases can infect healthy individuals living in specific regions. One area of high infection in the US is in the Southwest desert. In this region Coccidioidomycosis is a major concern for California farmers. Coccidioidomycosis, also known as Valley Fever, is a disease contracted by about 14,000 people every year in the United States that can cause fatigue, coughs, fevers, shortness of breath, headaches, night sweats, muscle aches, joint pain, and rashes. Symptoms persist for a few weeks to months.

Natural disasters can spur fungal infections such as the Joplin tornado [Benedict et al., 2018, Etienne et al., 2012] and hurricane Harvey [Chow et al., 2019]. In these places of mass destruction coupled with flooding early diverging fungi like *Rhizopus* and *Mucor* produce many asexual spores that are then spread by wind or drafts infecting open wounds [Neblett Fanfair et al., 2012]. Battlefield injuries are also highly susceptible to fungal infections due to dirty, heavy clothing and outdoor environments in contact with wounds from explosive devices [Tribble and Rodriguez, 2014]. *Actinomucor elegans* infection lead to its first reported fatality due to injuries sustained in Kuwait [Tully et al., 2009]. A variety of

mucromycetes have been reported to infect humans over the last 100 years (*Rhizopus species* 233 cases, *Mucor species* 85, *Cunninghamella bertholletiae* 34, *Apophysomyces elegans* 27, *Absidia species* 25, *Saksenaea species* 6, *Rhizomucor pusillus* 19), the majority of cases are caused by *Rhizopus*. Other early diverging fungi can cause disease as well (*Entomophthora species* 13, *Conidiobolus species* 10, *Basidiobolus species* 9) [Roden et al., 2005]. Fungi like *R. microsporus* are a growing problem in the health community and are not susceptible to voriconazole and fluconazole [Caramalho et al., 2017]. While these diseases are uncommon they are difficult to treat and can take months to cure, if the patient survives that long. The most effective of three known antifungals that was shown to be effective against these fungi was amphotericin B. Unfortunately, Amphotericin B is very old, introduced 50 years ago, demonstrating the lack of antifungals available to combat these infections. In addition,

Fungal pathogens have an even greater impact on plants. There are nearly 19,000 known fungal plant pathogens causing many disease symptoms like leaf spot, rust, wilt, blight, coils, scab, gall, canker, damping-off, root rot, mildew, dieback, and death (Jain et al. 2019). This variety of disease symptoms usually arise as a result of the plant and fungal interaction and are a testament to the many fungal infection strategies. Some fungi do very little damage to plants, some quickly kill the plant. Fungal biotrophic pathogens keep the plant alive during infection however can reduce yield by living with the plant and utilizing its nutrients. Necrotrophic fungi, on the other hand, or kill crops and then consume nutrients. Regardless of how the pathogen infects the result of disease is reduced return for many farmers throughout the world, especially as climate change continues to create drought and stress crops [Lindsey Pedroncelli, 2019].

Many different fungi are pathogenic to major food staples, some of the most infamous include *Magnaporthe oryzae, Puccinia graminis, and Ustilago maydis.* These pathogens can cause major losses in production. In rice 10-35% of yield can be lost due to Rice blast *Magnaporthe oryzae* [Talbot, 2003]. Wheat has a Basidiomycete pathogen called *Puccinia graminis* [Leonard and Szabo, 2005], and in Kansas alone, a major wheat producer of the United States, rust disease can account for over 20% of yield losses [Gaelle F. Hollandbeck and Todd, 2019]. The emergence of UG99 threatens the worlds wheat production as 90% of the wheat varieties grown are not resistant as of a few years ago [Singh et al., 2011]. Corn smut, *Ustilago maydis*, which replaces the kernels will large galls filled with fruiting bodies causing around 20% crop loss [Godfray et al., 2016]. To prevent these infections antifungals such as azoles (Imazalil, oxpoconazole, triflumizole, diniconazole, epoxiconazole, flutriafol), succinate dehydrogenase inhibitor (Carboxin, Benodanil, Thifluzamide, Furametpyr), methyl benzimidazole carbamate (Benomyl), Anilinopyrimidine (Cyprodinil), Qo inhibitor (Azoxystrobin) are typically applied through soil treatments, seed treatments or foliar sprays [Brauer et al., 2019]. Azoles (and Morpholines) are a class of antifungals that inhibit the production of ergosterol synthesis and important compound in the fungal cell membrane [Campoy and Adrio, 2017]. Succinate dehydrogenase inhibitor reduces respiration of fungi by binding to the ubiquinone-binding site of mitochondria [Avenot and Michailides, 2010]. Methyl benzimidazole can prevent microtubule assembly in the fungal hyphae and stop growth [Davidse, 1986]. Anilinopyrimidine inhibits methionine synthesis (an important amino acid) and secretion of hydrolytic enzymes [Chapeland et al., 1999].

However, these do not provide protection for fruits after they are harvested. In addition to human disease, *Rhizopus* is a common postharvest rot of many fruits and vegetables such as stone fruit, grapes, berries, tropical fruits, tomatoes, and tubers [Coates and Johnson, 1997] and can reduce the store shelf life of many other fleshy fruits and vegetables as well [Wang et al., 2013b]. For post harvest disease increased levels of CO_2 or lowering of the temperature help to slow the rotting and proliferation of *Rhizopus* and other fungal rotters while the food is in storage. Recently, more work is being done looking at how different organisms like *Bacillus* can work to help prevent *Rhizopus* postharvest rot [Wang et al., 2013b].

Some of the most famous microbiology work has revealed the role of microbial compounds, such as Penicillin [Fleming Alexander and Wright Almroth Edward, 1922] or homoserine lactone and quorum sensing [Bassler, 1999, Atkinson and Williams, 2009]. Since then a great deal of work has been done to study microbial interaction such as the production of specialized metabolites like volatile and water-soluble compounds for communication [Schmidt et al., 2019]. Another study showed that bacteria use fungal hyphae as a highway, specifically utilizing the moist mucoromycete hyphae to travel great distances [Zhang et al., 2018]. Additionally, bacteria have been shown to domesticate fungi by supplying a necessary protein, only produced by the bacteria, to the fungal host to allow asexually reproduction ensuring they pass on with the next generation of fungi [Partida-Martinez et al., 2007].

1.3 Bacterial Tactics for Dealing with Fungi

Bacteria and fungi are found ubiquitously and in constant contact, found on every continent, helping form the foundations of nutrient cycling, from saprophytic to pathogenic they utilize many energy sources most other organisms cannot. Working against each other for at least the last billion years [Berbee et al., 2017], bacteria and fungi have developed an arsenal of tools and chemicals for warfare. Microbial attacks on fungi take many forms such as competition for rare nutrients (Siderophores), cell wall attack (Azoles, Polyenes, Capsofungin acetate), and toxification. The bacteria of genus *Serratia* have been studied extensively for agriculture and medicinal research and to explore this rhizobacteria's ability to inhibit fungal growth. *Serratia* research has revealed that it has many metabolites and proteins that have antifungal properties such as chitinases [Wang et al., 2013a], prodigiosin [Darshan and Manonmani, 2015], and other components of the type-6 secretion system [Trunk et al., 2018].

Microbes like bacteria and fungi must compete for space and nutrients in many different environments and iron is a limiting resource for many microbes. A common technique for excluding enemies is to have superior sequestration of iron [Griffin et al., 2004]. Siderophores, proteins that help in sequestration of iron, are ubiquitously produced by microbes [Saha et al., 2013], and are key to the critical process of iron acquisition from the environment [Angerer et al., 1992]. Weaponizing siderophores, similar to the "Trojan horse" microbes can poison enemies. They do this by coupling a siderophore with an antimicrobial compound, to sneak in toxins. Some examples of this phenomenon are used as antibiotics albomycins, ferrimycins, danomycins, and salmycins [Miethke and Marahiel, 2007].

The fungal cell wall is partly made up of chitin polymers and is integral for retaining cytoplasm and hyphal morphology. Chitinases, are enzymes that can cleave β(1,4)-linked homopolymer of N-acetylglucosamine that makeup cell walls [Lenardon et al., 2010]. Bacterial-produced chitinases have been studied as they produce many chitinases that may be used in the breakdown of chitinous cell walls in insects and fungi [Duzhak et al., 2012, Parani et al., 2011, Downing et al., 2000, Meziane et al., 2006]. Another mechanism for cell wall disruption is through the destruction of another important fungal cell wall component β(1,3)-glucan mediated by caspofungin acetate. Caspofungin, isolated from *Glarea lozoyensis*, has been shown to be an effective treatment for humans with a variety of fungal infections. It inhibits the function of the enzyme β(1,3)-D-glucan synthase, preventing cell wall construction [McCormack and Perry, 2005, Letscher-Bru and Herbrecht, 2003]. Production of ergosterol, a fungi-specific cholesterol present in the cell membrane, is inhibited by azoles. These are the most common type of antifungal and have been around since the 1970s [Vicente et al., 2003]. They bind to 14-demethylase CYP51 (a member of the cytochrome P450 family) stopping the ergosterol biosynthetic pathway, unfortunately, fungal resistance to azoles is achieved through amino acid changes in the CYP51 [Price et al., 2015].

Using all of these mechanisms and more in tandem can help prevent simple mutations from lending resistance to fungi. In fact, bacterial biocontrols often utilize many antagonistic methods at once, making them a great tool for growers. Inoculation techniques for biocontrols can be simple and includes bacterialization (coating of seeds), soil inoculation in pots or in the field [Souza et al., 2015] facilitate the introduction of these beneficial

microbes to a variety of agricultural crops. Many species are currently used for biocontrols such as *Bacillus, and Serratia* and research has shown interesting mechanisms they deploy to fight fungal pathogens. *Bacillus spp* have many non-ribosomal peptide synthetases to help metabolize a wide variety of antimicrobials making it a popular biocontrol [Fira et al., 2018]. Among the antimicrobials are bacteriocins which can disrupt targets in non-self cell walls, as well as fatty acid chains, and can act as surfactants to destroy cell walls [Shafi et al., 2017]. *Bacillus* can be used to help trees battle Polyphagous Shot Hole Borers' fungal gardens, and is effective at inhibiting many fungal pathogens such as *Fusarium euwallaceae* [Na, 2016]. *Serratia spp* generally produce chitinases that digest fungal cell walls [Chen et al., 2017, Duzhak et al., 2012, Gutiérrez-Román et al., 2012]. This rhizobacterium promotes plant growth as well as defends it from fungal pathogens through stimulating plant defense by priming a plant for an attack, an important biocontrol technique for combating disease. Additionally, *Serratia* has the biosynthesis pathways for indole acetic acid which acts as a growth stimulant for plants [Selvakumar et al., 2008]. *Serratia* has even been shown to secrete a potent anti-oomycete compound, Oocydin [Srobel et al., 1999]. Recently this bacteria's type vi secretion system has been shown to play an important role in fighting a pathogenic yeast [Trunk et al., 2018]. While the function of the protein is unknown knockouts of the protein and the syringe delivery system disrupted *Serratias* ability to outcompete the yeast in the same media. Recent research has shown that *Serratia* has mycophagous abilities against many types of fungi including *Rhizopus* [Hover et al., 2016].

While these methods, metabolites, and proteins are effective during direct interactions, microbes have also developed the ability to defend their turf from a distance. Small

organic compounds with low vapor pressure volatilize and can serve as fungal inhibitors and can even cause morphological changes to hyphae as with *Burkholderia tropica* volatiles and *Fusarium culmorum* and *F. oxysporum* [Tenorio-Salgado et al., 2013]. These transcriptome profiles can lead to potential roles/targets of the volatile compounds. For example, using transcriptional response data of Penicillium, volatiles from *Saccharomyces cerevisiae* were found to affect the transcription of tRNAs [Liu et al., 2014]. Volatiles foster indirect interactions between microbes and perceiving volatiles can help microbes gather information about the environment, these signals are called infochemicals [Wheatley, 2002]. Plant volatiles can be an effective method of controlling postharvest rot (*Altenaria alternata, Botrytis cinerea, Rhizopus stolonifer, Aspergillus niger,* and *Fusarium sp.* on many types of fruit [Taghavi et al., 2018]. *Botrytis cinerea* sporulation in grape bunches was reduced by over 50% using thyme oil or massoialactone from *Cryptocarya massoia* [Walter et al., 2001]. *Aspergillus* and *Rhizopus* growth was prevented using chitosan and *Origanum vulgare L.* (oregano) essential oil [dos Santos et al., 2012]. At 500 ppm volatiles of Lemongrass essential oil were able to prevent spore germination as well as spore production of *Colletotrichum coccodes, B. cinerea, Cladosporium herbarum, R. stolonifer,* and *A. niger* on potato dextrose agar. The lemongrass volatile profile was analyzed with gas chromatography coupled with mass spectrometry and revealed it was composed of many terpenoids such as limonene (4%), Citronellal (1%), Neral (32%) and Geranial (41%) [Tzortzakis and Economakis, 2007].

While biocontrols such as *Pseudomonas, Bacillus,* and *Serratia*, can help defend plants or prevent infection, little is known about what kind of volatiles they produce and their effect on potential pathogens in the soil. *Pseudomonas fluorescens, P.*

putida, and *P. chlororaphis*, are ideal biocontrols because they grow quickly, persist in the plant rhizosphere, compete with other microbes, and produce many antimicrobials [Weller, 2007]. One of these antimicrobials is phenazine-1-carboxylic acid and other derivatives, 2,4-diacetylphloroglucinol, pyrrolnitrin, and/or pyoluteorin which help to suppress disease through preventing the formation of biofilms, and the quick growth of *Pseudomonas* helps them competitively colonize and survive in the rhizosphere microbiome [Weller, 2007, Mazzola et al., 1992, Yu et al., 2018]. *Serratia* can produce many volatiles commonly associated with enterobacteria, but many studies stop short of exploring the uniqueness of the *Serratia* profile and its applications. Although volatiles produced by plants and other microbes are now being shown to inhibit fungal growth, they rarely are analyzed. One comprehensive study, however, found that *Bacillus* and *Pseudomonas* produced many different volatiles such as sulfides, ketones, and benzenes, that inhibited the growth of *Fusarium sp.*, *C. gloeosporioides*, and *P. cinnamomi*. Unfortunately though, many studies have shown the effect of volatiles but fail to identify the compounds.

Other studies identify the compounds but do not explore the applications. The studies that have identified compounds have shown that many bacteria create their own unique profiles of volatiles. This illustrates the bounty of antimicrobials that could be present in such a variety of volatile profiles. In fact, the profiles are so different that many researchers in the field believe that microbes can be identified through them. Which is why the microbial volatile database, mVOC2, was developed. It is a curation of the volatile profiles published for bacteria or fungi [Lemfack et al., 2018]. Although the abundance

and presence of many volatiles is still variable and further data and exploration of more conditions is necessary. 1.1

A few studies analyzing *Serratia spp* volatile profiles show that they produce dimethyl disulfide [Groenhagen et al., 2013, Kai et al., 2010], ketones (2-nonanone, 2-heptanone, 2-undecanone) [Popova et al., 2014], and sodorifen (a *Serratia* specific volatiles with an identified biosynthesis pathway) [Weise et al., 2014]. A study looking at the *Serratia* volatile profile explored the ability of Arabidopsis to sense a rhizobacterial partner. They found that there were metabolic shifts due to the volatiles, but it was not clear why or how [Wenke et al., 2019].

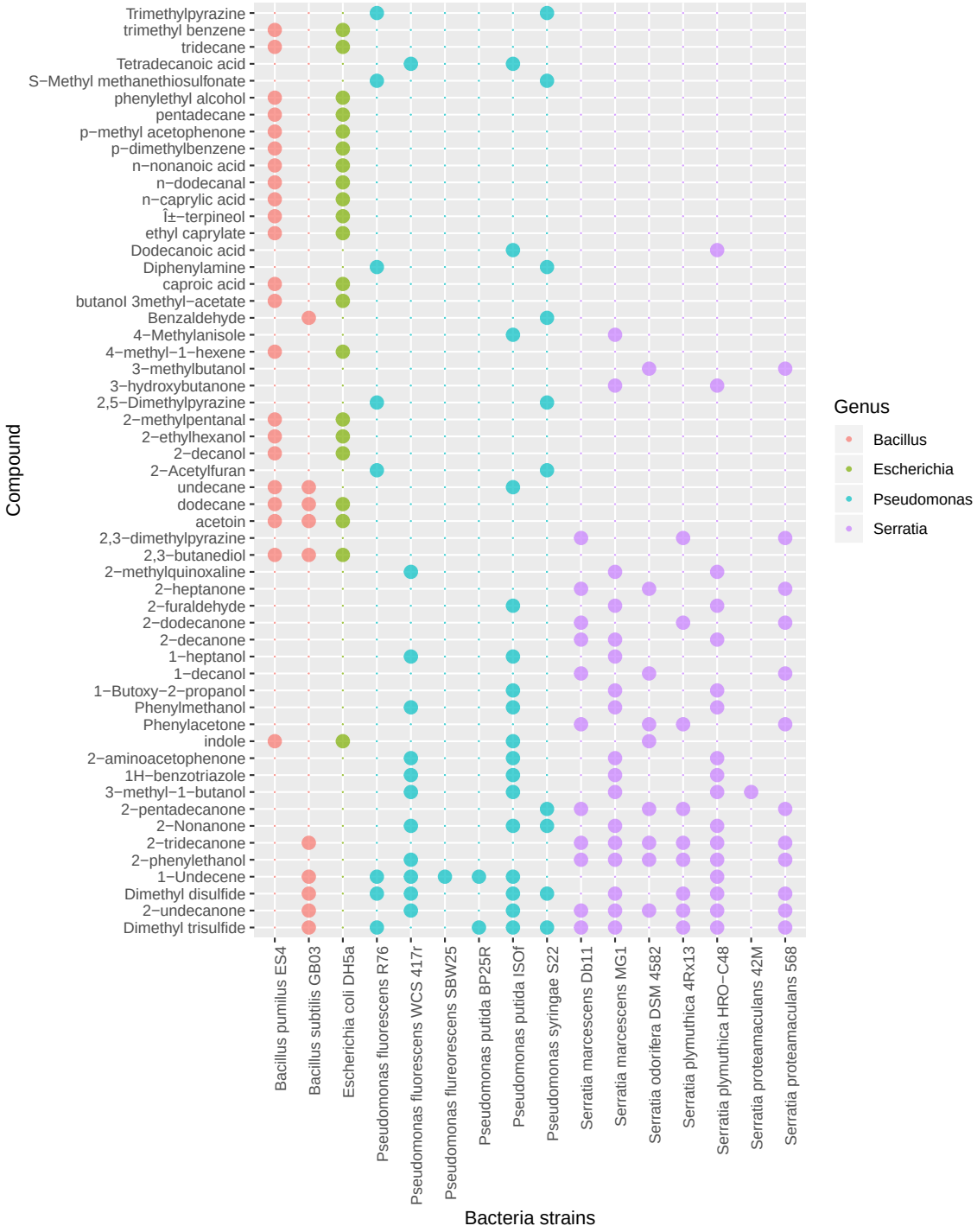
1.4 Looking to the Future of Microbial Volatiles Research and Applications

Small organic compounds that volatilize and diffuse throughout the environment provide a method for microbes to have long-distance communication [Briard et al., 2016]. This long-distance communication may be useful to stop fungal infections from a distance.

Currently, the recommended practice to target these human pathogens and prevent resistance development is to combine therapies [Beardsley et al., 2018]. This mimics the volatiles produced by bacteria and plants as they typically have several antifungal compounds which could prove to be a strategy for reducing instances of resistance in fungi. Additionally, using volatiles as antifungals farmers may be able to combat fungicide pollution.

Bacterial-fungal interactions via volatiles is a new an emerging field with many unforeseen applications and lessons to be learned. However, the advancement of this research and its applications may be able to help address antifungal resistance, reduce symptoms and fatalities, and improve crop yields in the future.

Figure 1.1: Bacterial volatiles reported by the mVOC database for four genra of bacteria



Chapter 2

Characterization of the Volatile Profile of *Serratia marcescens, S. proteamaculans* and *Bacillus subtilis*

2.1 Abstract

I have demonstrated that the bacteria (*Serratia marcescens*, *Serratia proteamaculans*, and *Bacillus subtilis*) produce small molecular weight volatile compounds that inhibit or slow fungal growth. I found bacterial volatile profiles are fungistatic and inhibit the growth of several tested fungi. These include the zygomycete fungus and common post-harvest pathogen, *Rhizopus stolonifer* (black bread mold), the genetic model organism

Neurospora crassa and several other ascomycete and zygomycete fungi. Chemical analysis by mass spectrometry demonstrated that the bacterial cultures produce multiple volatile compounds, a subset of which are unique to each species. Based on profiling of the volatiles, I identified compounds to test individually for their specific activity on fungal growth. This work demonstrates that bacteria can produce small, volatilized compounds that are fungistatic and potentially fungicidal.

2.2 Introduction

Interactions between fungi and bacteria range from mutualistic to antagonistic, relying heavily on sensing and reacting to each other's presence. Advances in imaging and next-generation sequencing are revealing many niches where fungal and bacterial communities coexist, communicate and cooperate. In fact, in the last five years over 300 studies have shown how bacteria and fungi interact [Deveau et al., 2018]. Metagenomic analyses show organisms' presence or absence leading to an understanding of microbes involved in community assembly, such as the symbioses of cyanobacteria and fungi to form lichens [Meiser et al., 2017]. Competitive interactions have also been studied with this approach, such as populations among bacteria and fungi in the topsoil [Bahram et al., 2018], or community dynamics of microbes in extreme environments [Gonzalez et al., 2018]. However, studying metabolites reveals the mechanisms underlying the dialog between fungal and bacterial taxa. For example, the bacterial effectors Tfe1 and Tfe2 are produced by *Serratia marcescens* in response to the presence of *Candida albicans*, a common human fungal pathogen. These effectors are among the first discovered showing that bacteria can sense fungi and deploy

effector proteins to subdue fungi. In this instance, the consequence of the effectors are to reduce the fungal population by two orders of magnitude under lab conditions [Trunk et al., 2018]. Due to competition for space and resources, bacteria and fungi recognize each other and can attempt to obtain more resources through the use of chemicals to out-compete their microbial rivals. The natural ability of many microbes to produce and secrete potent anti-fungal compounds has led to the commercialization of certain microbes as biological control agents. For example, preparations of *Serratia plymuthica* are marketed as a strawberry disease biocontrol (Rhizostar ®, Tradecorp) that reduces Verticillium wilt by up to 18.5% and *Phytophthora cactorum* root rot by up to 33.4% [Kurze et al., 2001]. The molecular mechanism of fungal repression by this bacteria is not known but was proposed to involve multiple secondary metabolites such as prodigiosin, siderophores, haterumalides, and the production of degradation enzymes glucanases, chitinases, and proteases. Metabolites and enzymes can be detected in the soil near plant roots at elevated levels after the addition of *S. plymuthica*. As part of the arms race between partners, having the capability to perceive, avoid, or respond to bacteria would be an advantage for fungi that encounter these microbes in the soil. The fungus *Aspergillus nidulans* activates secondary metabolites after physical contact with *Streptococcus* which could potentially serve as a defense. These metabolites are similar to lecanoric acid which has been proposed to inhibit ATP production [Schroeckh et al., 2009]. Interestingly these metabolites were also shown to be expressed during lichen symbiosis and serve a role in selecting compatible partners.

Being able to communicate with other microbes is not always about warfare, sometimes they can lead to a partnership as is with the case of *Burkholderia* and *Rhizopus*. The

toxin Rhizoxin supports the destruction of roots when fungi infect rice and was initially thought to be produced by the identified fungus *Rhizopus oryzae* [Partida-Martinez and Hertweck, 2005]. However, further work found that *Burkholderia* bacteria inhabiting *Rhizopus* hyphae encoded the biosynthetic pathway for Rhizoxin, making this partnership key in rice seedling blight. It is important to note that this level of intimate communication is not uncommon for early lineages of fungi, such as *Rhizopus*. In fact, research has shown that many harbor ecto- and endohyphal bacteria that assist in asexual reproduction, nitrogen, and carbon utilization [Uehling et al., 2017, Partida-Martinez et al., 2007, Li et al., 2017, Hoffman et al., 2013, Desirò et al., 2018, Shaffer et al., 2017, Pawlowska et al., 2018]. Understanding these relationships are important as findings have yielded many benefits including furthering our understanding of nutrient cycling in ecosystems, fermented foods and beverages, and antibiotics [Frey-Klett et al., 2011]. Another form of long-distance communication between fungi and bacteria takes place through volatiles [Briard et al., 2016] which can alter morphology and biomass. These studies and more illustrate the potential for bacteria producing antifungal metabolites to serve as important components of fungal disease management.

Bacteria producing metabolites	**Fungi/Oomycete inhibited by bacterial metabolites**	**Reference**
Bacillus megaterium	*Aspergillus flavus*	[Mannaa et al., 2017]
Bacillus subtilis	*Aspergillus niger*, *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fonsecaea pedrosoi*, *Fusarium oxysporum*, *Monilinia fructicola*, and *Penicillium expansum*	[Popova et al., 2014] [Machado et al., 2010] [Minerdi et al., 2009] [Vespermann et al., 2007]
Serratia proteamaculans	*Helminthosporium sativum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*	[Gkarmiri et al., 2015] [Woodhams et al., 2017]
Serratia plymuthica	*Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*, *Phytophthora cactorum*, *Rhizoctonia solani*, *Verticillium dahliae*	[Zhang et al., 2018] [Srobel et al., 1999] [Woodhams et al., 2017] [Dhar Purkayastha et al., 2018] [Vleesschauwer, 2007]
Serratia marcescens	*Batrachochytrium dendrobatidis*, *Batrachochytrium salamandrivorans*, *Candida albicans*, *Diaphorina citri*, *Didymella applanata*, *Lasiodiplodia theobromae*, *Pythium ultimum*, *Phytophthora parasitica, Phytophthora cinnamomi, Phytophthora citrophora, Saccharomyces cerevisiae, Curvularia eragrostidis, Pestalotiopsis theae, Colletotrichum camelliae, Lasiodiplodia theobromae, Rhizoctonia solani*	[Frey-Klett et al., 2011] [Vleesschauwer, 2007] [Hu et al., 2018] [Duzhak et al., 2012] [Someya et al., 2005] [Schulz-Bohm et al., 2017] [Ossowicki et al., 2017]

Much of the research on bacterial antifungals has focused on compounds produced during direct bacteria-fungal interactions that rely on organism contact, but bacteria also produce complex blends of volatiles that can diffuse in the soil and have activity over a broader spatial range [Garbeva et al., 2014, Schulz-Bohm et al., 2017, Ossowicki et al., 2017, Schmidt et al., 2015]. Bacteria-produced volatiles may be harmful to fungi and other organisms or may serve as infochemicals, prompting receivers to initiate physiological processes to avoid or protect against nearby bacteria that will soon be in their vicinity [Gkarmiri et al., 2015]. For example, *Serratia plymuthica* activates a biosynthetic pathway for sodorifen in the presence of *Fusarium culmorum*. Sodorifen is a terpene thought to be responsible for defense against fungi although the target is still unknown [Garbeva et al., 2014, Schmidt et al., 2019]. Clearly, there is a need to further characterize the role of chemical communication between bacteria and fungi, as it has many applications that can be applied to medicine and agriculture. For example, when the volatile producing bacteria are colonizers of plant surfaces (roots, leaves, stems, and fruits), volatile effects that operate at some distance from the plant could confer an added layer of protection by deterring fungal growth. This can complement the protective effects of bacteria as physical barriers to fungal root colonization [Vespermann et al., 2007, Neupane et al., 2015, Kai et al., 2010, Hoffman et al., 2013].

I explored the volatile-mediated (indirect) antifungal effects using two *Serratia* species, (*S. marcescens* and *S. proteamaculans*) alongside *Bacillus subtilis*, known to produce pathogen inhibiting volatiles and a known and trusted biocontrol species [Fiddaman and Rossall, 1994]. *Serratia* species are referred to in the literature as rhizobacteria, mean-

ing they can colonize plant roots and confer advantages to the host [Dhar Purkayastha et al., 2018]. Serratia is also known to produce blends of volatiles containing compounds with effects on growth, antibiotic production and gene expression of neighboring fungi [Garbeva et al., 2014, Weise et al., 2014, Schulz et al., 2010]. Most Serratia-produced volatiles have not been specifically tested for inhibitory effects on fungal pathogens of plants, even though Serratia are commonly found associated with a variety of plant surfaces [Ordentlich et al., 1987, Dhar Purkayastha et al., 2018]. In this study, we show that fungal growth is differentially inhibited by the odors of two different Serratia species, quantify differences in volatile emissions, and demonstrate the activity of specific volatiles against fungal pathogens. Our results suggest that *Serratia* inhibition of fungal colonization operates through both direct and indirect (volatile-mediated) mechanisms and that Serratia colonization of plant surfaces may, therefore, confer protection through multiple pathways.

2.3 Materials and Methods

2.3.1 Strains

I tested indirect antifungal effects of two Serratia species (*Serratia marcescens* (*S. marcescens*) - Lab strain ADJS-2C_Red [Aryal et al., 2017] and *Serratia proteamaculans* (*S. proteamaculans*) - Lab strain BW106 [Zhang et al., 2018] and compared these to a bacterial species with known indirect antifungal effects (*Bacillus subtilis* (*B. subtilis*) - Strain E9 [Na, 2016].

I evaluated activity against 8 diverse fungi: *Actinomucor elegans* (NRRL 1706), *Alternaria infectoria* (Lab strain: BD1-7), *Aspergillus fumigatus* (Af293 / CBS 101355) a

common fungal pathogen of humans (Lopes et al. 2013), *Basidiobolus ranarum* (Lab strain: AG-B5), *Conidiobolus rhysosporus* (ARSEF 448), *Mucor circinelloides* (CBS 277.49), *Rhizopus stolonifer* (NRRL 66455), and *Neurospora crassa* (FGSC 4289).

2.3.2 Inhibition assays with live bacterial cultures

Inhibition by volatiles was measured using a "donut" plate assay which consists of a 100mm diameter Petri dish with a smaller 60mm Petri dish lid placed inside physically separating the bacterial media in the outside ring and fungal media on the inside ring. A volume of 15ml of Luria-Bertani (LB) media (10g Peptone, 10gNaCl, 5g Yeast Extract, 7.5g Agar, and 500ml Water) was pipetted into the outer ring of a 100mm Petri dish with a 60mm Petri dish lid placed inside. Then 8ml of Malt Yeast Extract Agar (MEYE) (1.5g Yeast Extract, 1.5g Malt Extract, 5g Dextrose, 7.5g Agar, 500ml Water) was added to the 60mm Petri dish lid. For *Neurospora* Vogel's media was used instead `http://www.fgsc.net/methods/vogels.html`. 1ml of overnight bacterial culture (single colony picked with sterile toothpick inoculated into 25ml LB without agar in 50ml conical tube, shaken overnight at 100 RPM at 28°C). Optical Density was measured at the absorbance at 700 nm (OD 700) to avoid the red prodigiosin [Haddix et al., 2008], OD 700 for overnight samples of *B. Subtilis,* and *Serratia* was 1.5, Supplemental Data. 1 ml was inoculated onto the outer LB ring and allowed to grow for 24 or 48 hours at 28°C. Fungi were grown to sporulation/conidiation (1 week) at which point the spores were collected with a sterile toothpick then stored in a 1.5ml microcentrifuge tube with 1ml of autoclaved water at 4°C. If fungi do not sporulate under lab conditions 1cm x 1cm mycelial plugs were harvested from one-week-old MEYE plates and stored in autoclaved water at 4°C. Mycelial plugs

(*Basidiobolus* and *Conidiobolus*) or 1000 spores/conidia (*Actinomucor, Mucor, Rhizopus, Aspergillus, Neurospora*) were added to the media in the central 60mm Petri dish lid and incubated at 25°C. The diameter of fungal mycelia were measured 24, 48, 72, and 96 hours after fungi were inoculated. Each experiment had three technical replicates of one to three biological replicates for each condition.

2.3.3 Quantification and identification of bacterial volatiles

One colony of each bacterial species was picked and grown overnight in liquid LB media as described above, then 1 ml of the overnight culture (OD 700 - 1.5) was inoculated onto three plastic 60mm Petri dishes incubated for 48 hours at 28°C. Volatiles were collected from bacterial cultures using a pull-only collection system. *B. subtilis*, was used as a positive control because it has volatiles with anti-fungal activity in measurable quantities. For negative controls, we collected volatiles from blank Petri dishes and Petri dishes with LB media. Petri dishes with cultures, or negative controls, were enclosed in 350ml Mason Ball jars with airtight Teflon lids having two Swagelok connection ports. One port was fitted with a charcoal filter (copper pipe filled with activated carbon) to remove odors from incoming air, while the other was fitted with an adsorbent trap (0.25 inch glass tube filled with 40 mg HayeSepQ beads 80-100 mesh size) to collect volatile emissions. On the end of the adsorbent trap a vacuum hose was attached and air pulled through the trap at a rate of 0.5L/min for 6 hours at 25°C. Volatiles were eluted into vials by passing 150μ l Dichloromethane spiked with 4ng/ul nonyl-acetate, 2 ng/ul octane through each trap and pushing into the vial using a gentle stream of nitrogen. Samples were stored at -80°C until analysis by gas chromatography and mass-spectrometry. Three biological replicates

of technical replicates yielded 9 total samples. Samples were analyzed using a Thermo FisherTRACE 1300 gas chromatograph (GC) linked to a TSQ Duo TripleQuadrupole mass spectrometer (MS) operating in a single quadrupole mode. The GC was fitted with a 30m TG-5MS column (Thermo Fisher),0.25mm diameter with a stationary phase of 0.25um. The inlet temperature was set to 220 °C and operated in splitless mode. Helium was used as a carrier gas delivered via constant flow at a rate of1.2ml/min. The transfer line to the MS was held at 280 C and the ion source was operated at 250 °C. The instrument was tuned to proper settings for electron ionization mode. MS detection was performed by scanning atomic masses from 30-500 at a scan rate of 0.2 seconds. One microliter of the sample was injected using an autosampler, volatilized in the inlet, and recollected on the column, which was held at 40 C for one minute following injection. Following this, the column temperature was increased linearly by 8 C/min up to 280 C, then held for one minute, after which data recording for that sample was terminated. The instrument was cooled to 40 C for the next sample run. Spectral outputs were evaluated using the Chromeleon 7 software. For all experimental samples (Serratia species and *B. subtilis*) Microsoft Excel table outputs of each peak retention time and area were generated along with putative identifications based on comparison to spectra in the NIST library. These were compared to outputs for both negative controls to identify compounds originating from the Petri dish and media, both of which could contribute some volatiles to the blend. Matches between negative control contaminants and peaks in the experimental samples were confirmed by spectral comparison. Any trace contaminants from column and septum bleed were also removed. The reduced list of compounds emitted by experimental treatments was then

examined for matches to known spectra in the NIST library. Compounds with Reverse Match Factors (RSI) of 85% or higher, and which were released consistently across samples within a treatment, were retained for further quantification, analysis, and examination as pure compounds in various concentrations.

2.3.4 Volatile quantification and analysis

Peaks in the total ion chromatogram were integrated and areas were used to calculate the total quantity of volatile present in the entire sample (representing compound sampled over the 6 hour collection period). Calculations were performed relative to the internal standard (nonyl acetate) peak area and concentration (4ng/uL). Compounds with less than RSI of 850 (85% confidence prediction) were not included in the analysis. The list of compounds detected and their amounts can be found in Table 2. 2. Compound IDs and amounts from this table were used for subsequent analysis with the R package, MetaboAnalyst 4.0 (R version 3.6.0). MetabolAnalyst analyses compared volatile emissions among treatments on a whole-blend level, as well as individual compounds. Autoscaling centered around the volatile production average was used to make all metabolites comparable. Analysis of Variance followed by posthoc Tukey tests identified volatiles emitted in significantly different quantities among the bacterial strains. The Random forest plot using 5000 generations of trees identified individual compounds with potential activity. Non-metric multidimensional scaling (NMDS was performed with the function metaMDS from the vegan package. Code used for analysis with MetaboAnalyst and NMDS can be found on Github. (`https://github.com/Derreckadam/Volatiles_Rmd`) or Zenodo

https://zenodo.org/badge/latestdoi/187256381 Below is a table of compounds and the amount detected 2.1.

2.3.5 Microscopy:

1000 *R.stolonifer* spores were suspended in autoclaved water, as previously described, and were plated onto 8 ml MEYE media with agar in a 60 mm Petri dish and allowed to grow for 12 hours at 25° then treated for 1 hour with volatiles. In volatile treated samples 10μl of the tested compound was added to a 1cm x 1cm piece of filter paper approximately 2 cm away from the *R. stolonifer* mycelium. The hyphae were then excised from the media and examined/imaged with an Amscope Compound Microscope with a 40X Phase Objective lens.

2.3.6 Pure Volatiles Assay

Tropone (Sigma-Aldrich 252832), 5-Methyl-2-furyl methanol (Sigma-Aldrich CDS003383), Lepidine (Sigma-Aldrich 158283), 2,5-Dimethylpyrazine (Acros Organics AC174520050), 2-Undecanone (Sigma-Aldrich U1303), Anisole (Acros Organics AC153920050), and Dimethyl trisulfide (Tokyo Chemical Industry Company D3418), were tested for growth inhibition on fungi. 10mg of the compound were pipetted onto a 1cm x 1cm filter paper in the outside ring of a donut plate with 1000 spores of *R. stolonifer* on MEYE in the central Petri dish. After 24 hours the fungal mycelium diameter was measured. Each compound had three replicates.

2.3.7 Strawberry Assay

Strawberries were examined for colonization and quantified via spore density by placing a 1 cm by 1 cm cube of strawberry inside of a donut plate with 48-hour old *S. marcescens* or with a 1cm x 1cm piece of filter paper with 10μl of 2-Undecanone. Strawberries (Monterey cultivar) were grown by at UCANR South Coast REC, with no fungicides. The strawberries were inoculated with 1000 spores of *R.stolonifer* and incubated at 25°C for 48 hours in 12-hour day/night cycles. Strawberry pieces were collected in 50mL conical tubes with 25mL of water then homogenized. The supernatant was collected and spores were quantified with a hemacytometer. Each supernatant was quantified three times to find the average spores recovered. Each condition had at least three replicates.

2.4 Results

I found that when *Serratia marcescens* (*S. marcescens*) was streaked on the Petri dish with fungi, the fungal growth direction changed. 2.1.I The difference in growth area could be quantitatively assessed based on growth with bacteria streaked on either side compared to growth with no bacteria. Streak plates showed the ability of bacteria to inhibit fungal growth from a distance, however, it allowed for bacterial metabolites or proteins to diffuse through the media, potentially coming in direct contact with the fungal hyphae. An improvement to streaking media on the plate with the fungi was to plate the bacteria in a different compartment separated from the fungi in a donut plate assay. This set-up allows for the exchange of gases between the fungi and bacteria, but prevents direct contact and allows for two types of media to be poured (one tailored to the bacteria and the other tailored to

the fungi). This is useful for the use of antibiotics in the fungal media or fungi with special growth needs (such as Vogel's media and Neurospora). The donut assay, was developed to limit the diffusion of bacterial products in the media to fungal hyphae 2.1 and showed that *Serratia proteamaculans* (*S. proteamaculans*) was the strongest inhibitor of growth followed by (*S. marcescens*) then (*B. subtilis*). Growth of *Serratia* and *Bacillus* strains 24 hours before *R. stolonifer* was inoculated was enough time to see growth inhibition by most strains. At 48 hours growth was equally inhibited by all *Serratia* and *Bacillus* strains tested. 2.2 The donut plates showed that the volatiles of *S. proteamaculans* was more effective than *S. marcescens* or *B. subtilis* at inhibiting many different fungi. This is interesting because *B. subtilis* was previously shown to be effective at inhibiting Fusarium and was used as a positive control.

I tested *S. marcescens* and *S. proteamaculans* volatiles' ability to inhibit growth across many fungi from various phylogenetic clades using the donut plate assays. Initial testing of *Actinomucor elegans* (NRRL 1706) showed resistance to the volatiles of *S. marcescens*. *Alternaria infectoria* (Lab strain: BD1-7) is an ascomycete and known pathogen of wheat, an allergen, and an opportunistic human pathogen [Lopes et al., 2013]. Another ascomycete tested, *Aspergillus fumigatus* (CBS 101355), is an opportunistic pathogen and common genetic model organism. *Basidiobolus ranarum* (Lab strain: AG-B5), commonly lives in amphibian guts and can be an opportunistic human pathogen [Khan et al., 2001] (Khan et al. 2001; Gastrointestinal Basidiobolomycosis -...). *Conidiobolus rhysosporus* (ARSEF 448), is a representative of the Entomophthorales and it many other animal-associated and pathogenic fungi. *Mucor circinelloides* (CBS 277.49), is a well studied zygomycetous fungi

and opportunistic human pathogen. *Rhizopus stolonifer* (NRRL 66455) is responsible for the postharvest rot of many fruits and vegetables. Finally, a genetic model organism and ascomycete, *Neurospora crassa* (FGSC 4289) was evaluated.

For all strains the exchange of gases was enough to inhibit fungal growth at some time within 72 hours, except for *Actinomucor elegans*. *S. proteamaculans* followed by *S. marcescens* then *B. subtilis* had the highest inhibition of *R. stolonifer* growth. Aspergillus was the slowest spreading mycelium with only about 40% of the plate covered after 72 hours (Supplemental data, AllFungalGrowth.pdf).

In the first 24 hours *S. proteamaculans* inhibition of *R. stolonifer* and *Neurospora crassa* was about 62% and 67% respectively, but *Actinomucor elegans* was able to grow without inhibition. *Mucor circinelloides, Alternaria infectoria* and *Conidiobolus rhizosporus* showed lower inhibition around 15%-30%. 2.3 At 48 hours *Neurospora crassa* growth was the most affected of all the fungi tested. Six out of eight have significantly inhibited growth by *S. proteamaculans* volatiles. 2.4 *Aspergillus fumigatus* begins to grow and its growth is significantly impacted by the volatiles compared to untreated samples at 72 hours. 2.5

After establishing that fungal growth is inhibited in the presence of bacterial volatiles I used gas chromatography coupled with mass spectrometry to identify the compounds produced by our strains of bacteria. Based on previous literature we predicted that we would find 2-Undecanone and Dimethyl Disulfide [Popova et al., 2014]. However, many more compounds were detected as well. On average there were about 50 volatiles produced by each strain and on average 500ng of volatiles were collected per sample. Outlying samples

with less than 200 ng of metabolites detected were removed from the analysis (*B. subtilis*A,G and I, *S. marcescens*D,F,G and *S. proteamaculans*_I). 2.6 All of the volatiles produced were compared to all of the samples to find if there was a species specific volatile profile. There were many compounds produced by *B. subtilis* that were not in the *S. marcescens* and *S. proteamaculans* profile. Also, many compounds were found at different levels between the *S. marcescens* and *S. proteamaculans* profiles. 2.7

To analyze the volatiles, I restricted the results to only the volatiles with a confident prediction (Thermo Mass Spectrometry RSI Score) of over 85%. The most abundant compound in the *S. proteamaculans* profile was Dimethyl trisulfide. Anisole was abundant and solely produced by *S. marcescens*. *B. subtilis* had multiple highly produced compounds, 2-undeconol/one, and Butanoic acid. 2.8

Compounds detected in 4 or more samples of the same species were considered to be an element of the volatile profile. This resulted in a total of 29 compounds between the three species. *B. subtilis* had the most unique compounds, 11, not detected in the other two bacteria, and shared 2 in common. *S. proteamaculans* had 2 unique compounds and shared 7 with *S. marcescens*. *S. marcescens* had 6 unique compounds. 2.9 In general the *Serratia* strains had the most overlap in compounds compared to *Bacillus*.

Non-Dimensional Multivariate Scaling (NMDS) shows low stress and clearly separates each bacteria based on the differences in their volatile profile. 2.10

Using an internal standard amounts of volatiles were predicted, this left us with an approximate abundance of compounds. To further analyze the data we used MetaboAnalyst V. 4 [Chong and Xia, 2018, Chong et al., 2019], an R package for metabolite profiles. Comparisons were made between all three samples, *B. subtilis*, *S. marcescens* and *S. proteamaculans* to see if each species could be differentiated by volatile profiles. ANOVA analysis with a p-value cut off .05 and Tukey's/HSD analysis reveals 63 significantly differentially produced volatiles compounds. 2.11. *S. marcescens*, *S. proteamaculans*, and *B. subtilis* strain had different volatiles as well as different amounts produced 2.12 2.2

The analysis identified volatiles produced by *S. marcescens*, *S. proteamaculans*, and *B. subtilis*. We tested 2-Undecanone, Dimethyl Trisulfide, Anisole, Lepidine, 2,5-dimethylpyrazine as they were some of the highest produced volatiles from the bacteria. 2-Undecanone was chosen because it is a known antifungal [Popova et al., 2014], is used in food and fragrance, as well as an effective insect repellent approved for use with humans [TOXNET,Bohbot]. Dimethyl Trisulfide was tested based on the abundance in the *S. proteamaculans* profile and sulfur content. Anisole was solely produced by *S. marcescens* in high amounts. To test the volatiles we loaded the compounds onto a piece of filter paper on a glass microscope slide on the side of a Petri dish with media, spores/conidia were inoculated approximately 2 cm away on the media. The effective volatiles for preventing growth were 2-Undecanone, Anisole, and Dimethyl Trisulfide (Figure 2.13).

Microscopy phenotype with volatiles reflect the growth inhibition results. The process of vacuolation is seen commonly in fungi being treated with antifungal and indi-

cates high stress. Vacuolation was found in 2-Undecanone and Dimethyl Trisulfide samples (Microscope Images: Figure 2.14, 2.15), but not in Dimethyl Pyrazine, Anisole, or the control.

To test mycelial growth inhibition on substrate that *R. stolonifer* naturally grow on a strawberry rotting assay was conducted. *S. marcescens* could reduce the number of asexual spores produced compared to the untreated control. After seven days of incubation on strawberry 100 spores/μl of *R. stolonifer* were recovered, however, on the strawberries exposed to *S. marcescens* volatiles during the infection period only an average of 36 spores/μl were recovered. 2.16

2-Undecanone incubated with strawberry pieces prevented any spores from being recovered from the strawberry after the assay. This demonstrates that 2-Undecaone can inhibit fungal growth in environments apart from MEYE.

2.5 Discussion

This study is one of the most extensive looks at the volatiles produced by *Serratia* and their potential applications and it shows that there is definitive differences among species in volatile production. A reexamination of volatiles produced by *Serratia* reveals a larger profile and several antifungal compounds not previously reported. Importantly, I show that a known biocontrol, *B. subtilis*, has a distinct volatile profile, and in lab settings, is less able to inhibit fungal growth than our Serratia strains in these assays. In addition, I have shown that many compounds differ even between species of Serratia. This may help

inform work on metabolic pathway synthesis and learning how these metabolites are being produced as these two species have sequenced genomes and potential differences in gene content may create a starting point when studying at what genes are involved in metabolic volatile profiles.

Interestingly, *Serratia* and other bacteria tested had the ability to influence the behavior of fungi. With the streak plates the fungi grew parallel to the bacteria. This was compounded in the circular plate reducing fungal growth on all sides. This phenomenon required high populations of bacteria (at least 24 hours of growth) at optimal temperatures and humidity on artificial food substrate. More work is needed to study the bacterial-fungal volatile profiles in soil and if a zone of inhibition is also formed around plant roots. This work in an important first step in learning about the unique volatile profiles produced by the rhizobacteria, *Serratia* and their influence on fungal growth.

All three bacteria tested had a significant impact on various economically important fungi including *Alternaria, Aspergillus, Basidiobolus, Conidiobolus, Mucor, Neurospora, and Rhizopus*. However, preliminary data showed that *Actinomucor elegans* was resistant to the volatiles produced by bacteria at all time points tested. Further research is needed to explore the mechanisms that help this fungus grow in the presence of volatiles. Another interesting aspect of this analysis is at 72 hours *Basidiobolus* growth was elevated compared to the control. This is not unusual, as fungi and bacteria can utilize each other's metabolites for growth [Li et al., 2017], however, it does contradict the inhibition seen at 48 hours. This requires further work to understand how *Basidiobolus* was able to overcome

the volatiles and potentially utilize them for growth. Potentially, the compound Anisole does not affect the same cellular targets the same degree in the other fungi.

While many studies are beginning to incorporate microbial communities into their work, the volatile profiles of these communities are at times neglected but can clearly play a major role. The stunning amount of volatiles collected in our experiments, over a microgram of volatiles detected in just a small amount of the collected sample, demonstrates the intense production of these potential antifungals. Using metabolites from one microbe to inhibit the growth of another is not novel, but the volatile profiles examined here have shown that multiple antifungals are being produced simultaneously which may help mitigate the loss of efficacy due to the development of resistance to one compound. *N. crassa* and *Aspergillus* were shown to be more susceptible to the volatiles produced by the bacteria than *R. stolonifer* and *Mucor*. Understanding why these zygomycete fungi are able to grow better than the ascomycete in this work is important for battling infections in immunocompromised patients.

Several compounds stood out as highly produced including 2-Undecanone and other long-chain fatty acids, Anisole, Dimethyl Trisulfide, and Butanoic acid. Unfortunately, the pathways for these products are not known and therefore not possible to test gene disruptions of the pathway genes necessary to synthesize volatiles, however, next steps for investigating the key components of fungal inhibition would benefit from being able to manipulate or reconstruct the profile and change components similar to having a defined media for growth conditions. Limitations of this work is that I can only analyze the bacterial profile being created while bacteria are on LB media, hardly a realistic environmental

conditions. It would be interesting to see these same volatiles with antifungal properties are produced in a community of other rhizobacteria with various soil types, and temperatures, and with various plant partners to investigate if there is a core set of volatiles produced.

Studying the compounds separately from the others I showed that the growth repression could be inhibited by a few of the volatiles detected in the GC MS experiment. The microscopy of the fungal hyphae shows the tell-tale traits of stress with the vacuolation observed in Anisole and DMTS (and 2-Undecanone in *N. crassa*). 2-Undecanone was a weaker inhibitor of growth in *R. stolonifer* potentially due to its very short half-life of 1.2 days `https://toxnet.nlm.nih.gov/`, this makes this compound less than ideal for a long treatment period requirements but helpful for some conditions such as for strawberries that need to be sold without lingering effects of the antifungals [Yu et al., 2000].

Table 2.1: A list of compounds and the amount of ng detected by GCMS from each species of bacteria

Compounds	*B. subtilis*	*S. marcescens*	*S. proteamaculans*
1-(2-Thienyl)-1-propanone	0	0	2.79467881
1-Decanol	0	0	10.9127873
1-Hexanol	0	0	5.43858139
1-Nonanol	0	15.02649698	20.94301093
1-Phenyl-2-propanone	0	34.09383056	96.42582152
1,3-Cyclopentadiene, 5-methyl-	0	322.2523723	104.0489803

Table 2.1 continued from previous page

Compounds	*B. subtilis*	*S. marcescens*	*S. proteamaculans*
2-Decanol	14.96369334	0	0
2-Decanone	10.42702769	0	0
2-Dodecanol	21.43002897	0	0
2-Dodecanone	72.5479806	0	0
2-Heptanone	76.5308929	228.0751131	99.408633
2-Heptanone, 4-methyl-	11.71958342	0	0
2-Heptanone, 5-methyl-	36.084172	0	0
2-Heptanone, 6-methyl-	54.17496115	0	0
2-Methyl-3-isopropylpyrazine	2.73446588	0	0
2-Nonanol	69.71446456	0	0
2-Nonanone	102.9227555	0	0
2-Tridecanone	43.62448819	50.41455949	26.87876892
2-Undecanol	760.5468063	0	0
2-Undecanone	497.0801784	32.41560279	43.21653498
2,4,6-Cycloheptatrien-1-one	0	36.04270725	68.24085595
3-Aminoacetophenone	0	25.97663226	74.88776494
3-Hexanone OR Butanoic acid	44.04418243	0	0
5,9-Undecadien-2-one, 6,10-dimethyl-	0	19.3777489	15.66216462

Table 2.1 continued from previous page

Compounds	*B. subtilis*	*S. marcescens*	*S. proteamaculans*
Anisole	0	2376.470096	0
Benzene, 1-chloro-2-methoxy-	0	17.57447159	0
Benzene, 1-methoxy-4-methyl-	0	32.81009851	0
Benzophenone	0	20.12483271	24.88514826
Bicyclo[3.2.0]hept-3-en-2-one	0	51.5716149	54.9036736
Butanoic acid, 2-methyl-	877.7119068	402.007371	109.0467891
Butanoic acid, 3-methyl-	812.1207885	0	0
Butylated Hydroxytoluene	45.09446842	30.08036407	9.21248629
Caprolactam	0	60.51033257	32.06794772
Cyclohept-4-enone	0	19.63322924	0
Cyclohexanone	0	0	44.1630777
Cyclooctene	0	8.77933205	5.34563894
Dimethyl trisulfide	0	151.3286756	4845.387752
Formamide, N,N-dibutyl-	0	21.5325987	7.86612978
Phenol, 2-iodo-	0	21.47750406	0
Phenol, 2-methoxy-	0	547.9678892	0
Phenol, 2-methoxy-	2.64538343	0	0
Phenol, 3-ethyl-	0	4.09277948	0

Table 2.1 continued from previous page

Compounds	*B. subtilis*	*S. marcescens*	*S. proteamaculans*
Phenylethyl Alcohol	0	686.4932248	688.9438987
Prenol	2.68846647	0	0
Propanoic acid, 2-methyl-	569.7590386	0	0
Pyrazine, 2-ethyl-5-methyl-	0	48.90258571	77.79155381
Pyrazine, 2-ethyl-6-methyl-	0	9.48171744	70.05159363
Pyrazine, 2-methyl-5-propyl-	0	13.0701381	5.78911501
Pyrazine, 2,5-dimethyl-	0	3.66554648	3.06505137
Pyrazine, trimethyl-	0	0	61.8087536
Pyridine, 2,3,5-trimethyl-	0	4.35102547	0
Pyridine, 2,4,6-trimethyl-	0	3.26232318	2.91576189
Pyridine, 3-methyl-	5.41787381	0	0
Quinazoline, 4-methyl-	0	5.35866995	5.76001544
Quinoline, 3-methyl-	0	11.46393388	11.03075385
Quinoline, 4-methyl-	0	0	4.27276454
S-Methyl methanethiosulfinate	0	8.70268463	28.43815404
Unknown methyl ketone 2	190.2187317	0	0
Unknown methyl ketone 3	130.1119881	0	0
Unknown methyl ketone 4	6.21509891	0	0

Table 2.1 continued from previous page

Compounds	*B. subtilis*	*S. marcescens*	*S. proteamaculans*
unknown straight-chain alcohol 1	44.72740519	0	0
Unknown straight-chain alcohol 3	35.60332759	0	0

Table 2.2: List of Compound Comparisons and their resulting p-value from ANOVA

Compound	p-value	FDR	Tukey's HSD
2-Nonanol	1.07E-07	6.28E-06	*Smar-Bsub*; *Spro-Bsub*
4-Butoxy-2-butanone	1.36E-07	6.28E-06	*Smar-Bsub*; *Spro-Bsub*
2-Undecanone	2.05E-07	6.30E-06	*Smar-Bsub*; *Spro-Bsub*
2-Nonanone	2.80E-07	6.43E-06	*Smar-Bsub*; *Spro-Bsub*
2-Heptanone, 4-methyl-	1.21E-06	1.70E-05	*Smar-Bsub*; *Spro-Bsub*
3-Hydroxybutyric acid, t-butyl ester	1.22E-06	1.70E-05	*Smar-Bsub*; *Spro-Bsub*
4-Acetyl-1-methylcyclohexene	1.29E-06	1.70E-05	*Smar-Bsub*; *Spro-Bsub*
2-Und ecanol	1.90E-06	2.19E-05	*Smar-Bsub*; *Spro-Bsub*
23H-Furanone...-	5.12E-06	5.05E-05	*Spro-Bsub*; *Spro-Smar*
Phenol, 2-methoxy-	5.49E-06	5.05E-05	*Smar-Bsub*; *Spro-Bsub*
Bicyclo3.2.0hept-3-en-2-one	6.91E-06	5.78E-05	*Smar-Bsub*; *Spro-Smar*
Benzene, 1-methoxy-4-methyl-	9.97E-06	7.65E-05	*Smar-Bsub*; *Spro-Smar*

Table 2.2 continued from previous page

Compound	p-value	FDR	Tukey's HSD
Benzene, 1-chloro-2-methoxy-	1.25E-05	8.87E-05	*Smar-Bsub*; *Spro-Smar*
Unknown straight-chain alcohol 3	1.44E-05	8.88E-05	*Smar-Bsub*; *Spro-Bsub*
Allyl 2-ethyl butyrate	1.45E-05	8.88E-05	*Smar-Bsub*; *Spro-Smar*
unknown straight-chain alcohol 1	3.04E-05	0.00017506	*Smar-Bsub*; *Spro-Bsub*
2-Methyl-3-isopropylpyrazine	3.72E-05	0.00020119	*Smar-Bsub*; *Spro-Bsub*
Phenylethyl Alcohol	8.58E-05	0.00043849	*Smar-Bsub*; *Spro-Bsub*
Anisole	9.78E-05	0.00045149	*Smar-Bsub*; *Spro-Smar*
2-Heptanone, 5-methyl-	9.81E-05	0.00045149	*Smar-Bsub*; *Spro-Bsub*
unknown methyl ketone 1	0.0001546	0.00065059	*Smar-Bsub*; *Spro-Bsub*
Benzophenone	0.00015558	0.00065059	*Smar-Bsub*; *Spro-Bsub*
2-Furanmethanol, 5-methyl-	0.00023314	0.00093258	*Spro-Bsub*; *Spro-Smar*
Unknown methyl ketone 2	0.00025718	0.00098586	*Smar-Bsub*; *Spro-Bsub*
2-Dodecanone	0.00028566	0.0010512	*Smar-Bsub*; *Spro-Bsub*
Cyclohept-4-enone	0.00046197	0.0016347	*Smar-Bsub*; *Spro-Smar*
2-Decanone	0.00065154	0.0022201	*Smar-Bsub*; *Spro-Bsub*
2-Hydroxy-3-hexanone	0.00068939	0.0022256	*Smar-Bsub*; *Spro-Bsub*
a-Phellandrene	0.00072258	0.0022256	*Smar-Bsub*; *Spro-Bsub*
2-Heptanone, 6-methyl-	0.00072575	0.0022256	*Smar-Bsub*; *Spro-Bsub*

Table 2.2 continued from previous page

Compound	p-value	FDR	Tukey's HSD
2-Dodecanol	0.0009426	0.0027974	*Smar-Bsub*; *Spro-Bsub*
Dimethyl trisulfide	0.001078	0.0030994	*Spro-Bsub*; *Spro-Smar*
5,9-Undecadien-2-one, 6,10-dimethyl-	0.0011758	0.003278	*Smar-Bsub*; *Spro-Bsub*
Unknown methyl ketone 0	0.0013269	0.0035903	*Smar-Bsub*; *Spro-Bsub*
1-Phenyl-2-propanone	0.0015493	0.0040725	*Spro-Bsub*
3-Aminoacetophenone	0.0017133	0.0043785	*Spro-Bsub*
Unknown aromatic 1	0.0020006	0.0049744	*Smar-Bsub*; *Spro-Bsub*
Unknown methyl ketone 4	0.0026005	0.0062033	*Smar-Bsub*; *Spro-Bsub*
Cyclooctene	0.0026297	0.0062033	*Smar-Bsub*; *Spro-Bsub*
Phenol, 2-iodo-	0.0031128	0.0071595	*Smar-Bsub*; *Spro-Smar*
2-Propionyl-6-methyl-3,4-dihydropyran	0.0042669	0.0095114	*Smar-Bsub*; *Spro-Smar*
Cyclopentadecanone	0.0043788	0.0095114	*Smar-Bsub*; *Spro-Smar*
23H-Furanone, dihydro-3,5-dimethyl-	0.0044455	0.0095114	*Smar-Bsub*; *Spro-Smar*
S-Methyl methanethiosulfinate	0.0055491	0.011132	*Spro-Bsub*
Unknown aromatic 2	0.0055842	0.011132	*Smar-Bsub*; *Spro-Bsub*
1,3-Cyclopentadiene, 5-methyl-	0.0055897	0.011132	*Smar-Bsub*; *Spro-Smar*
1-Nonanol	0.0056872	0.011132	*Spro-Bsub*
3-Hexanone OR Butanoic acid	0.0061133	0.011717	*Smar-Bsub*; *Spro-Bsub*

Table 2.2 continued from previous page

Compound	p-value	FDR	Tukey's HSD
Caprolactam	0.0076408	0.014346	*Smar-Bsub*
unknown straight-chain alcohol 2	0.0084836	0.01561	*Smar-Bsub*; *Spro-Bsub*
Alpha selinene	0.011406	0.020576	*Smar-Bsub*; *Spro-Bsub*
Methyl 2-methoxypropenoate	0.01168	0.020665	*Smar-Bsub*; *Spro-Bsub*
Phenol, 2-methoxy-	0.013774	0.023719	*Smar-Bsub*; *Spro-Smar*
Pyrazine, 2-methyl-5-propyl-	0.013922	0.023719	*Smar-Bsub*
2,4,6-Cycloheptatrien-1-one	0.016019	0.026795	*Spro-Bsub*
Phenol, 2-ethyl-6-methyl-	0.01848	0.030359	*Smar-Bsub*; *Spro-Bsub*
Butanoic acid, 3-methyl-	0.019024	0.030706	*Smar-Bsub*; *Spro-Bsub*
Cyclohexane, isothiocyanato-	0.020579	0.032642	*Spro-Bsub*; *Spro-Smar*
2,5-Hexanedione	0.024903	0.038832	*Smar-Bsub*; *Spro-Bsub*
2-Decanol	0.026074	0.039325	*Spro-Bsub*
Butylated Hydroxytoluene	0.030632	0.045454	*Spro-Bsub*
Unknown sesquiterpene	0.033191	0.04847	*Spro-Bsub*

Figure 2.1: I.) *R. stolonifer* grown for 48 hours with *S. marcescens* streaked on both sides. II.) Donut plates with *R. stolonifer* inside III.) *R. stolonifer* covered preventing volatiles from entering the inner plate. IV.) Normal growth of *R. stolonifer* without *S. marcescens*. n=3

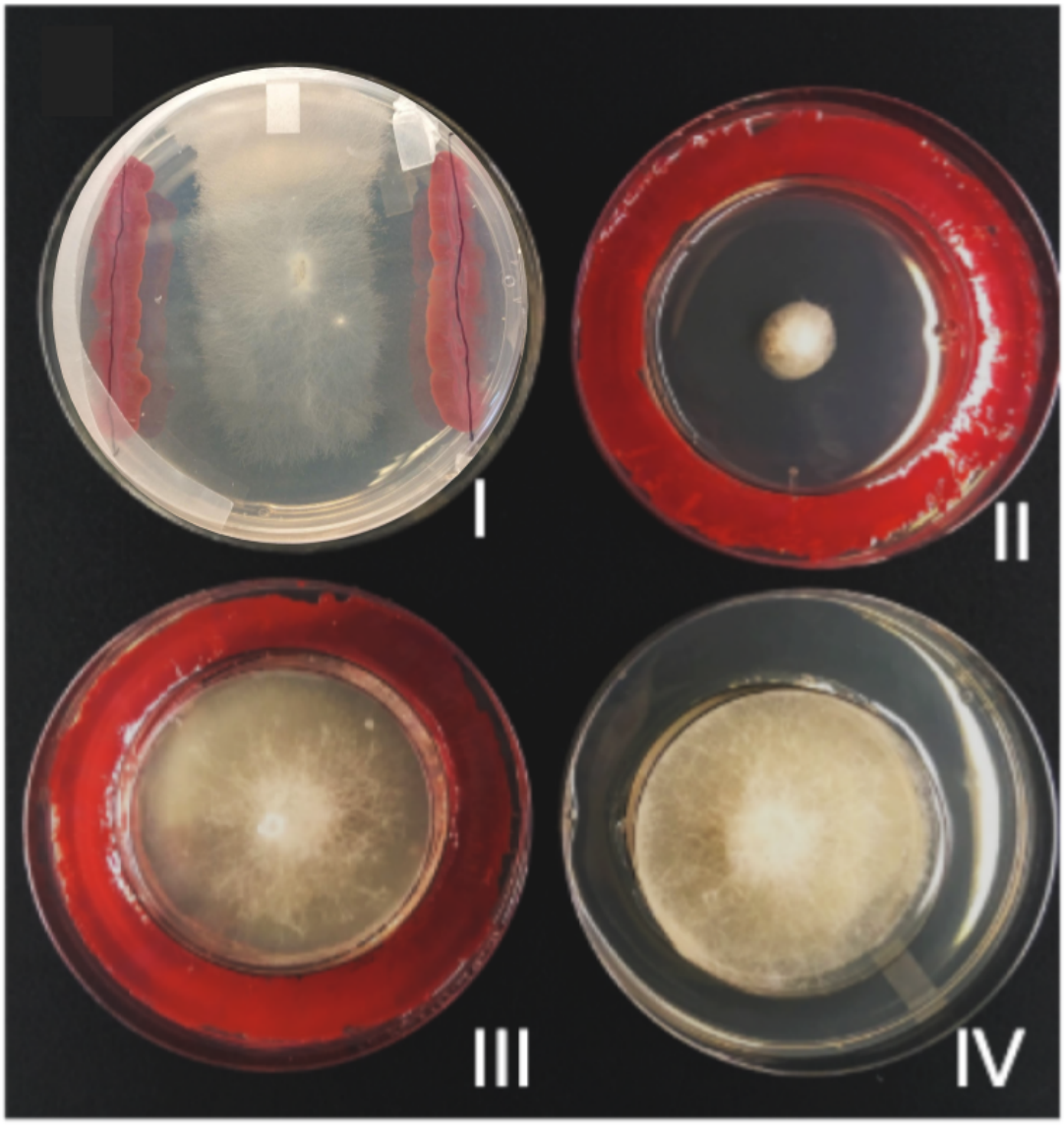


Figure 2.2: Bacteria grown for 48 hours before *R. stolonifer* were inoculated prevented mycelial growth. n=3

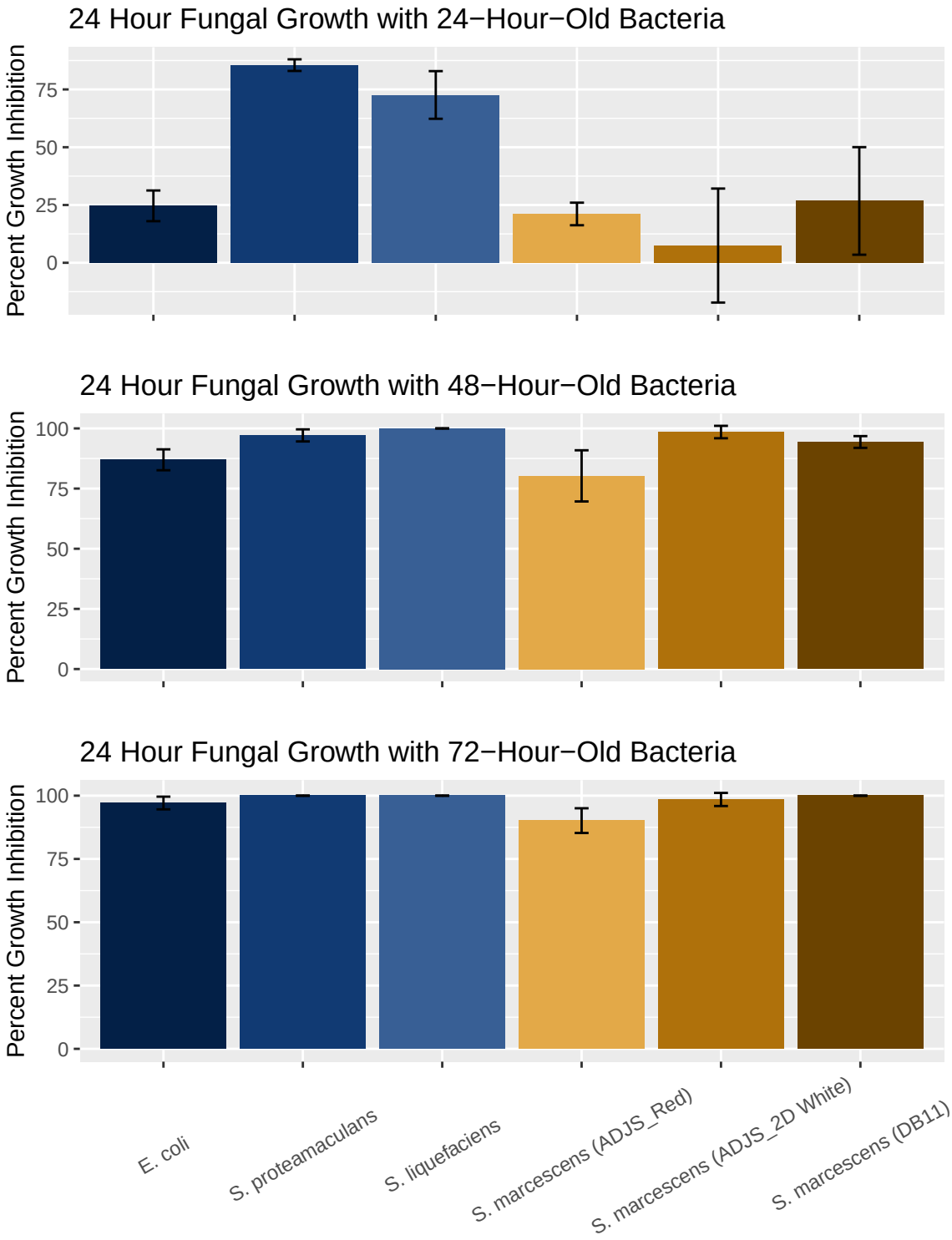


Figure 2.3: *Alternaria, Conidiobolus, Mucor, Neurospora, and Rhizopus* are significantly inhibited after 24 hours exposure to bacterial volatiles compared to control conditions. n=3

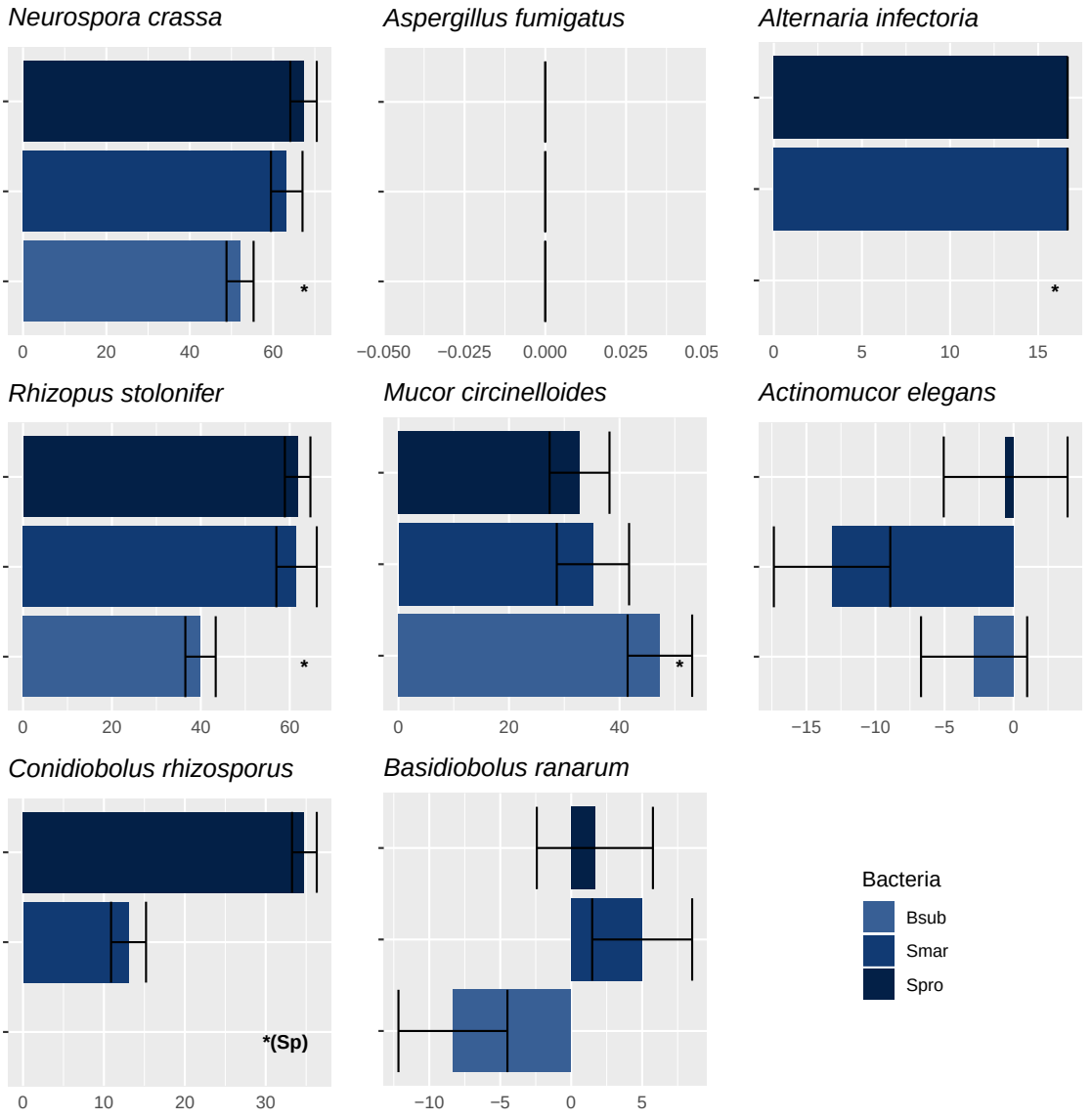


Figure 2.4: *Alternaria, Conidiobolus, Mucor, Neurospora, and Rhizopus* are significantly inhibited after 48 hours exposure to bacterial volatiles compared to control conditions. n=3

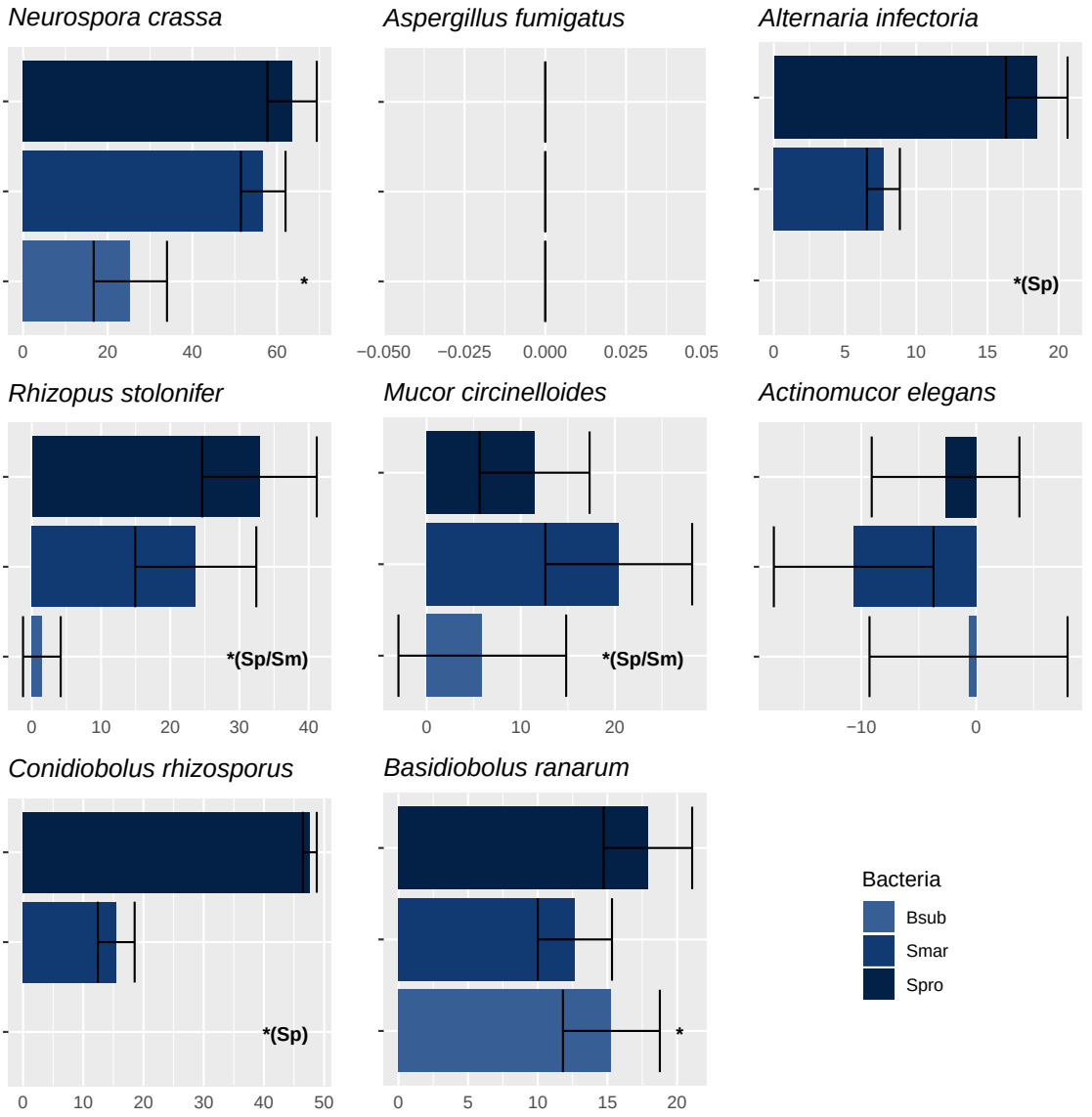


Figure 2.5: *Alternaria, Conidiobolus, Mucor, Neurospora, and Rhizopus* are significantly inhibited after 72 hours exposure to bacterial volatiles compared to control conditions. n=3

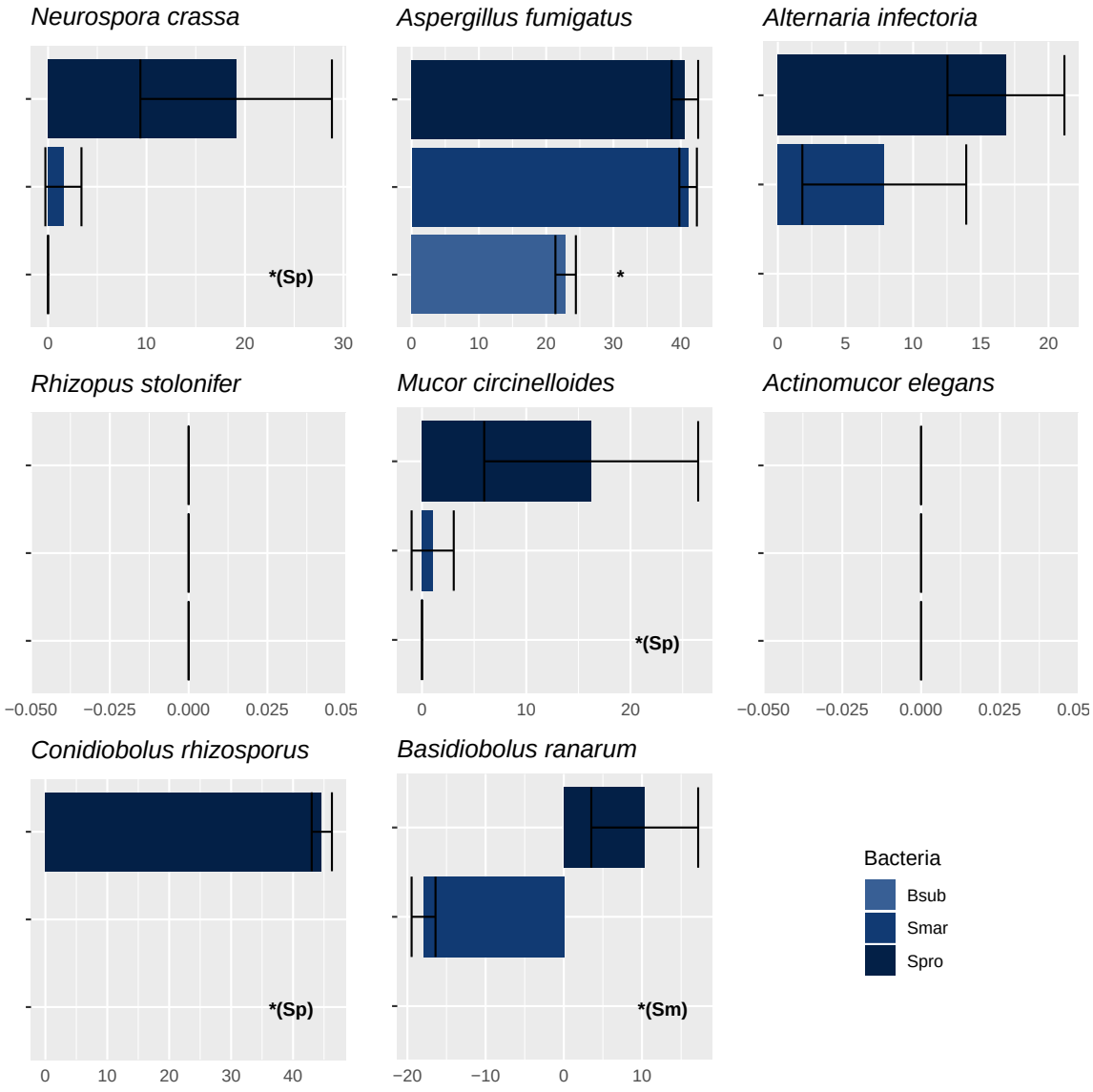


Figure 2.6: Total ng of detected volatiles from each bacterial sample in GCMS

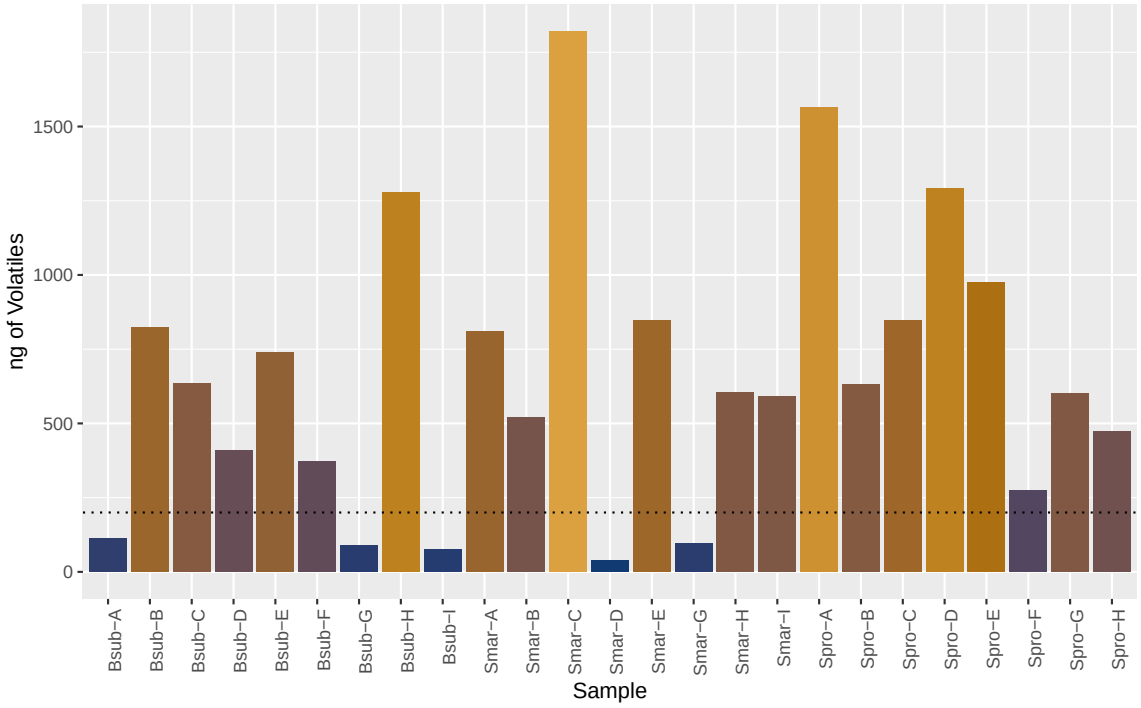


Figure 2.7: Volatiles by Samples shows the diversity and number of volatiles detected from each sample.

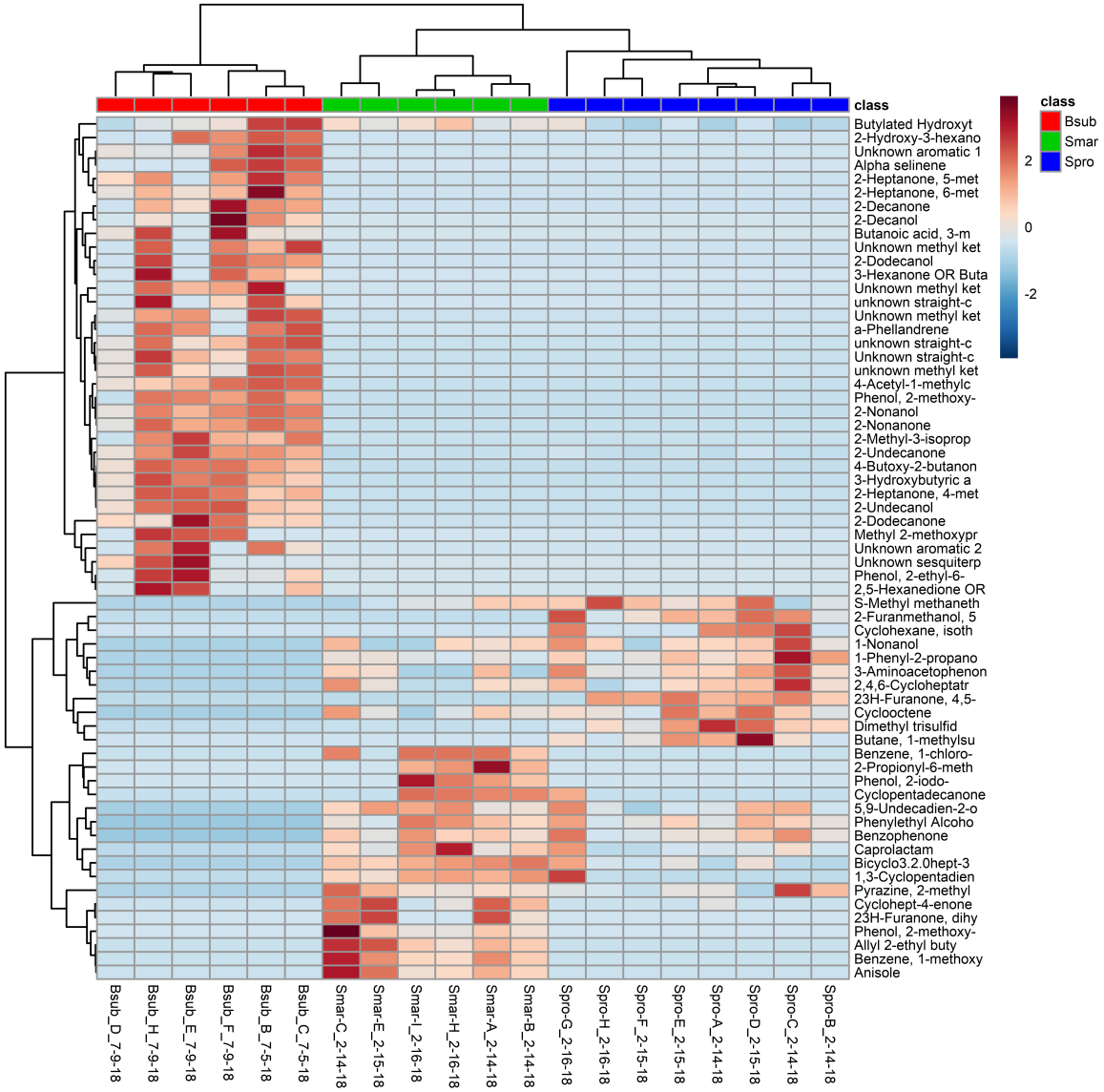


Figure 2.8: The sum of the most abundant volatiles identified from each bacteria from *B. subtilis, S. marcescens,* and *S. proteamaculans.*

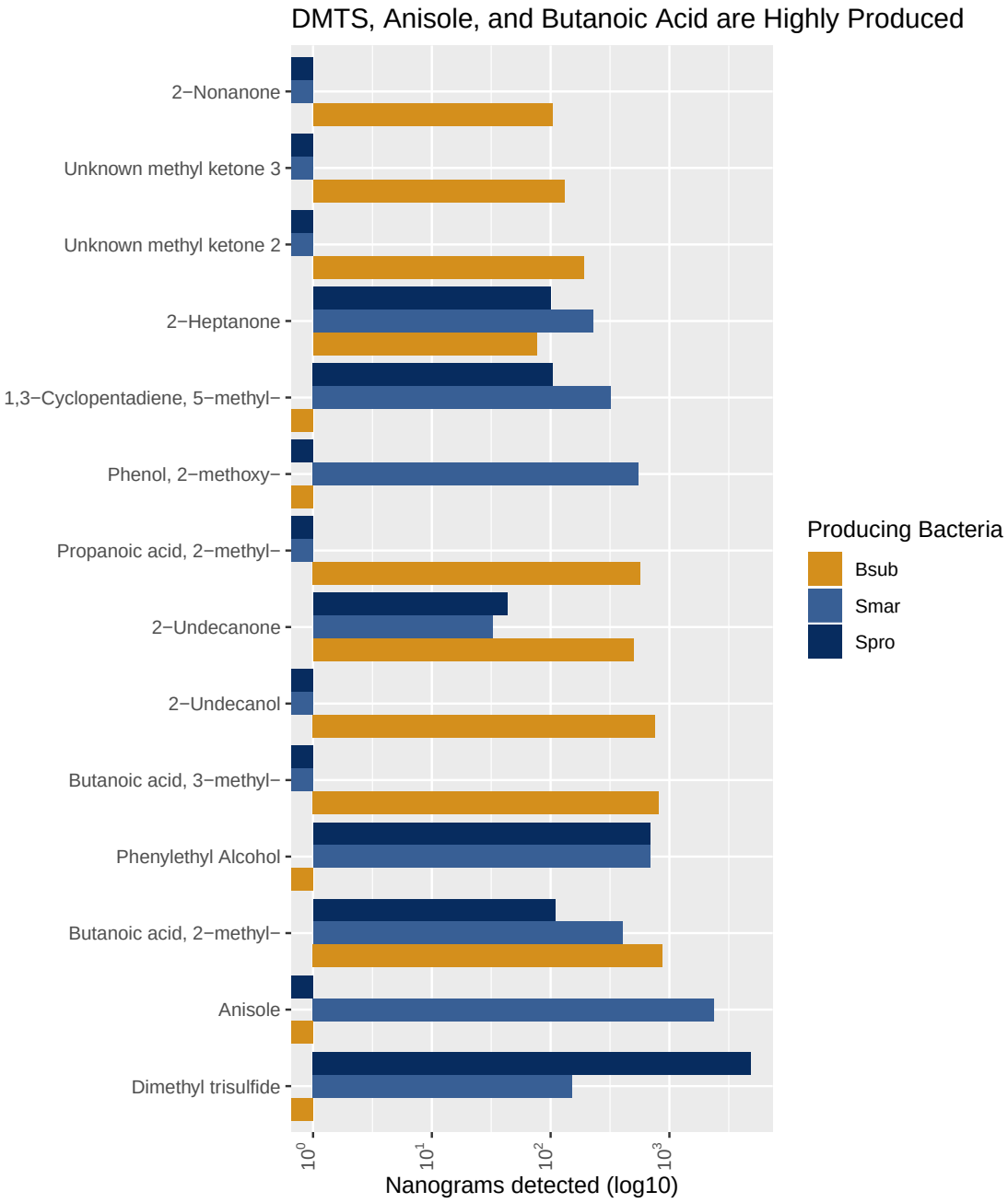


Figure 2.9: Comparison of the volatiles of *Serratia* and *Bacillus*

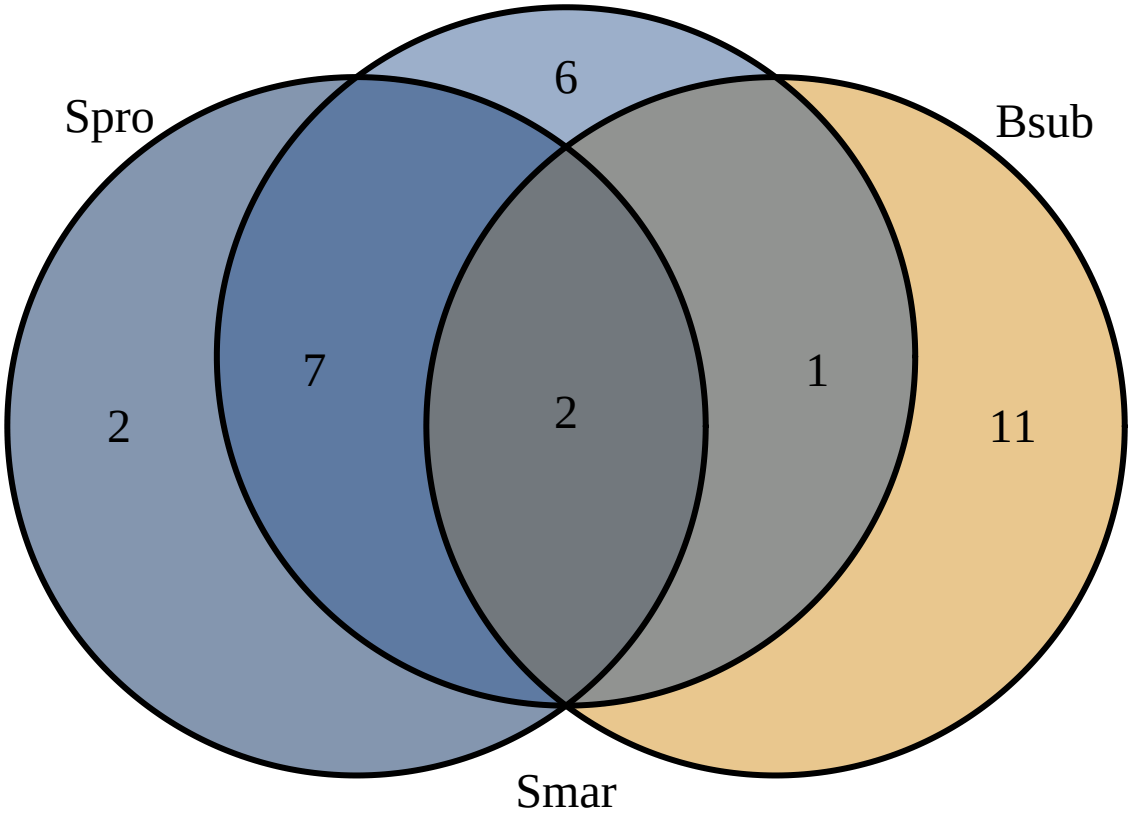


Figure 2.10: Bacterial volatiles variation and compound presence distinguishes species. High sepearation and near zero stress test result, 0.07344734.

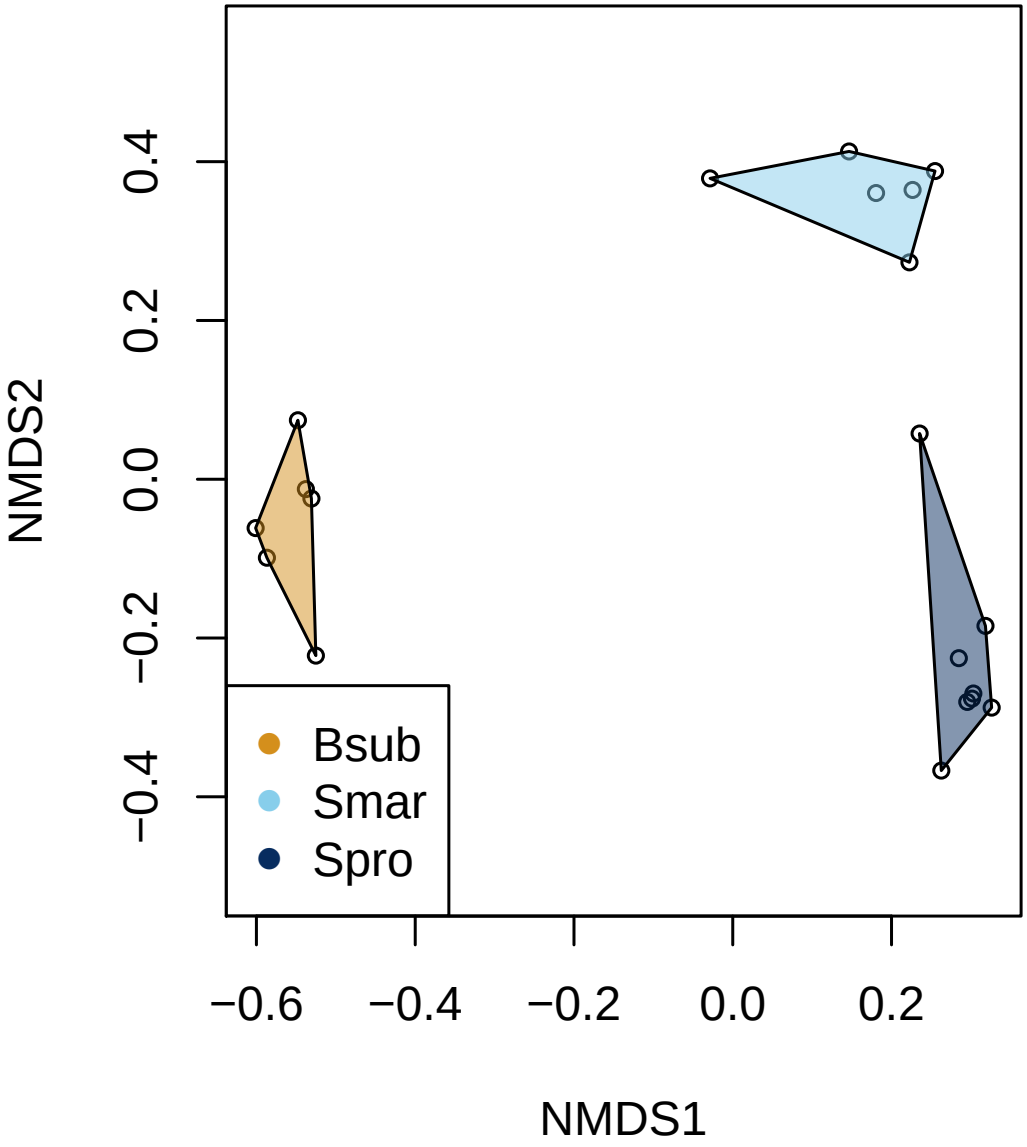


Figure 2.11: ANOVA with Post-hoc Tukey test reveals 63 bacterial volatiles with different production between the three species

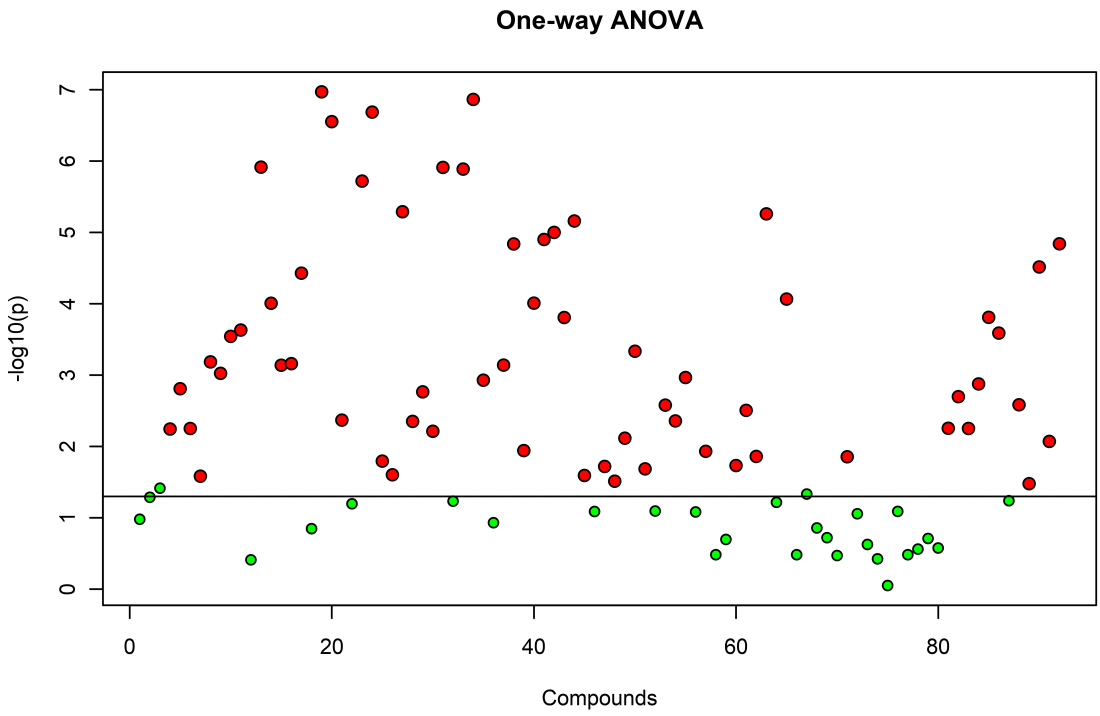


Figure 2.12: A few bacterial volatiles with greater production than the other two species

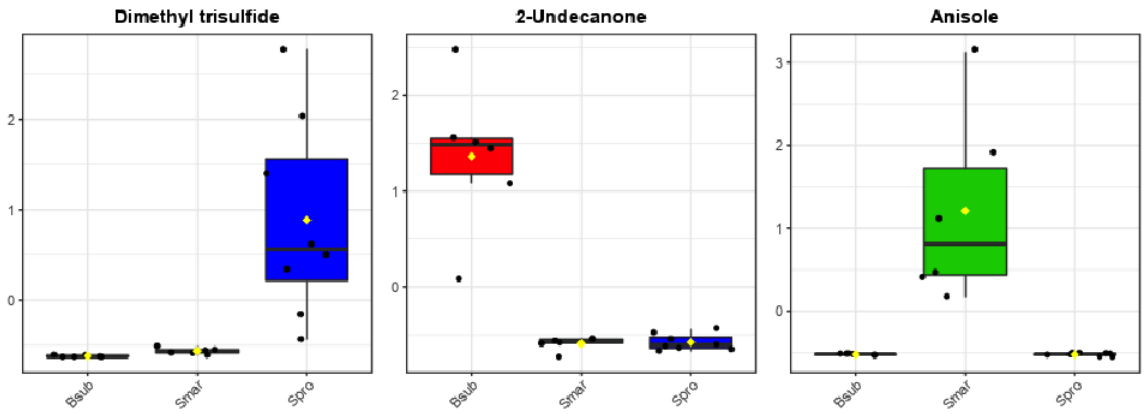


Figure 2.13: *R. stolonifer* germination and growth from spores in the presence of 10 mg of select bacterial volatiles. n=3

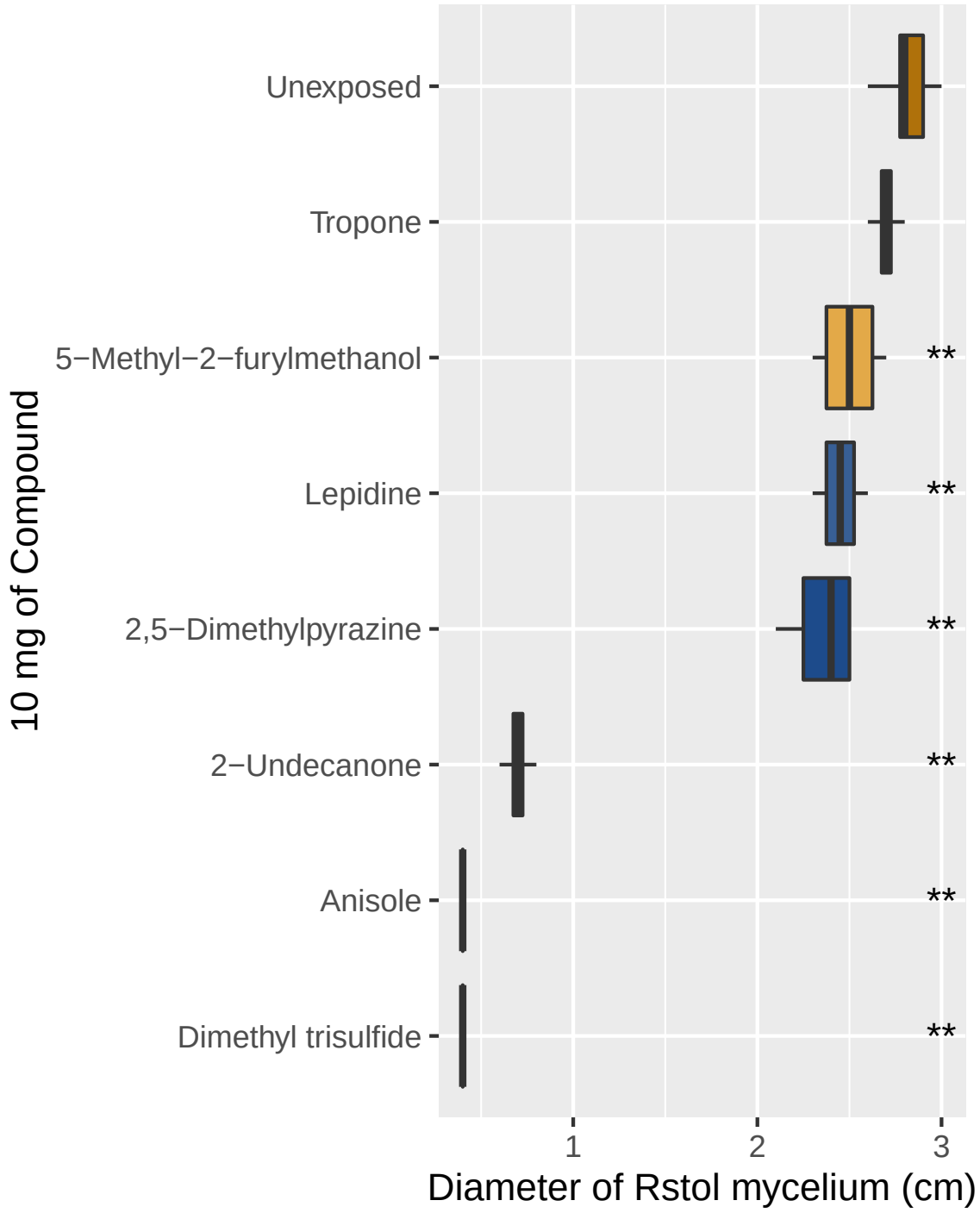


Figure 2.14: 40x Phase view A. Control Condition - *R. stolonifer* hyphae unexposed to volatiles. B. Dimethyl Pyrazine previously shown to not affect fungal growth. C.2-Undecanone D. Anisole caused moderate vacoulation E. Dimethyl Trisulfide. n=3 for each condition

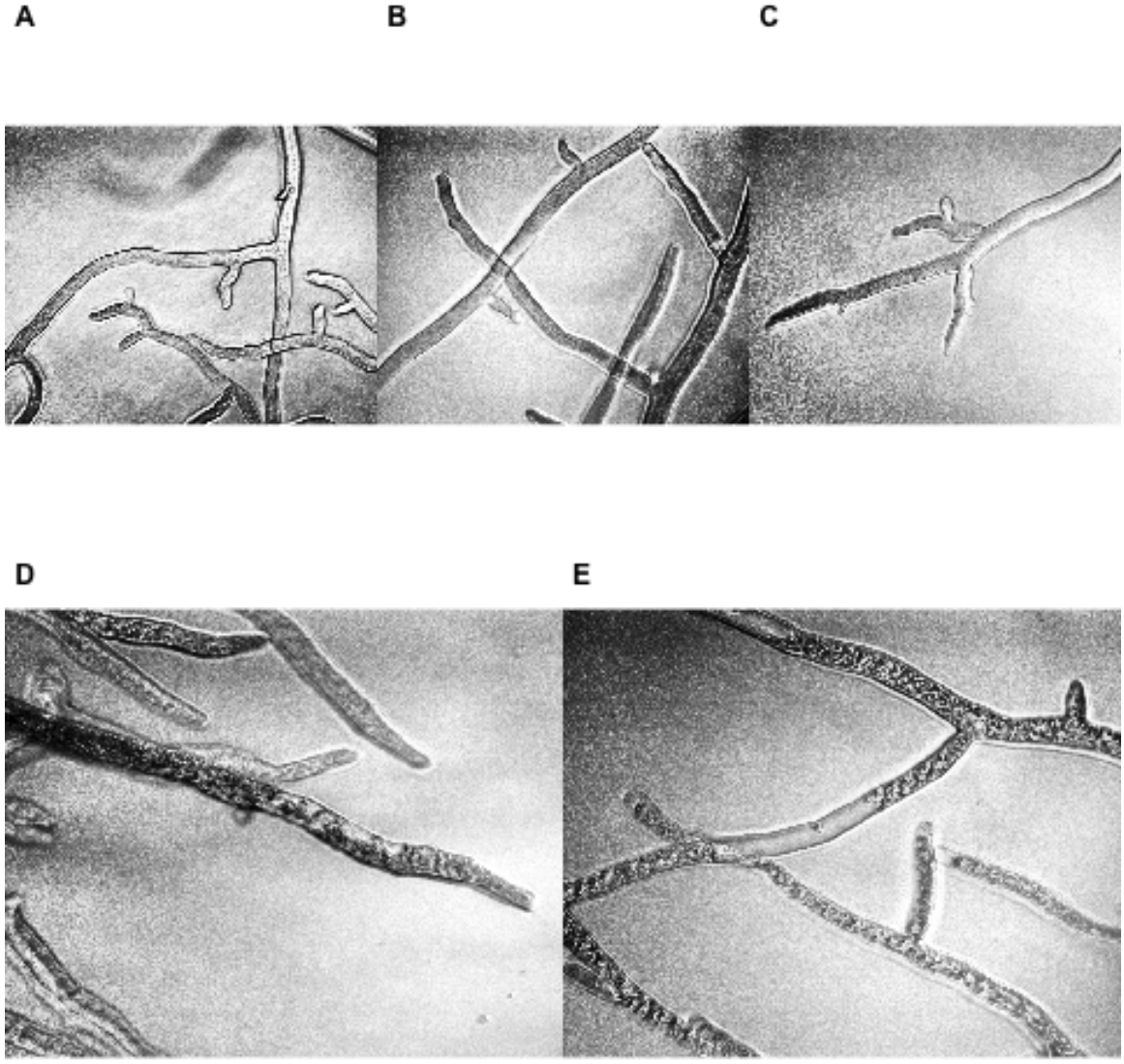


Figure 2.15: 40x Phase view A. *Neurospora crassa* control B. *Neurospora crassa* with 2-undecanone n=3 for each condition

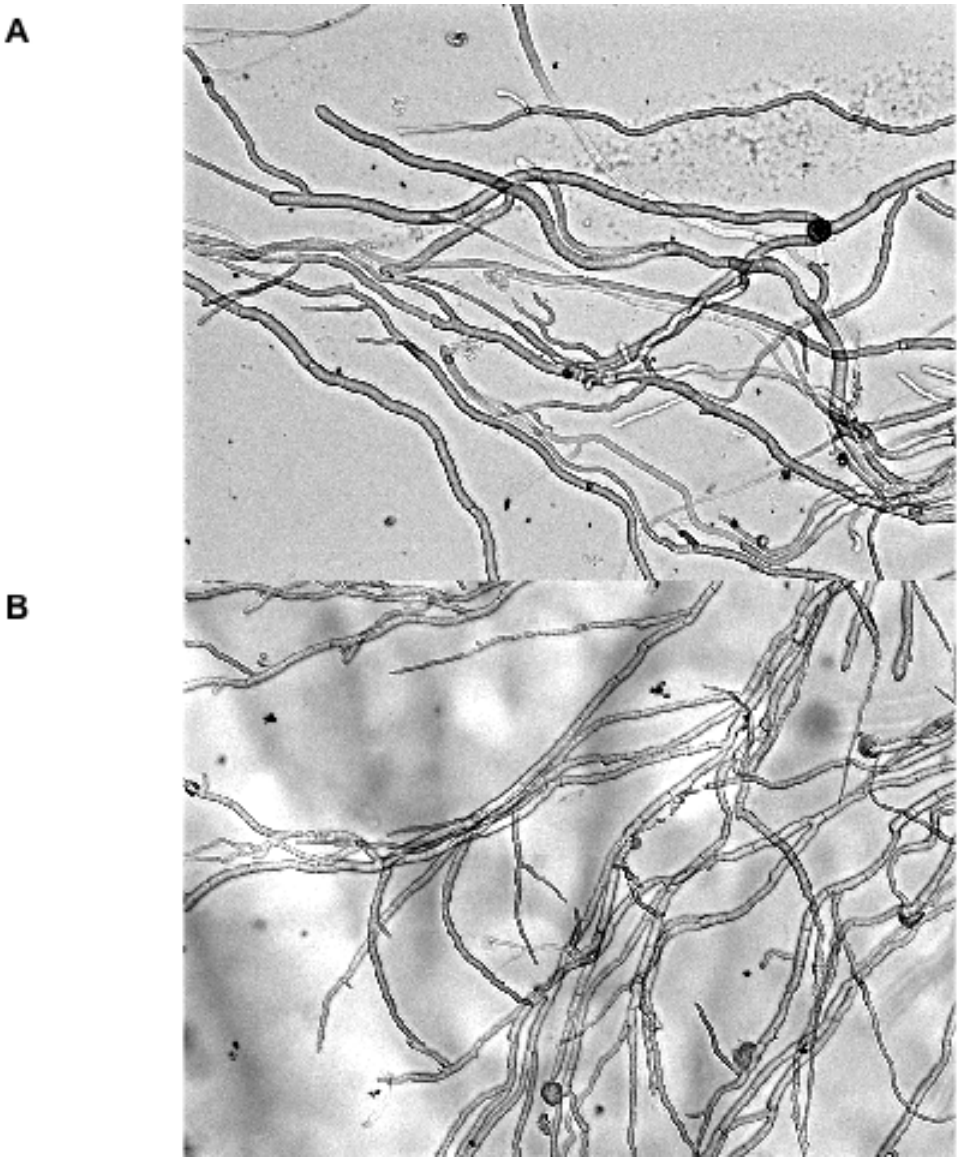
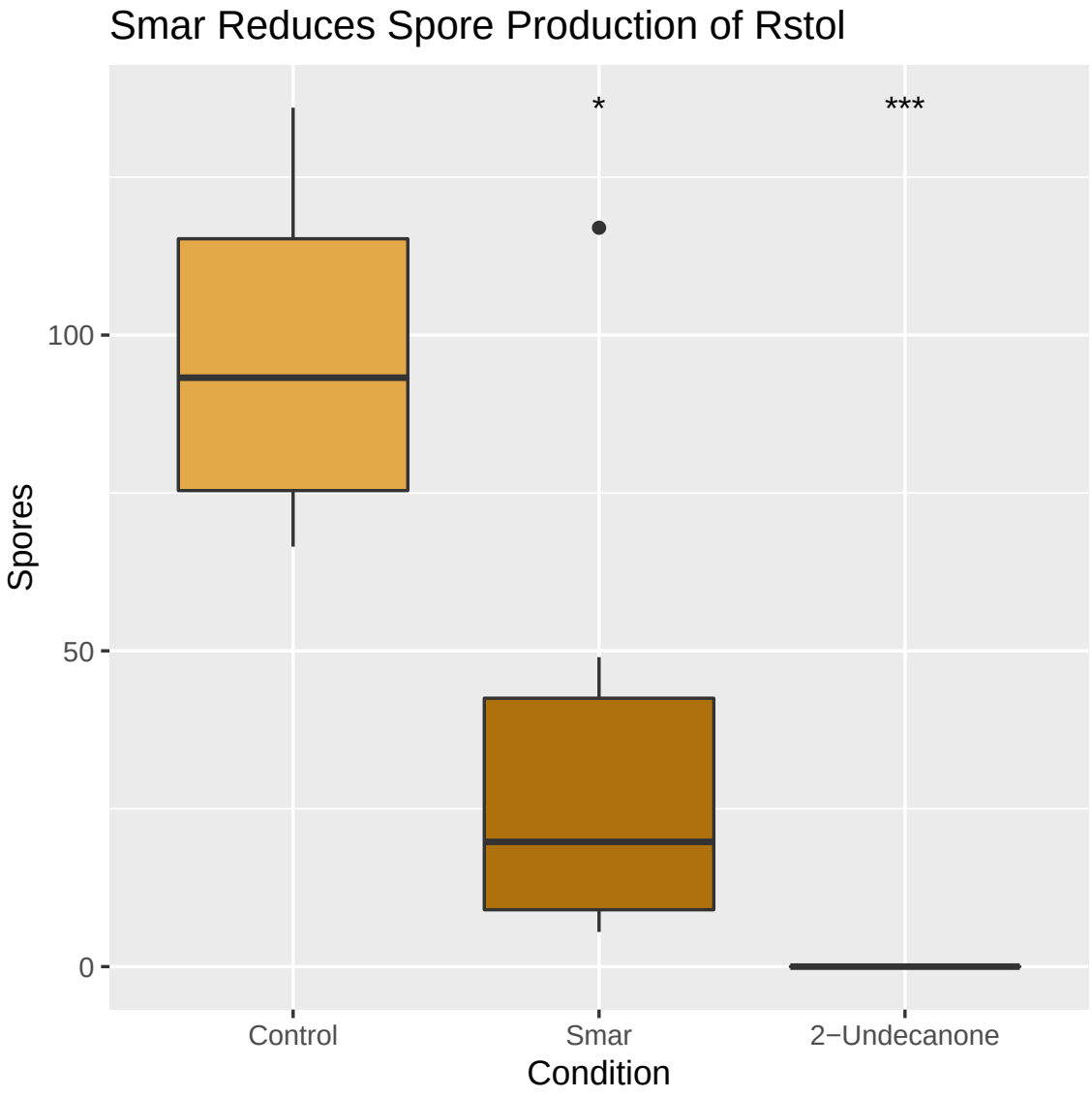


Figure 2.16: Strawberries grown with *S. marcescens* had less spores of Rsol compared to control, n=3



Chapter 3

Individual Bacteria-Derived Volatiles Inhibit Fungal Growth and Elicit a Transcriptional Response in *Neurospora crassa* and *Rhizopus stolonifer*

3.1 Introduction

Understanding microbial community interactions can provide insight into how the community functions as a whole, how these communities are formed and in turn, allows us to utilize these communities for the protection of agricultural crops. A form of long-distance

interactions can take place through small volatile organic compounds. These volatiles can be produced by bacteria and have detrimental effects on their fungal counterparts. Discovering these mechanisms and molecules that enable microbes to inhibit fungal competitors can provide new leads for compounds for therapeutics and improvements in agriculture proteins.

The development of new strategies and fungal inhibition and fungicides are spurred on by the devastation brought on by fungal pathogens. Fungi kill nearly 100,000 Americans every year [Stein et al., , Ballou, 2017]. Mucoromycosis is rare but exceptionally fatal killing 30-50% of patients depending on the species [Roden et al., 2005]. Despite the severe disease and death in humans caused by fungi very little is known about how other organisms fight with these microbes and how these pathogens perceive and interact with other microbes.

One disease management strategy to inhibit the growth of fungi in agriculture is through the application of biocontrol strains of bacteria and fungi. These are microbes that associate with plants or soils and can reduce or inhibit pathogenic microbe populations. *Pseudomonas* strains isolated from potato have been shown to inhibit *Phytophthora infestans* in dual culture assays in the lab [Hunziker et al., 2015]. Common soil-dwelling bacteria belonging to the genera *Serratia*, *Achromobacter*, *Bacillus* and *Stenotrophomonas* have been shown to colonize the hyphae of *Fusarium oxysporum* and attenuate its pathogenicity. Cured of its microbes the strain becomes virulent again [Minerdi et al., 2009, Minerdi et al., 2008]. These Rhizobacteria prevent fungal growth and are important plant partners and help promote growth. The bacteria *Serratia plymuthica* and *Stenotrophomonas maltophilia* can serve as beneficial partners with tobacco [Sharifi and Ryu, 2018].

Some rhizobacteria can produce aerosolized fungistatic compounds. A large variety of volatile compounds have been classified from bacteria such as acids, alcohols, lactones, ketones, amides, pyrazines, sulfur compounds, aromatic compounds from many soil-dwelling bacteria such as *Serratia*, *Xanthomonas*, *Bacillus*, *Pseudomonas*, and *Burkholderia* [Schulz et al., 2010, Kanchiswamy et al., 2015]. These volatile profiles are specific enough that the mVOC database was created to store key signatures for microbes [Lemfack et al., 2014, Lemfack et al., 2018]. A study of nearly 50 *Actinomyces* strains revealed they each had unique volatile profiles and the ability to inhibit pathogenic *Pseudomonas* [Choudoir et al., 2019]. In fact, many of these profiles have compounds that are effective antifungals, for example, hydrogen cyanide was detected in *Pseudomonas* strains and shown to be effective at preventing the growth of *Pythium, Fusarium,* and *Rhizoctonia* [Ossowicki et al., 2017]. Bean plant rhizobacteria volatiles were tested for their ability to inhibit the growth of fungal pathogens and light microscopy of *Sclerotinia sclerotiorum* revealed extensive vacuolization of hyphae, a common symptom induced by stress from antifungals [Giorgio et al., 2015]. Many studies have shown the power of volatile profiles in inhibiting fungal growth but few have examined the fungal response or the potential targets.

A common target of antifungals is the cellular membrane. Ergosterol is a key cholesterol in fungal cell membranes, and not in plants or animals, making it a key target for antifungals [Sangamwar et al., 2008]. Azoles are inhibitors of lanosterol 14-alpha-demethylase a crucial enzyme for converting lanosterol to ergosterol, preventing its production. Morpholines also inhibit ergosterol biosynthesis, but through 14-reductase and δ4-, δ8-isomerase interference [Horsburgh and Kirkpatrick, 1983]. Polyenes like amphotericin B,

nystatin, and natamycin forms a complex with ergosterol creating ion leakage events in the cell membrane [Gallis et al., 1990]. After damage, permeable cell membranes can permit free radicals to enter and cause oxidative stress, such as observations of *Saccharomyces cerevisiae* undergoing cell wall damage [de Souza Pereira and Geibel, 1999], or chitosan inducing an oxidative stress response in *Neurospora crassa* [Lopez-Moya et al., 2016]. Polyenes derived from *Streptomyces* Important targets for antifungal include: the fungal cell wall, efflux pumps, protein synthesis, and microtubules. There are multiple differences between fungi and animal cells and an important one is the cell wall. It can be disrupted through its major macromolecular components (chitin, B-glucan, and mannoproteins) [Etienne et al., 2012]. Efflux pumps are important for dumping toxins that are being used to kill it, however, some compounds like quinones can reduce the expression of transcripts for these pumps [Xie et al., 2016]. Protein synthesis in fungi is dependent on an additional elongation factor, EF-3, making this another important target [Sasnauskas et al., 1992]. Also, amino acid analogs have been found to be toxic [Capobianco et al., 1993]. Fungi (with the exception of many Mucoromycota fungi) cannot grow in the presence of griseofulvin (benomyl) as microtubule aggregation is inhibited [Randal M. Hauptmann, 1985].

Another compound that may affect cell wall stability is 2-Undecanone, an 11 carbon methyl ketone produced by many plants and bacteria. While it has been used as an insect repellent and known to inhibit fungi the exact mechanism is unknown. A recent study looked at the response of *Trichoderma* to three and 12-hour exposure to undecanoic acid introduced to the media [Mendes et al., 2018]. This study showed that alternative splicing of genes may be important for responding to Undecanonic acid. *Trichoderma* attempted to

detoxify the compound through lipid metabolism. The damage done to the cell wall was shown through biochemical assays looking at ergosterol, this explains the observed oxidative stress response. Finally, a glycogen synthase, phosphoglucomutase, used in cell wall biosynthesis, exhibited a significant difference in exon 4 usage following undecanone treatment. Interestingly, while the response to undecanoic acid seems incredibly fortuitous it would see that fungi may have to deal with their own fatty acid volatiles. A *Penicillium sp.* volatile profile changed rapidly after several generations of domestication cheese. In a matter of 10 generations it changed in color and showed a significant increase in its 2-Undecanone levels which led to a new flavor profile for the cheese.

Reactive oxygen species created inside fungal hyphae are another effective mechanism to inhibit fungal growth. Hydrogen sulfide was used to prevent *Aspergillus* and *Pencillium* and can create oxygen radicals in the hyphae [Fu et al., 2014]. A common bacteria volatile, dimethyl trisulfide, was shown to be effective at inhibiting *Colletotrichum gloeosporioides* on mango. Treated with dimethyl trisulfide the /textitColletotrichum had severe damage to the cell wall and internal organelles [Tang et al., 2019].

Along with these mechanisms some antifungals have been shown to be effective at increasing the potency of drugs with which they are coupled. Anisole, a compound we have shown recently to be produced by a lab strain of *Serratia marcescens*, has a very similar structure to many known antifungal quinones. These compounds have been shown to work in tandem with other drugs to increase their effectiveness or restore it from resistant fungi [Xie et al., 2016].

Very little is known about how fungi sense the presence of bacteria. Gaunine nucleotide binding protein coupled receptors (GPCRs) are important for perceiving the outside environment such as light, nutrients, chemical signals, [Cabrera et al., 2015] but have never been shown to be important in bacterial fungal interactions. 34% of drug targets are GPCRs in humans, making this a well studied system in mammals [Hauser et al., 2017]. In in GPCRs the N terminus binds to ligands from the environment then a conformational shift in the transmembrane domain leads to the G-protein complex (made up of an alpha, beta, and gamma subunit) phosphorylation of guanosine diphosphate to guanosine triphosphate, which goes on to complete the signal transduction cascade. There are around 10 classes in fungi in general, however some lineages have have less [Brown et al., 2018]. One class, PTH11, is important in virulence in Pizizomycotina further illustrating the importance of these proteins.

Despite the vast phylogenetic distances of bacteria and fungi they seem to have established a chemical *lingua franca* in chemical compounds. For example, fungi can detect many quorum sensing molecules produced by bacteria, and respond by reducing growth, inducing the formation of biofilms, and undergoing morphogenesis [Dixon and Hall, 2015]. This knowledge is particularly powerful given that these small diffusible compounds may be helping to prevent biofilm formation in the lungs of cystic fibrosis patients with *Aspergillus fumigatus* infections [Mowat et al., 2010]. The metabolites that microbes detect and exude are part of an interkingdom language of communication that allows information exchange about their environment and how to interact with it [Schmidt et al., 2017]. Despite the fact that nutrient availability can affect the volatile profile, there are still key components

that have been proposed to be used in the diagnosis Aspergillosis [Heddergott et al., 2014]. Another analysis looked at 151 microbial volatile profiles and has shown that many of these microbes can be distinguished from each other down to the Genus level [Misztal et al., 2018].

3.2 Methods

3.2.1 Strains

The strains used to calculate EC50 values, study gene expression, and find orthologs were *Neurospora crassa* (FGSC 4289) (*N. crassa*) and *Rhizopus stolonifer* (NRRL 66455) (*R. stolonifer*) and *Serratia marcescens* (lab strain: ADJS-2C Red). *R. stolonifer* was grown on Malt Yeast Extract Agar (MEYE) (1.5g Yeast Extract, 1.5g Malt Extract, 5g Dextrose, 7.5g Agar, 500ml Water), Smar was grown on Luria-Bertani (LB) media (10g Peptone, 10gNaCl, 5g Yeast Extract, 7.5g Agar, and 500ml Water) was added to the 60mm Petri dish lid. For *Neurospora* Vogel's media was used instead (125 g Na3 Citrate 2-H2O), 250 g KH2PO4-anhydrous, 100 g NH4NO3-anhydrous, 10 g MgSO4-7 H2O, 5 g CaCl2-2 H2O, 5mL Trace Element Solution (5 g Citric acid-1 H20, 5 g ZnSO4-7 H20, 1 g Fe(NH4)2(SO4)2-6 H20, .25 g CuSO4-5 H20, 0. 25 g MnSO4-1 H20, 0. 05 g H3BO3-anhydrous, 0. 05 g Na2MoO4-2 H20), 2.5 mL Biotin Solution (5.0 mg biotin in 50 mL distilled water)

3.2.2 Assessing Fungal Sensitivity to Compounds

Testing Abundance Compounds Dimethyl Trisulfide (Fisher, AC415030050), Anisole (Sigma-Aldrich 296295), and 2-Undecanone (Sigma-Aldrich U1303), Tropone (Sigma-

Aldrich 252832), Lepidine (Sigma-Aldrich 158283), and 2,5-Dimethylpyrazine (Acros Organics AC174520050) were found to be abundantly produced by *Serratia marcescens, Serratia proteamaculans,* and *Bacillus subtilis.* The following is a table showing the compound, its average percent presence in the profile, and the bacteria that produced it. 3.1

Table 3.1: Compounds with their percent of abundance in the total volatile profile.

Compounds	***B. subtilis***	***S. marcescens***	***S. proteamaculans***
Dimethyl trisulfide	0	2.855258029	73.41496594
Anisole	0	44.83905842	0
2-Undecanone	11.04622619	0.611615147	0.654795985
Tropone	0	0.68005108	1.033952363
Lepidine	0	0	0.064738857
2-ethyl-5-methyl-Pyrazine*	0	0.922690296	1.178659906

*tested pyrazine was actually 2,5-dimethyl Pyrazine, as this was the original compound we predicted during the first analysis of the volatile profiles (Feb 2018).

10 mg of each of these volatiles were pipetted onto a 1cm x 1cm filter paper in the outside ring of a donut plate with 1000 spores of *R. stolonifer* or 1000 conidia of *N. crassa* on MEYE or Vogel's in the central Petri dish. After 24 hours the fungal mycelium diameter was measured. Each compound had three replicates.

Assessing Volatile effect on Hyphal Growth 20mL of Agar + MEYE or Vogels Media (for *N. crassa*) in a 100mm diameter Petri dish with a circle of cellophane

covering the surface was inoculated with 10μl of spore/conidial suspension of approximately 500 spores/μl (as assessed by hemocytometer) was added to each plate, and allowed to incubate for 16 or 36 hours at 25°C for *R. stolonifer* and *N. crassa* respectively. The cellophane was used to allow for easier removal for the RNA extraction experiments, and to keep the hyphae at the surface of the media. The liquid compounds of Dimethyl Trisulfide (Fisher, AC415030050), Anisole (Sigma-Aldrich 296295), and 2-Undecanone (Sigma-Aldrich U1303) and a blend of all three compounds (1:10:20) were diluted in 95% ethanol (all compounds were soluble in ethanol) in series of series of dilutions from 0 to 1000 times diluted. A volume of 10 μl of each diluted compound (or pure ethanol for the control) was pipetted onto a 1cm by 1cm piece of filter paper on top of a 1.5 by 1.5cm piece of aluminum foil placed on the opposite side of the 100mm Petri dish with the germinated spores of *N. crassa* or *R. stolonifer*. It should be noted that the ethanol control had no effect on the hyphal growth as compared to samples with no ethanol as a control. The aluminum foil prevented the compound from coming into contact with the media and diffusing toward the spores and the filter paper facilitated even and equal volatilization of the compounds from the same amount of surface area (1cm^2). This ensured that the compounds only interaction with the spores was from aerosolized volatiles. The plates were incubated in 12 hour light and dark conditions at 25°C, and growth was photographed at a height of 20 cm with a 14-megapixel camera then quantified by the change in pixels of the mycelium using ImageJ software.

Assessing Volatile Effect on Germination In my previous work *Neurospora* was shown to be more sensitive to 2-Undecanone than textitRhizopus so I tested Neurospora

spore germination in the presence of volatiles was assessed in a similar assay to the hyphal growth assay to see if it also could prevent germination. A known number of conidia (500) were plated on Vogels media, then a 10mg solution of a liquid compound (one of the pure volatiles produced by bacteria as identified by GCMS) was added on top of Vogels media with agar, then the conidia and compounds were mixed and spread over the surface with a cell spreader. The Petri dishes were then sealed with a double layer of Parafilm to ensure that no holes would allow for the exchange of fresh air. After 12 hours the germinated conidia were counted by dissecting microscope.

3.2.3 Growth Rate Inhibition Calculation:

To establish the concentration of 2-Undecanone, DMTS, and Anisole at which there was 50% hyphal growth rate (GR50) of *R. stolonifer*/ *N. crassa*, the growth measurements were calculated with linear regression tool, GRmetrics (v 1.10.0) package in R (v 3.6.0). The reason GR50 was used over the effective concentration where 50% of growth was prevented (EC50) was that GR50 is normalized for growth rate for the different fungi. For example, a slow-growing fungus may seem to have a higher resistance to a drug than a fast-growing one just by looking at the EC50 values although the biological effect is the same. After the GR50 was calculated the volume of chemical aerosolized in the headspace was calculated by using the area minus the volume of media. Volume of Petri dish = $\pi r^2 h$, $r = 50cm$, $h = 15cm$. (Total Volume − Media Volume) = Headspace Volume.

$$31cm^3 - 18cm^3 = 13cm^3$$

So the volatile/unit headspace was calculated as:

(GR50 of Volatileul)/13cm^3

3.2.4 Measuring Volatiles' Potential for Inhibition of G-protein Coupled Receptor Subunit alpha KO Mutants in *Neurospora crassa*

Mutants (Gna1 - NCU06493 , Gna2 - NCU06729, Gna3 - NCU05206, Gnb1 - NCU05206) were obtained from the lab of Katherine Borkovich [Ivey et al., 1999, Yang et al., 2002, Baasiri et al., 1997] and were grown on Vogel's media with either *Serratia marcescens* in a "donut" plate assay or with filter paper with 10mg of 2-Undecanone on Vogels as described below. *Serratia* volatile profile inhibition ability was measured using a "donut" plate assay which consists of a 100mm diameter Petri dish with a smaller 60mm Petri dish lid placed inside physically separating the bacterial media in the outside ring and fungal media on the inside ring. A volume of 15ml of LB media was pipetted into the outer ring of a 100mm Petri dish with a 60mm Petri dish lid placed inside. Then 8ml of Vogel's media was pipetted into the center plate. Fungi were grown to conidiation (1 week) at which point the spores were collected with a sterile toothpick then stored in a 1.5ml microcentrifuge tube with 1ml of autoclaved water at 4°C until use. 1 ml of overnight bacterial culture (single colony picked with sterile toothpick inoculated into 25ml LB without agar in 50ml conical tube, shaken overnight at 100 RPM at 28°C) was inoculated onto the outer LB ring and allowed to grow for 48 hours at 28°C. *N. crassa* spores were inoculated onto the center plate and allowed to grow for 24 hours then the diameter of the mycelia were measured. There were three technical replicates. The growth rate inhibition was calculated by the following equation:

$$\frac{GrowthRateofControl(cm^2) - GrowthRatewithSmar(cm^2)}{GrowthRateofControl(cm^2)} = GrowthRateInhibition$$

To test 2-Undecanone (Sigma-Aldrich U1303) for growth inhibition on fungi. 10mg of the compound was pipetted onto a 1cm x 1cm filter paper in the outside ring of a "donut" plate with 5000 conidia of *N. crassa* on Vogel's media in the central Petri dish. After 24 hours the fungal mycelium diameter was measured. There were three technical replicates.

3.2.5 RNAseq Experiment Set-up

100 mm Petri dishes were filled with 20mL of MEYE/Vogels media with agar then a sheet of cellophane cut to the size of the Petri dish was placed on the surface to allow for quick collection of RNA from fungal tissue. 10 μl of 5000 spores/μl of *R. stolonifer/N. crassa* were inoculated in a 7 cm long line on opposite sides of a 100 mm Petri dish, the center point of the lines was set approximately 1.5 cm in from the perimeter of the Petri dish. The fungi were then incubated for 16 hours at 25°C, in the dark. The filter paper was inoculated with 2-Undecanone, Dimethyl Trisulfide or a blend at equal potency levels using the EC50 values (2 mg of 2-Undecanone, 1 mg of Anisole, and .01 mg of DMTS). Lids were taken off to allow gas exchange on all samples including the controls, then a thin sheet of aluminum foil with a strip of filter paper (1 cm wide) was added down the middle between the *R. stolonifer/N. crassa* lines. In the experimental condition, the filter paper was inoculated with 10 μl of 2-Undecanone, DMTS, or a blend and allowed to incubate for 1 hour.

3.2.6 Tissue Collection and RNA Extraction for Transcriptional Profiling

The hyphae were collected from the cellophane (Bio-Rad, Cat No. 1650963) and transferred to liquid nitrogen within 5 seconds of the initial disturbance. The tissue was then ground into a fine powder in a mortar and pestle. 1 mL of Trizol (Invitrogen) was used to resuspend the nitrogen frozen and ground fungal tissue. 0.5 mL of isopropanol was added and incubated for 10 minutes to precipitate the RNA. The RNA was then centrifuged for 10 minutes at 12,000 g at 4°C. The pellet was gently washed with 1 mL of 75% ethanol then centrifuged for 5 minutes at 7500 g at 4°C. RNA pellets were desiccated at 65°C for 5 minutes and resuspended with 50 μl nuclease-free water. Then quantified and quality checked by Nanodrop 2000 (Thermo), all samples have greater than 500 ng/μl and 260/230 ratio of over 1.8 and 260/280 ratio over 2.

3.2.7 RNA Sequencing

250-300 base pair cDNA library was prepared and sequenced by Novogene (Novogene, China) using NEB Next Ultra RNA Library Prep Kit. The 150PE non-stranded library was then sequenced by Illumina NovaSeq 6000 (Illumina, USA). Each sample had at least 6 G of reads and over 20 million reads. For *N. crassa* there were 3 samples of each condition Control and 2-Undecanone. For a second sequencing event 3 Controls, DMTS and Blend were sent. For *R. stolonifer* there were 3 samples of 2-Undecanone and DMTS.

3.2.8 Generating Read Counts

Using Kallisto (v 0.46.0) [Bray et al., 2016] the transcriptomes of *Neurospora crassa* OR7A strain FGSC 4289 and *Rhizopus stolonifer* NRRL 66455, downloaded from FungiDB

and the Joint Genome Institute (August 2019), were indexed with kmer size of 31. Then the reads of each transcriptomic sample were quantified with the following parameters of Kallisto quant: fragment length = 300, with standard deviation from that length = 30, with read bias correction.

3.2.9 EdgeR Differential Expression Analysis

The kallisto count data was passed to EdgeR [Robinson et al., 2010] (v 3.26.8) to generate the DEG list. The DEG list was created using the following parameters: a False Discovery Rate (p-value) cut-off of ¡.01 and Log Fold Change (LFC) of 1 (2 fold or greater up or down expression change), using trimmed mean of M-values to normalize. Each condition of 3 samples were all compared to the controls (3 for *R. stolonifer*, and 6 for *N. crassa*) to find DEG from control conditions. Degust (v 4.1.1) [Powell et al., 2019] was used to visualize transcripts and QC RNA read data script and figures in supplemental data.

3.2.10 Assigning GO terms to DEGs

The Gene Ontology tables for *N. crassa* were downloaded from Fungidb, for release-46 *N. crassa*ORA7 (Nov 4 2019) and the for *R. stolonifer* they were downloaded from the Joint Genome Institute (November 2019) for Rhizopus stolonifer NRRL 66455 v1.0. There were 2796 unique GO terms for *N. crassa* and 5866 proteins had a GO term assignment. *R. stolonifer* had 2152 GO terms and 6512 proteins assigned to them. The GO terms were assigned to the DEG list using the R packages, AnnotationDbi [Herv Pags, 2017] (v 1.46.1), GSEABase [Martin Morgan, 2017] (v 1.46.1), GOstats [Robert Gentleman, 2017] (v

2.50.0), the script can be found at `https://github.com/stajichlab/RNASeq_volatile_response`.

GO assignments for molecular function for DEGs of *N. crassa* are extracted from a computed and curated GO term table downloaded from Fungidb (Nov 2019), with a p-value of less than .05. These results were uploaded to REVIGO to generate the *N. crassa* GO plots and Euler diagrams in R using treemap (v 2.4-2) and VennDiagram (v 1.6.20) [Chen and Boutros, 2011].

3.2.11 Comparison of the Transcripts from *N. crassa* and *R. stolonifer*

Comparison of the shared response of two different organisms was assessed two ways, shared GO terms between the organisms' DEGs and orthologs of the proteins of said DEGs. The GO term list generated for each fungi at each condition was compiled into a list for a side by side comparison.

Using the proteomes of *N. crassa* and *R. stolonifer*, OrthoFinder (v 2.2.7) [Emms and Kelly, 2015] generated an ortholog table for genes between the fungi with predicted orthology. Each ortholog group had one or more genes assigned to it that were then parsed into separate rows. Then the DEGs from each condition were used as a query for this file to generate a list of orthogroups that could be compared between species.

After finding the DE genes in the various conditions a few genes of interest were examined further for their presence in other kingdoms. This was done using ortholog tables from FungiDB.

3.3 Results

3.3.1 Fungal Growth Inhibition by Single Volatiles

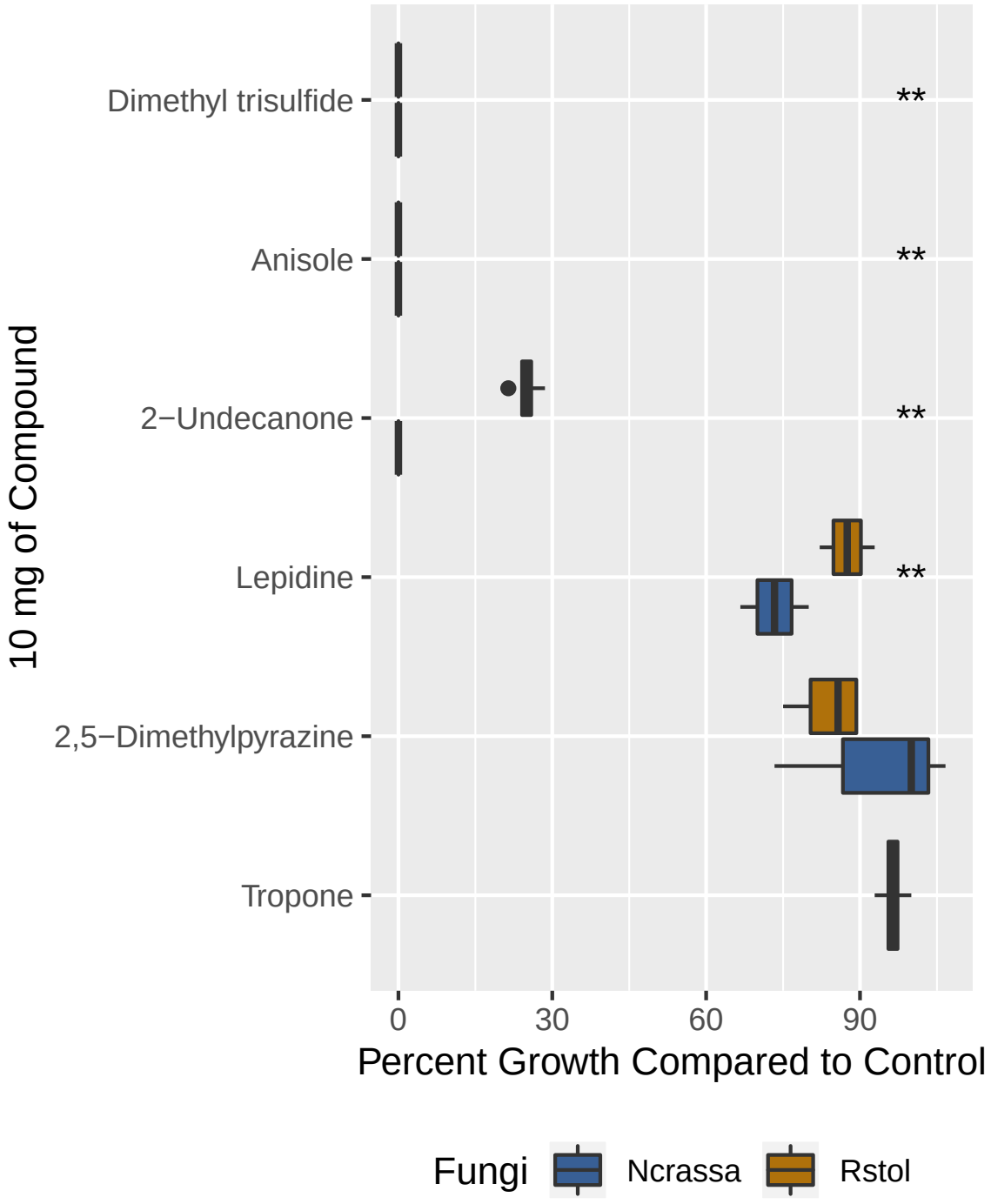
In chapter 2,GCMS analysis revealed that 2-Undecanone, Anisole, Lepidine, Tropone, 5-Methyl-2-furyl methanol, 2,5-Dimethylpyrazine, and Dimethyl trisulfide (DMTS) were identified as abundant compounds from the volatile profiles of *Serratia marcescens, Serratia proteamaculans*, and *Bacillus subtilis*. These compounds were tested for their ability to inhibit fungal growth 3.1. DMTS and Anisole could prevent spores from germinating for both *R. stolonifer* and *N. crassa*. 2-Undecanone could prevent growth in *N. crassa* but slowed growth in *R. stolonifer*. The other compounds tested showed little inhibition in growth.

3.3.2 EC50 Curves

I then tested serial dilutions of DMTS, 2-Undecanone, and the blend on *N. crassa* and *R. stolonifer* cultures to find the minimum effective concentration necessary to observe 50% growth inhibition (EC50).3.2

Effective concentration with 50% of the fungal growth of the control was calculated for each compound. For all the tested volatiles *N. crassa* had a lower tolerance for all volatiles compared to Rhizopus. Anisole and 2-Undecanone were not able to prevent *R. stolonifer* mycelia plugs from growing, therefore their EC50 could not be calculated but for DMTS the concentration for *R. stolonifer* spores was approximately 0.001 μl/cm^3 for *R. stolonifer* and .0008 μl/cm^3 for *N. crassa*.

Figure 3.1: Selected volatiles tested at 10 mg for fungal growth rate inhibition. n=4



DMTS was the most effective at inhibiting growth at the lowest concentration, followed by Anisole then 2-Undecanone. Blending all volatiles together did not inhibit better than any one compound alone, indicating that perhaps 2-Undecanone and Anisole were diluting the DMTS resulting in a weaker overall inhibition potential. The blend of volatiles made reduced the growth rate of *R. stolonifer* hyphae, but not to the degree of DMTS. *R. stolonifer* spores were more sensitive to volatiles than Mycelia, fig 3.1C. DMTS had a GR50 three times higher on spores than on hyphal tissue. For the *R. stolonifer* spores DMTS EC50 was over 1000 times greater than 2-Undecanone, over 100 times greater than Anisole, and twice as high as the Blend.

3.3.3 G-couple Protein Receptor, GnA1, is less sensitive to Smar Volatiles

One GPCR, GnA1 (NCU06493), had a lower percent inhibition than the WTA *N. crassa*. 3.3. The GPCR KOs were still inhibited by Smar volatiles however, GPCR alpha 1 subunit KO, GnA-1 (NCU06493), was able to grow better than the WT controls. This inhibition rate of GnA-1 was about 30% but the other strains and WT *N. crassa* had inhibition around 60%.

3.3.4 Fungal Transcriptomic Response to 2-Undecanone, DMTS and Blend

One hour of exposure to the volatiles lead to differential expression of around 20% of the *N. crassa* transcriptome. There were 1250, 1282, 1289 transcripts with higher levels than the control treatments from 2-Undecanone, DMTS, and the Blend treatments respectively and 1166, 771, and 1272 transcripts were down in the three treatments. Al-

though there were similar numbers of upregulated genes, they were not the same genes. 2-Undecanone and Blend had a very different set of DEG than DMTS. Overall from the three conditions 5,572 unique genes had greater than 1 LFC and a p-value of less than 0.01. 3.4 637 Genes are up in DMTS and not 2-Undecanone, the GO terms for these genes are oxidoreductase activity, acting on a sulfur group of donors and ion binding. In 2-Undecanone 586 genes are up and not in DMTS - some of these are specifically for lipid metabolic process.

The upregulated genes in the DMTS treatment had a higher average log fold change (LFC) level than 2-Undecanone. 3.5 DMTS has an average of 2.38 LFC above 1 LFC, and 652 genes about 2 LFC, compared to 2.05 LFC and 465 genes for 2-Undecanone. 2-Undecanone treatments had the most, lowest expression genes with 377 genes below 2 LFC and an average expression below 1 LFC of -1.92. DMTS treated fungi at 88 genes below 2 LFC expression and an average of -1.51 LFC below 1 LFC. 3.6

The 2-Undecanone and DMTS DEG response in *R. stolonifer* was different than *N. crassa*. 3.7 Only about 1,000 genes were differentially expressed in the 2-Undecanone treatment vs over 7,000 genes in the DMTS treatment, roughly 7% and over 40% of the transcriptome respectively. 7,080 unique genes were expressed 1 LFC or greater. 3.8

3.3.5 Comparing Expression of Ncrasssa and *R. stolonifer* with Orthogroups

To compare the transcripts of *R. stolonifer* and *N. crassa* we used the proteins encoded by the DEGs as a query to search an ortholog table with orthogroups shared between *R. stolonifer* and *N. crassa*. These orthogroup terms were compared to find conditions that shared potentially similar transcriptional responses. Each condition had many unique orthogroups but *R. stolonifer* DMTS and *N. crassa* 2-Undecanone DMTS with greater than 1 LFC down had 151 orthogroups, more than any other condition. 3.11 GO summary of biological process activities of this intersection showed that many of these genes are involved in transcription, RNA processes, transport, among other things listed in the following table, indicating that these two conditions had down-regulation of growth. 3.2

There were no orthologs found only in 2-Undecanone up-regulated genes between *N. crassa* and *R. stolonifer*. GO terms assigned to the DEGs were associated with response to light stimulus and quinone metabolic process. 3.3

Table 3.2: Biological processes gene ontology summary of upregulated genes in response to 2-Undecanone and DMTS in *N. crassa* and *R. stolonifer*, respectively, with orthologous proteins.

GOBPID	**p-value**	**Count**	**Size**	**Term**
GO:0051179	0.000351048	7	805	localization
GO:0051234	0.000531459	7	784	establishment of localization

Table 3.2 continued from previous page

GOBPID	p-value	Count	Size	Term
GO:0006810	0.00055266	7	782	transport
GO:0055085	0.000643245	1	377	transmembrane transport
GO:0051252	0.006786277	2	348	regulation of RNA metabolic process
GO:1903506	0.00787231	2	341	regulation of nucleic acid transcription
GO:2001141	0.00787231	2	341	regulation of RNA biosynthetic process
GO:0006355	0.00787231	2	341	regulation of transcription, DNA-templated
GO:0016192	0.011792156	0	175	vesicle-mediated transport
GO:0050896	0.014805552	42	2202	response to stimulus

Table 3.3: Biological processes gene ontology summary of upregulated genes in response to 2-Undecanone and DMTS in *N. crassa* and *R. stolonifer*, respectively, with orthologous proteins.

GOBPID	p-value	Count	Size	Term
GO:0009416	0.001185529	14	363	response to light stimulus
GO:0009314	0.00125056	14	365	response to radiation
GO:0005992	0.002310761	2	5	trehalose biosynthetic process
GO:0046351	0.002310761	2	5	disaccharide biosynthetic process

Table 3.3 continued from previous page

GOBPID	p-value	Count	Size	Term
GO:0009312	0.002310761	2	5	oligosaccharide biosynthetic process
GO:0006879	0.003431284	2	6	cellular iron ion homeostasis
GO:0055072	0.003431284	2	6	iron ion homeostasis
GO:0042180	0.005768486	3	24	cellular ketone metabolic process
GO:1901661	0.006277102	2	8	quinone metabolic process
GO:1901663	0.006277102	2	8	quinone biosynthetic process
GO:0006743	0.006277102	2	8	ubiquinone metabolic process

Interestingly, 75 orthogroups intersected for *R. stolonifer* DMTS Down and *N. crassa* DMTS Up. A biological process GO summary of these genes reveal that many of these genes are involved sexual reproductive processes.

DMTS UP conditions for both *R. stolonifer* and *N. crassa* had genes that were involved in detoxification, riboflavin biosynthesis, sulfur compound metabolic process, and many other compounds seen in the table below. 3.4

Table 3.4: Biological processes gene ontology summary of upregulated genes in response to DMTS with orthologous proteins in *N. crassa* and *R. stolonifer*.

GOBPID	**p-value**	**Count**	**Size**	**Term**
GO:0097237	1.26E-08	4	4	cellular response to toxic substance
GO:1901701	4.30E-07	4	7	response to oxygen-containing compound
GO:1990748	1.22E-06	3	3	cellular detoxification
GO:0046185	1.22E-06	3	3	aldehyde catabolic process
GO:0098754	4.85E-06	3	4	detoxification
GO:0006771	4.15E-05	3	7	riboflavin metabolic process
GO:0042726	4.15E-05	3	7	flavin-containing compound (metabolic)
GO:0042727	4.15E-05	3	7	flavin-containing compound (biosynthetic)
GO:0009231	4.15E-05	3	7	riboflavin biosynthetic process
GO:0042364	4.32E-05	4	19	water-soluble vitamin biosynthetic process
GO:0009110	5.36E-05	4	20	vitamin biosynthetic process
GO:0006767	6.56E-05	4	21	water-soluble vitamin metabolic process
GO:0006749	6.59E-05	3	8	glutathione metabolic process
GO:0006790	9.66E-05	6	70	sulfur compound metabolic process
GO:0016226	9.80E-05	3	9	iron-sulfur cluster assembly
GO:0031163	9.80E-05	3	9	metallo-sulfur cluster assembly

Table 3.4 continued from previous page

GOBPID	p-value	Count	Size	Term
GO:0070887	9.83E-05	28	1349	cellular response to chemical stimulus

GO Analysis Shows Condition-specific Responses

Overall, many GO terms were assigned to the DEGs for each condition for *N. crassa*, there were far fewer for *R. stolonifer*. 3.12 The following table is a summary of all the GO terms assigned to *R. stolonifer* DEGs. 3.5 Many GO terms assigned to *N. crassa* DEGs 3.15 overlapped for both the greater than 1 LFC DEGs and the lower than -1 LFC. 3.13 3.14 A summary of all the GO assignments shows that the Blend and 2-Undecanone treatments had more GO term assignments to the DEGs showed that *N. crassa* was turning off transporter activity, and slowing down processes involved in growth (rRNA metabolism, ribosome biogenesis, in the presence of 2-Undecanone, carboxylic acid transport - Fatty acid synthase is way down NCU07308. The GO terms associated with DMTS treatment focused on oxidoreductase activity, acting on a sulfur group of donors, sulfur is an acceptor.

Table 3.5: Summary of *R. stolonifer* Go Terms with p-values less than .05

GOMFID	**Count**	**Size**	**Term**	**Condition**	**DEG**
GO:0004602	9	24	glutathione peroxidase activity	DMTS	Up
GO:0016684	9	25	oxidoreductase activity...	DMTS	Up
GO:0016209	9	25	antioxidant activity	DMTS	Up
GO:0004601	9	25	peroxidase activity	DMTS	Up
GO:0004930	3	16	G protein-coupled receptor activity	UND	Up
GO:0004888	3	20	transmembrane receptor activity	UND	Up
GO:0016500	1	1	protein-hormone receptor activity	UND	Up
GO:0004936	1	1	alpha-adrenergic receptor activity	UND	Up
GO:0004937	1	1	alpha1-adrenergic receptor activity	UND	Up

Using curated GO assignments I improved the predicted functions of the DEG in *N. crassa*. 3.18

In the 2-Undecanone treatment, oxidoreductase activity is up as well as cell wall integrity. This may indicate that the 2-Undecanone as a detrimental effect on the cell wall and the fungi is responding to damage. In the down-regulated genes are GO terms

associated with growth such as RNA transcription, as well as many genes in similar semantic space as transporter activity, meaning dozens of go terms are indicating that transport is down.

This may be a result of a lack of response to the presence of the volatiles, allowing the fungi to "ignore" the bacteria.

DMTS had ion binding GO terms for the up-regulated genes. As well as up-regulation of glutathione transferases which a commonly associated with detoxification of xenobiotics in cells. [Allocati et al., 2018]. DMTS had transport activities associated with the down-regulated genes as well as RNA binding and tubulin and other growth associated functions.

The blend up-regulated genes included those found in DMTS and 2-Undecanone such as lyase activity. 2-Undecanone and the blend shared genes associated with lipid binding, tetrapyrrole binding, and photoreceptor activity. This is interesting as heme-binding homologs in Arabidopsis do not function as they do in human cell lines, rather than binding to heme, they serve as messengers between organelles, [Takahashi et al., 2008]. In fungi, tetrapyrroles are involved in light detection. There may be some overlap in response to volatiles and response to light [Herrera-Estrella and Horwitz, 2007], and utilizing similar pathways for signaling. Down-regulated genes had GO terms associated with growth and development and transmembrane transport. The transporters down-regulated in every condition may be the result of the fungi attempting to compartmentalize the chemical, preventing it from moving throughout the hyphae. While the blend had a similar transcriptomic profile to the 2-Undecanone it also had the compound Anisole which may contribute

to additional genes not found in the other profiles. 379 genes were downregulated in the blend treatment but not in 2-Undecanone or DMTS. These 379 genes were also involved in growth such as elongation factor activity, nucleotide-binding, and actin-binding. There were 291 genes that were upregulated in the blend and not in 2-Undecanone or DMTS, these were ligases and oxidoreductases. Additionally, there were transporter genes that were not present in the downregulated genes of DMTS and 2-Undecanone treatments and found 16 genes. 3.6 An interesting gene here is the multidrug resistance -12 and 15 protein, a type of ATP-Binding cassette (ABC) transporter which has been shown to be important in efflux, or dumping of toxins.

Table 3.6: 16 genes found down-regulated in blend condition but not in 2-Undecanone or DMTS, thought to be a result of Anisole treatment

Gene ID	Product Description
NCU00790	potassium transporter hak-1
NCU02263	SEC14 cytosolic factor
NCU03710	mitochondrial carrier-39
NCU04127	hypothetical protein
NCU04293	vesicle transport-9
NCU04656	MFS transporter
NCU04942	methionine permease

Table 3.6 continued from previous page

Gene ID	Product Description
NCU05519	MFS transporter
NCU06860	multi-drug resistance-15
NCU07247	DUF6 domain-containing protein
NCU07668	multi-drug resistance-12
NCU08199	6-hydroxy-D-nicotine oxidase
NCU08439	leptomycin B resistance protein pmd1
NCU08743	inorganic phosphate transporter
NCU08897	protein transporter SEC61 subunit alpha
NCU10239	hypothetical protein

3.3.6 Evaluating similarities between *N. crassa* and *R. stolonifer*

200 GO terms were assigned to the 6 different conditions for *N. crassa* at a p-value of less than .05. *R. stolonifer* had 16 GO terms associated with 3 out of 4 conditions. There were five overlapping GO terms predicted above 95% confidence from the DEGs between *N. crassa* and *R. stolonifer* occurring in the same condition.

20 genes involved in transporter activity were found in *N. crassa* with corresponding down-regulated DEGs in *R. stolonifer* during exposure to DMTS. Of these 20, two were Major facilitator superfamily transporters, which are important microbial transporters

specifically found in Fungi. Additionally, 16 transport-associated genes were found with the blend treatment with an assortment of MFS and ABC transporters being down-regulated.

3.4 Discussion

DMTS and Anisole were the strongest inhibitors of growth from these assays, but Tropone, 2,5-Dimethyl Pyrazine, and Lepidine were all weak inhibitors of growth. This demonstrates that even at very high levels not all volatiles in the bacterial profile have inhibitory properties.

Studying GPCRs role in detecting volatiles showed that the loss of function of the GPCR alpha subunit does not prevent inhibition. The GPCR alpha subunit is important connection between the transmembrane domain and the other GPCR subunits. Of the three proteins alpha subunits have been created in *N. crassa*, on gna1 seemed to have decreased sensitivity to 2-Undecanone. This may be due to the fact that this mutant has higher oxidoreductase activity compared to WT [Yang and Borkovich, 1999], however, gene expresssion did not significantly change when exposed to the volatiles. Further work needs to be done to understand the role of this subunit in volatile response.

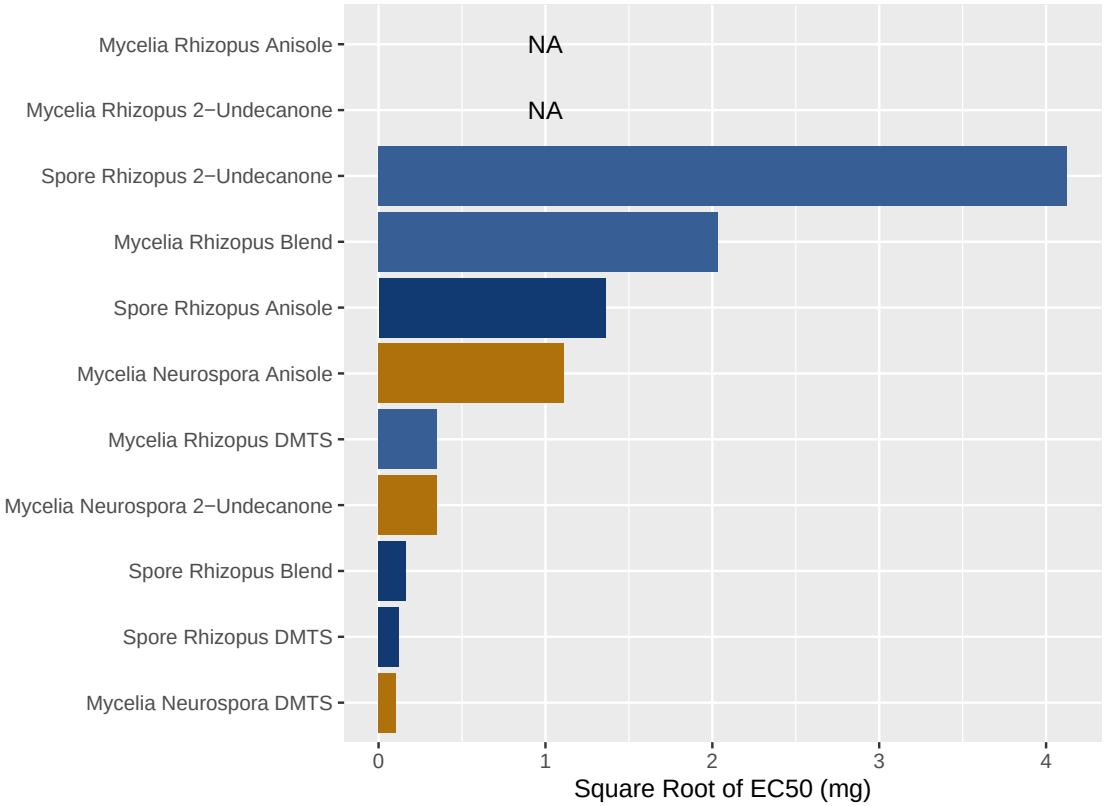
From both *N. crassa* and *R. stolonifer* we see that 2-Undcanone downregulated genes that might stop the spread of volatiles throughout the mycelium like transporters while at the same time working to metabolize the fatty acids from two undecanone or utilize the sulfur as an elector receptor. DMTS response increased gene expression that may be helping the fungi reduce oxidative stress from damaged cell walls.

Due to the importance of transporter activity in efflux and detoxification the down-regulation of so many transporters was unexpected. However, this may be only the initial stage of the detoxification which has been characterized in fungi as being the modification phase [Sang et al., 2018]. The fungi may be attempting to metabolize the various compounds before flushing. Our work has highlighted the initial response of fungi to volatiles, a 1-hour snapshot, but there may be additional responses later on. More time points might help to show more genes in the GO term background.

While the curated GO terms for *N. crassa* greatly helped to identify the processes going on in the fungi, more work needs to be done in other fungi. The work done in Ascomycetes has helped to annotate what might be going on in the economically important fungi *R. stolonifer*. However, further exploration of non-model organisms will help to shed light on the unique responses used by Mucromycete fungi challenged with fungistatic compounds.

Unfortunately, I was never able to test 2-Methylbutanoic acid. This compound had extremely high levels of production in *B. subtilis* and also present in the other *Serratia spp.* It was only found in the last iteration of analysis of the GCMS profile.

Figure 3.2: EC50 Curve of each volatile



Experiment	EC50 in mg
Mycelia Rhizopus 2−Undecanone	NA
Mycelia Rhizopus Anisole	NA
Mycelia Rhizopus Blend	4.13862846
Mycelia Rhizopus DMTS	0.12294225
Spore Rhizopus 2−Undecanone	17.02173226
Spore Rhizopus Anisole	1.85003378
Spore Rhizopus Blend	0.02626635
Spore Rhizopus DMTS	0.01479548
Mycelia Neurospora 2−Undecanone	0.12125993
Mycelia Neurospora Anisole	1.23651149
Mycelia Neurospora DMTS	0.01139336

Figure 3.3: 4 GPCRS mutants growth compared to growth with Serratia at 24 hours

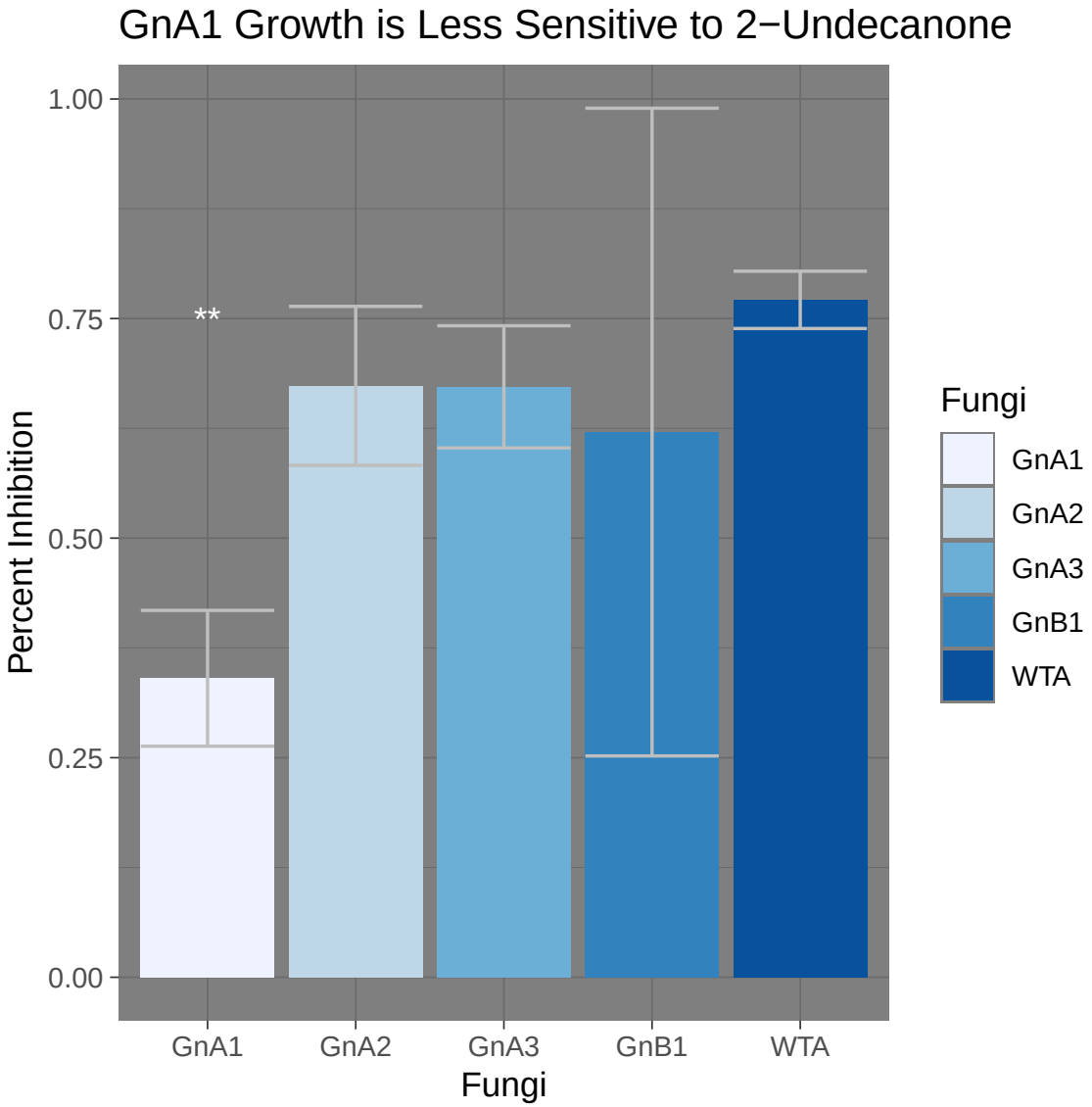


Figure 3.4: Transcripts with less than .01 p value and over 1 LFC expression than the control condition in *N. crassa* treated with DMTS, 2-Undecanone and blend

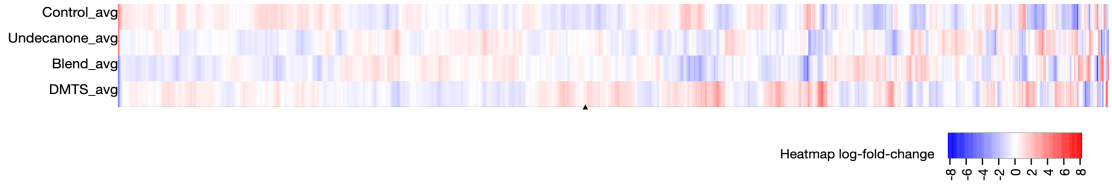


Figure 3.5: Transcripts showing DMTS-elicited response in *N. crassa* has more and higher expressed genes than 2-Undecanone

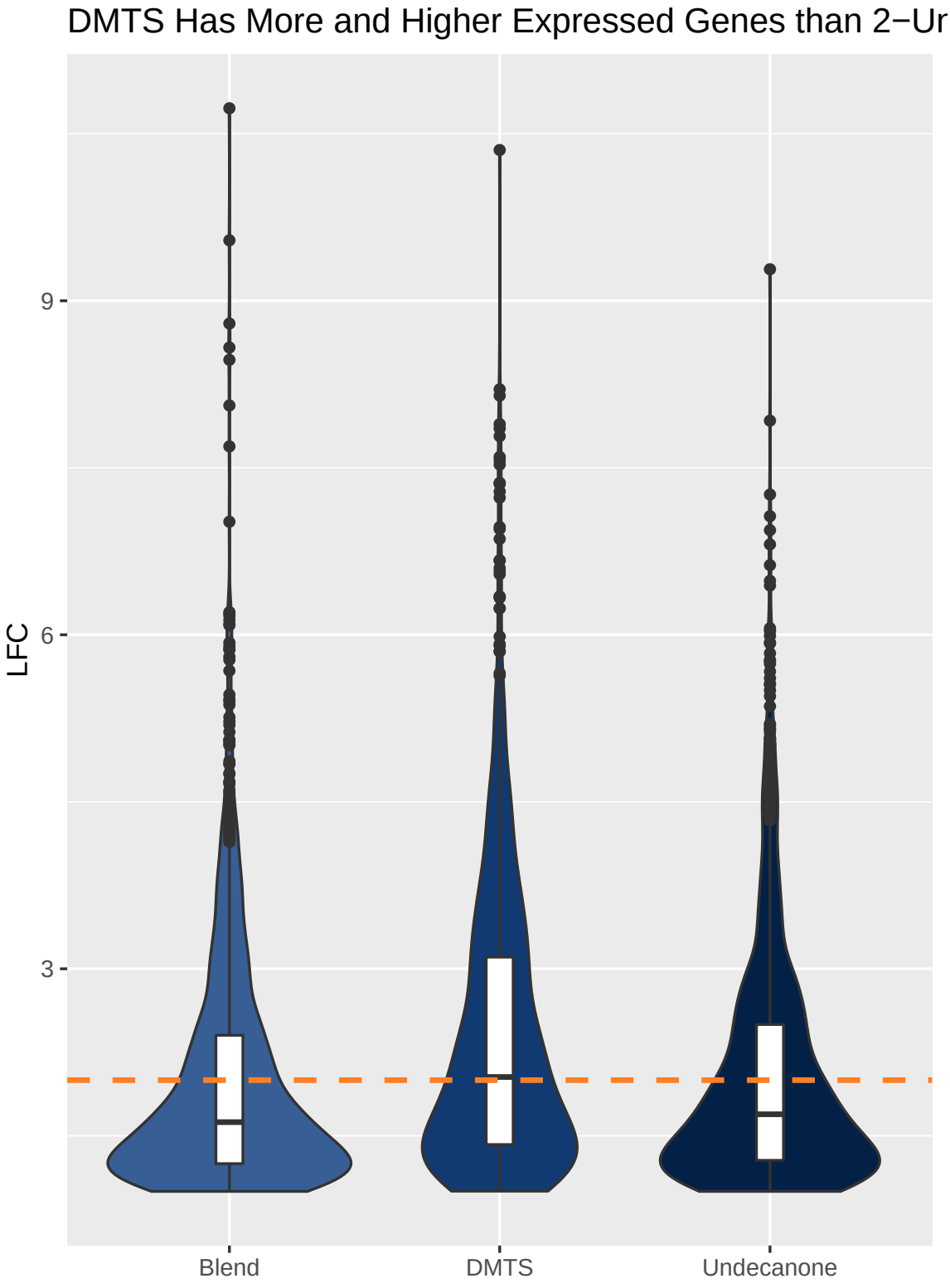


Figure 3.6: Transcripts showing blend and 2-Undecanone response in *N. crassa* has more lower expressed genes than DMTS

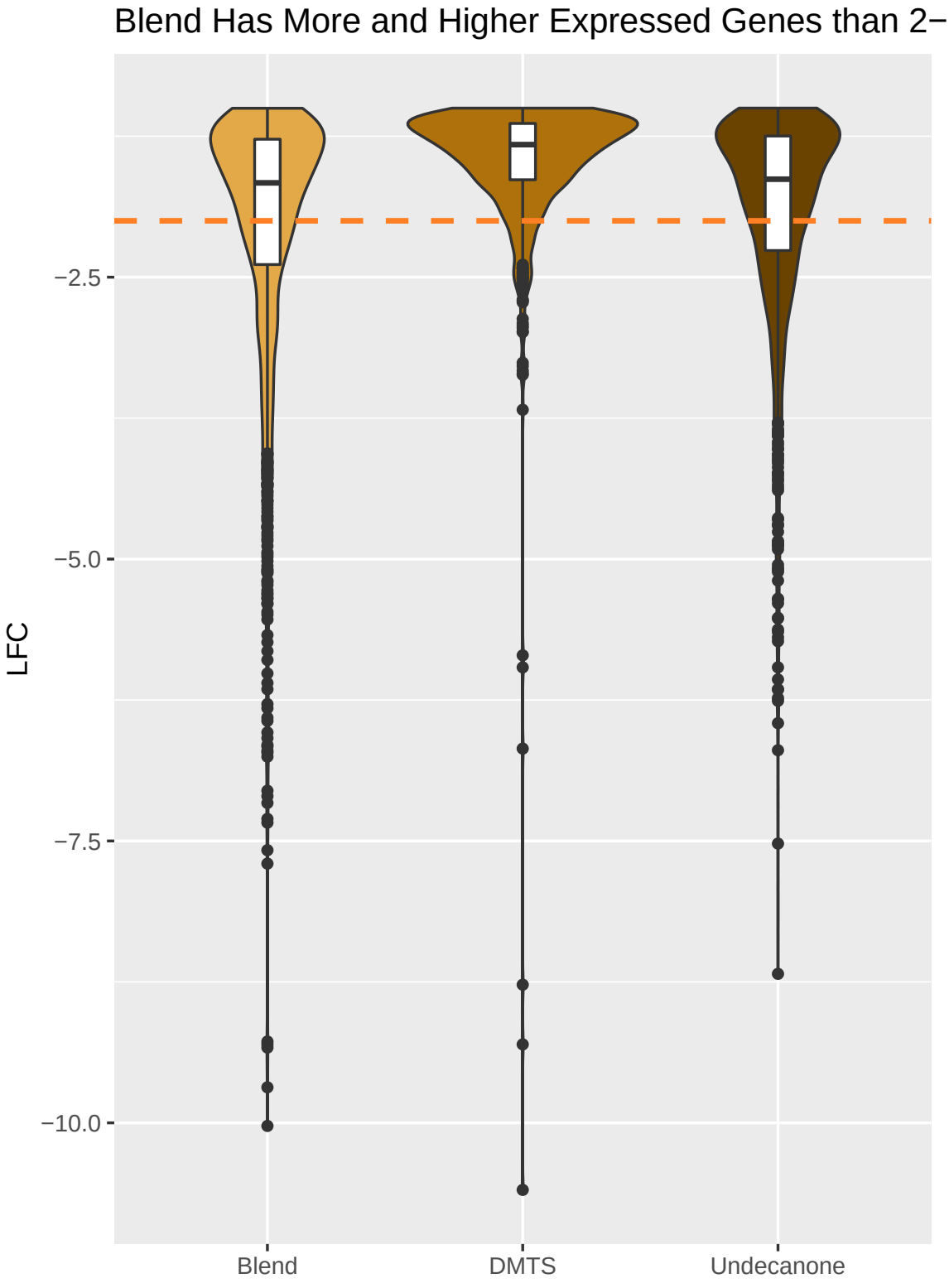


Figure 3.7: Transcripts showing difference in DEGs between *N. crassa* and *R. stolonifer* and the different conditions

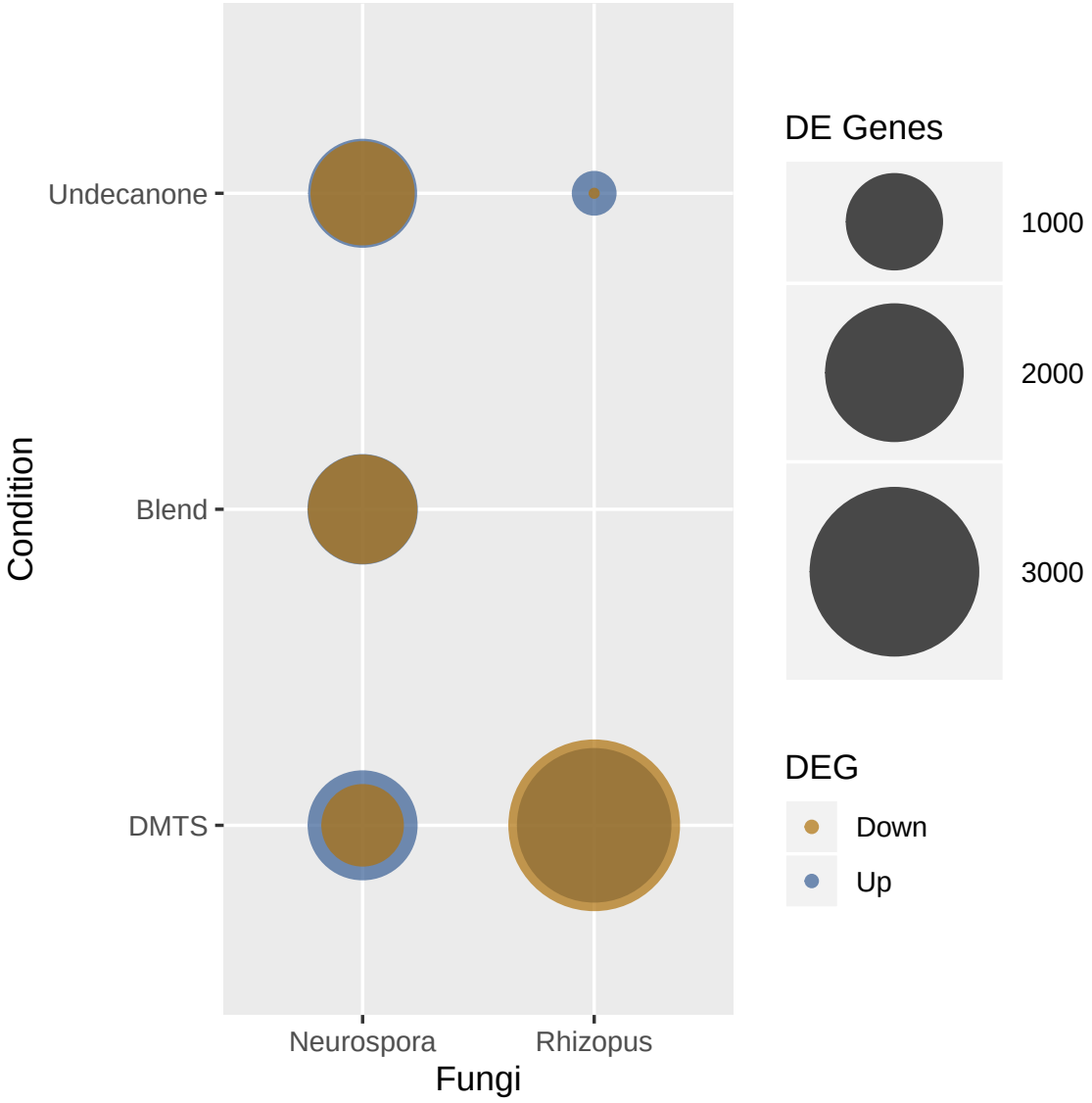


Figure 3.8: Transcripts with less than .01 p value and over 1 LFC expression in DMTS and 2-Undecanone treatments of *R. stolonifer*

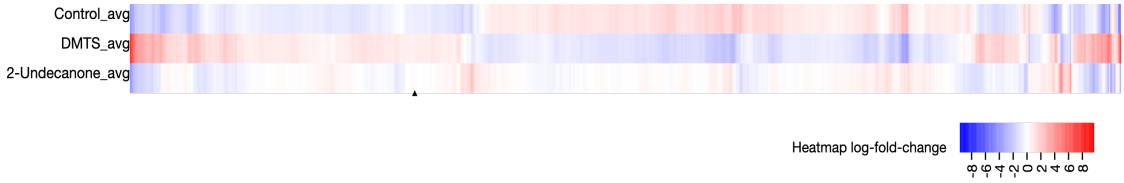


Figure 3.9: Transcripts showing DMTS elicited response has similar expression level of up-regulated genes compared to 2-Undecanone

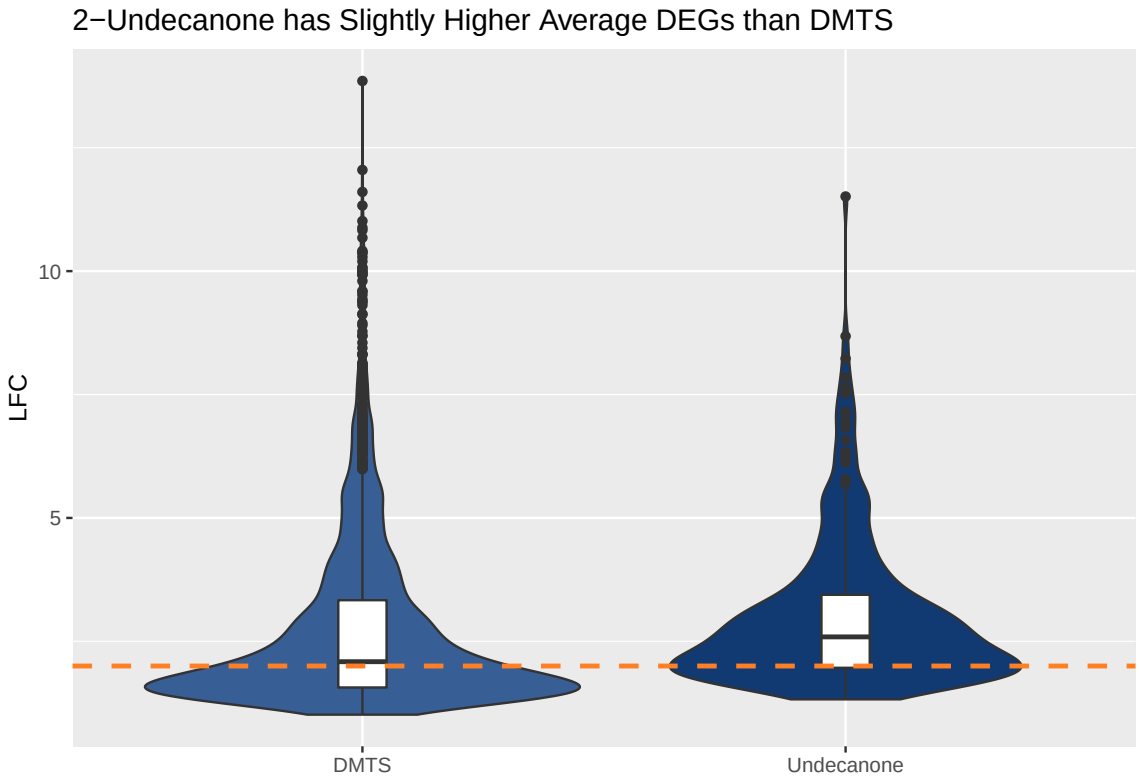


Figure 3.10: Transcripts showing 2-Undecanone response has slightly lower average expression genes less than -1 LFC than DMTS

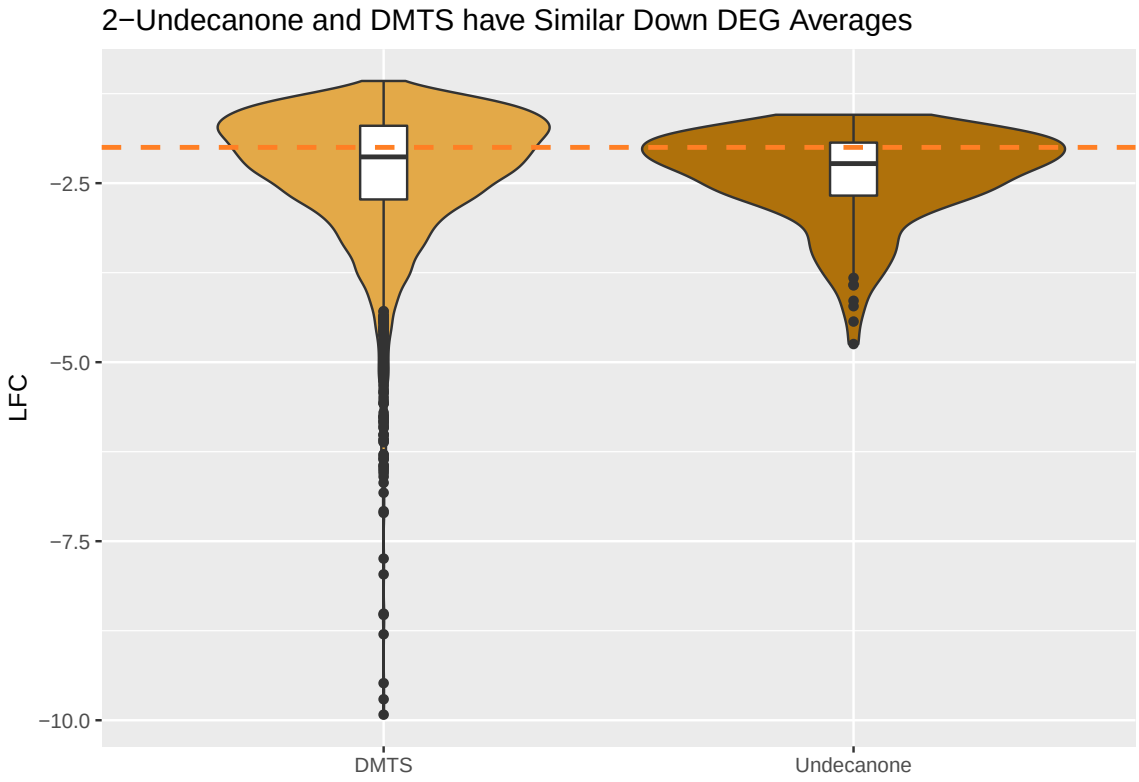


Figure 3.11: *N. crassa* and *R. stolonifer* DEGs that Share Transcripts with Orthologous Proteins

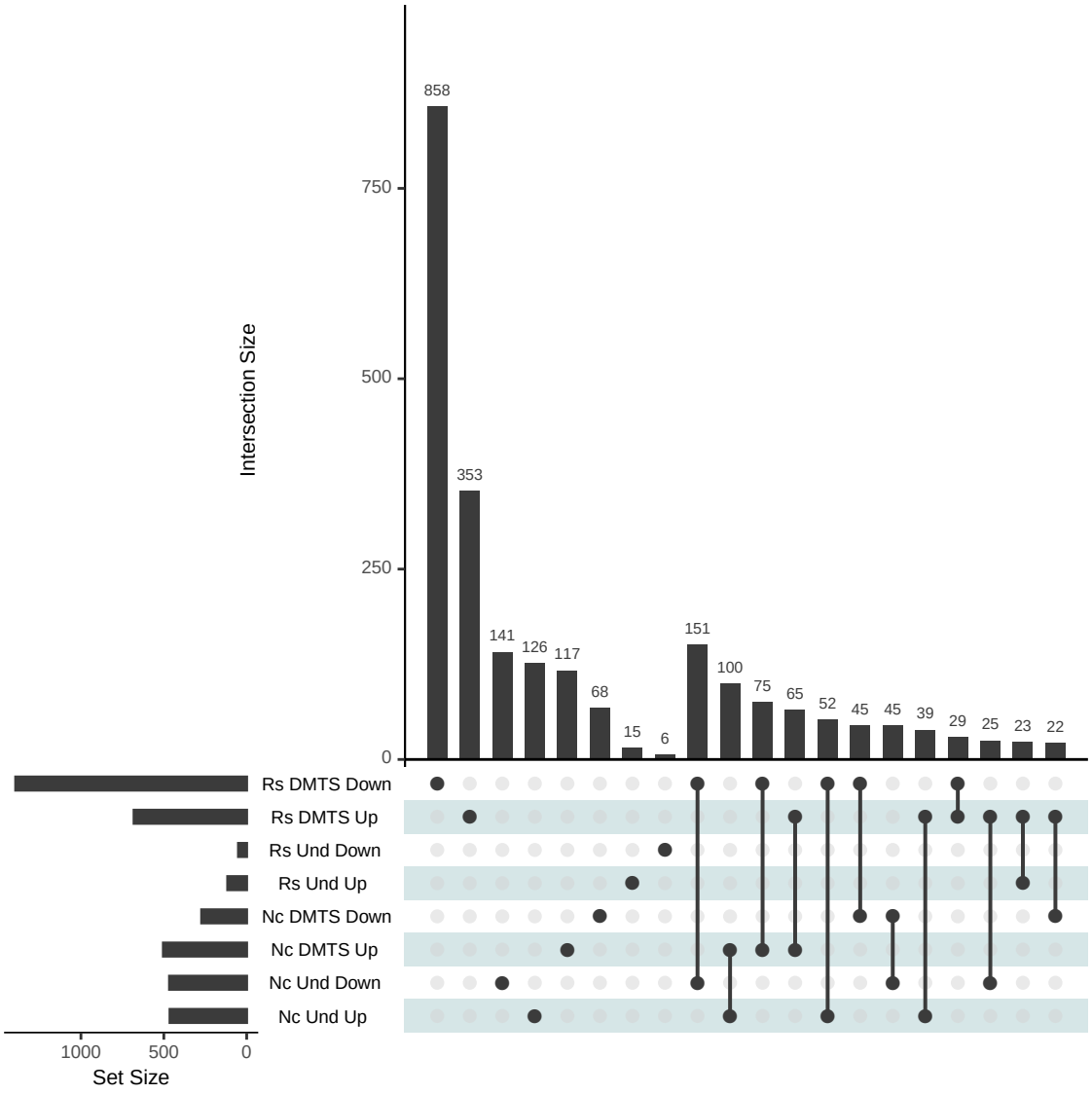


Figure 3.12: Analysis of Genes Ontology assignments for all DEGs with greater than 1 LFC and p-value less than .05

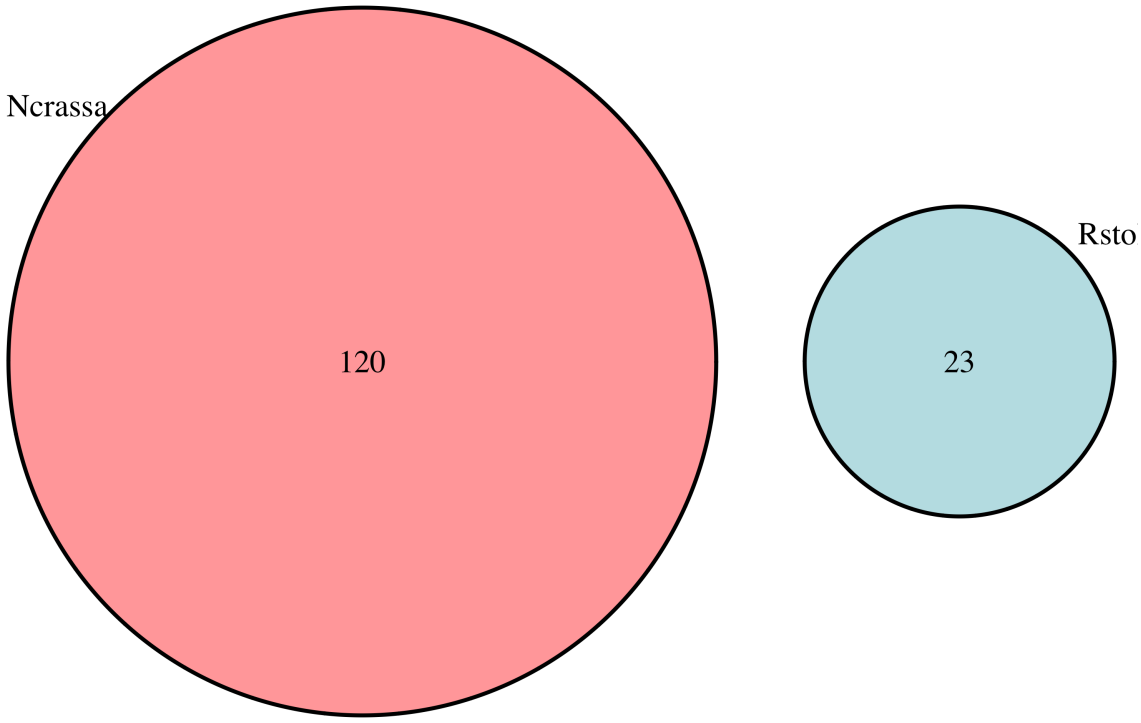


Figure 3.13: Analysis of Genes Ontology assignments for all DEGs with greater than 1 LFC

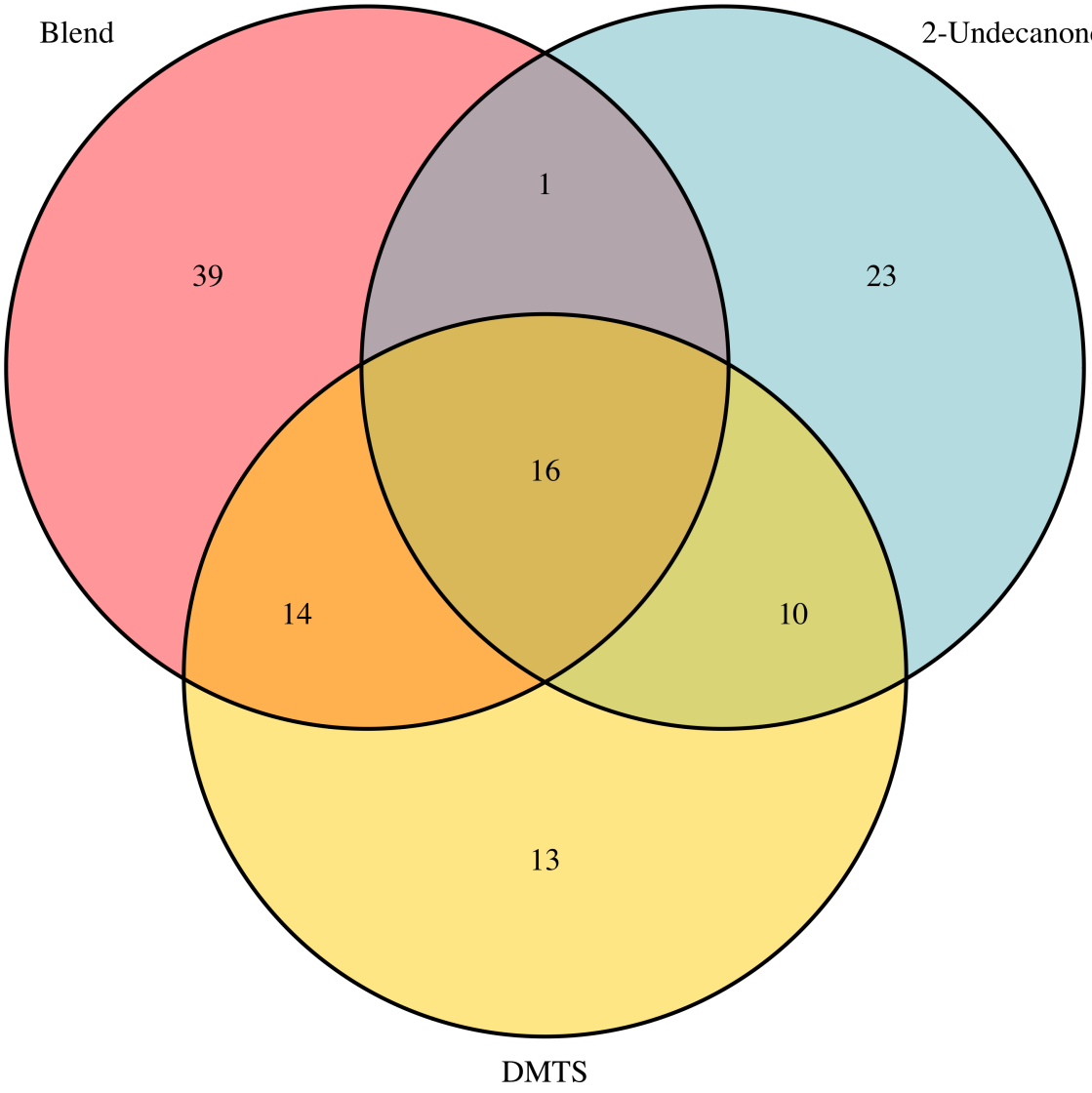
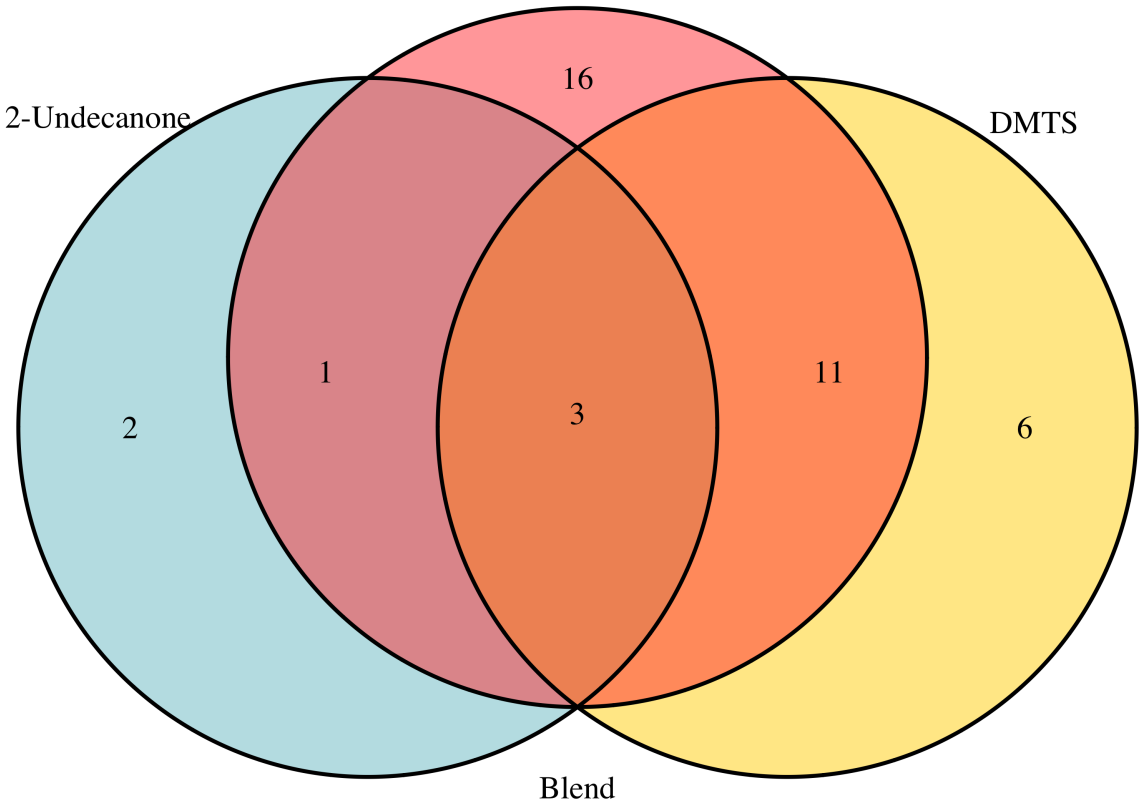
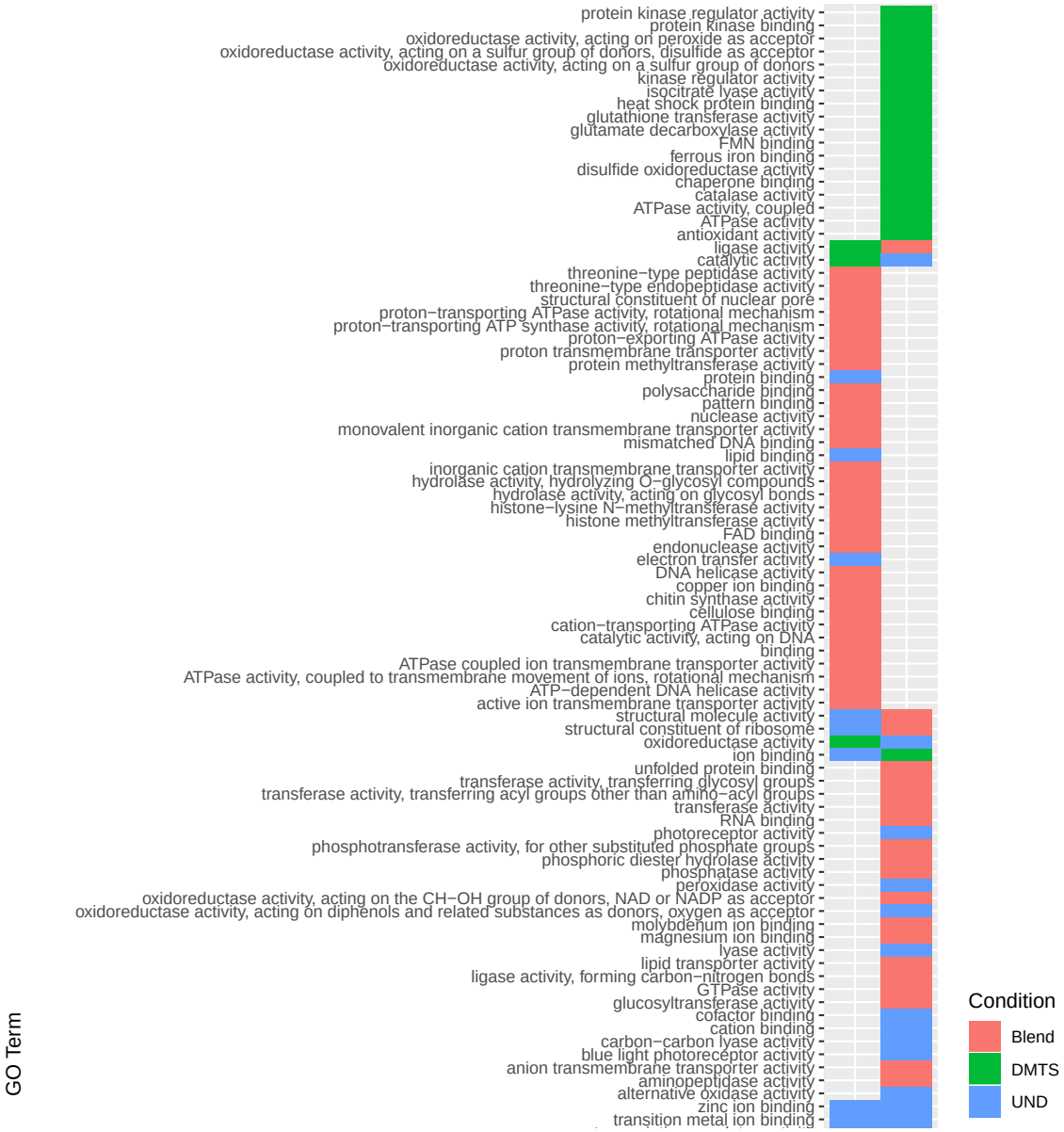


Figure 3.14: Analysis of Genes Ontology assignments for all DEGs with lower than -1 LFC




GO Term
protein kinase regulator activity
protein kinase binding
oxidoreductase activity, acting on peroxide as acceptor
oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor
oxidoreductase activity, acting on a sulfur group of donors
kinase regulator activity
isocitrate lyase activity
heat shock protein binding
glutathione transferase activity
glutamate decarboxylase activity
FMN binding
ferrous iron binding
disulfide oxidoreductase activity
chaperone binding
catalase activity
ATPase activity, coupled
ATPase activity
antioxidant activity
ligase activity
catalytic activity
threonine−type peptidase activity
threonine−type endopeptidase activity
structural constituent of nuclear pore
proton−transporting ATPase activity, rotational mechanism
proton−transporting ATP synthase activity, rotational mechanism
proton−exporting ATPase activity
proton transmembrane transporter activity
protein methyltransferase activity
protein binding
polysaccharide binding
pattern binding
nuclease activity
monovalent inorganic cation transmembrane transporter activity
mismatched DNA binding
lipid binding
inorganic cation transmembrane transporter activity
hydrolase activity, hydrolyzing O−glycosyl compounds
hydrolase activity, acting on glycosyl bonds
histone−lysine N−methyltransferase activity
histone methyltransferase activity
FAD binding
endonuclease activity
electron transfer activity
DNA helicase activity
copper ion binding
chitin synthase activity
cellulose binding
cation−transporting ATPase activity
catalytic activity, acting on DNA
binding
ATPase coupled ion transmembrane transporter activity
ATPase activity, coupled to transmembrane movement of ions, rotational mechanism
ATP−dependent DNA helicase activity
active ion transmembrane transporter activity
structural molecule activity
structural constituent of ribosome
oxidoreductase activity
ion binding
unfolded protein binding
transferase activity, transferring glycosyl groups
transferase activity, transferring acyl groups other than amino−acyl groups
transferase activity
RNA binding
photoreceptor activity
phosphotransferase activity, for other substituted phosphate groups
phosphoric diester hydrolase activity
phosphatase activity
peroxidase activity
oxidoreductase activity, acting on the CH−OH group of donors, NAD or NADP as acceptor
oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor
molybdenum ion binding
magnesium ion binding
lyase activity
lipid transporter activity
ligase activity, forming carbon−nitrogen bonds
GTPase activity
glucosyltransferase activity
cofactor binding
cation binding
carbon−carbon lyase activity
blue light photoreceptor activity
anion transmembrane transporter activity
aminopeptidase activity
alternative oxidase activity
zinc ion binding
transition metal ion binding
Condition
Blend
DMTS
UND

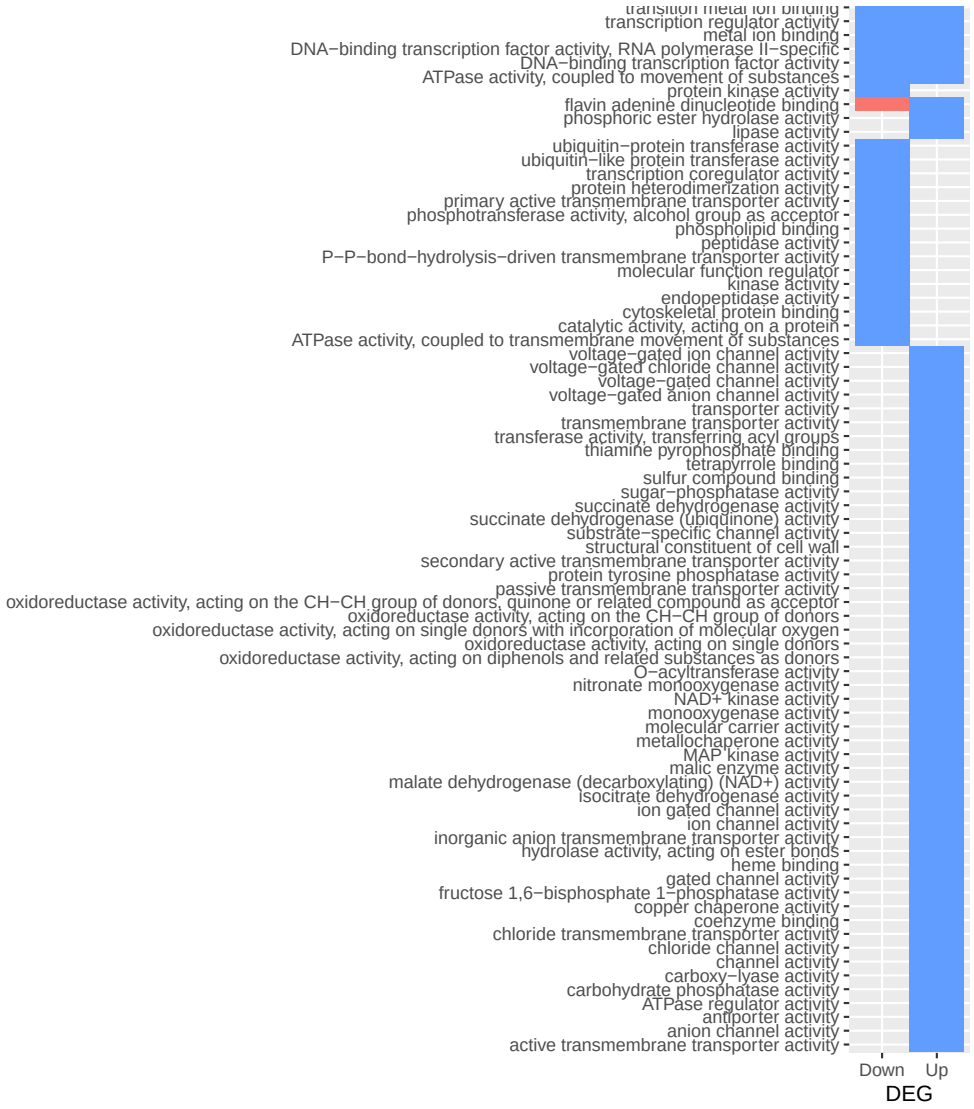


Figure 3.15: Genes Ontology terms with DEG for all conditions

Figure 3.16: Genes Ontology terms of genes with increased expression

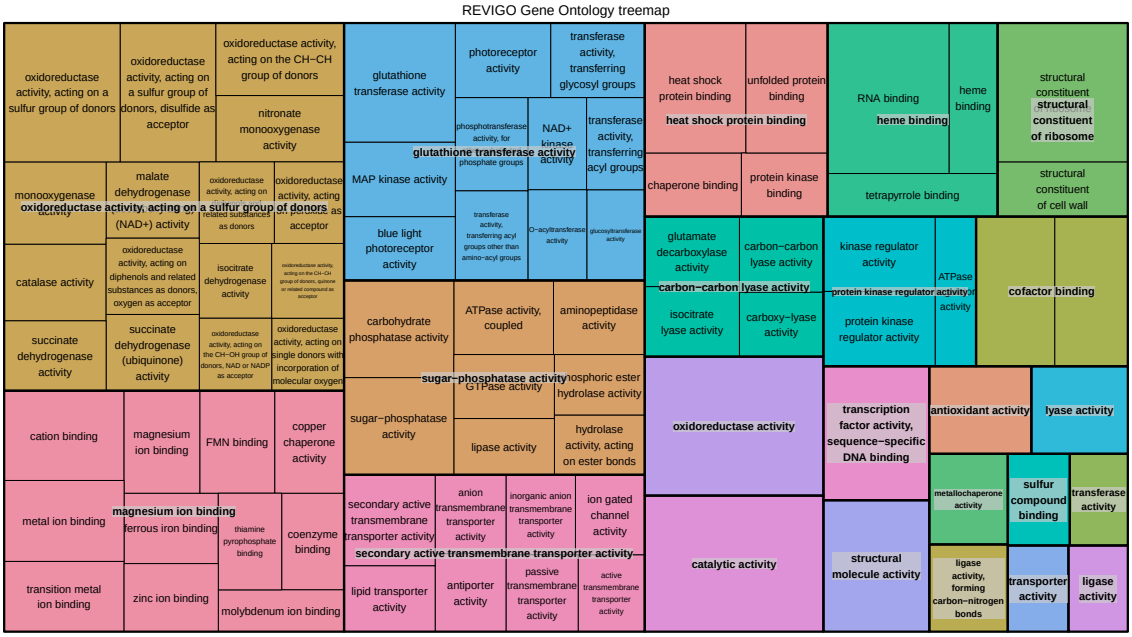


Figure 3.17: Genes Ontology terms of genes with decreased expression

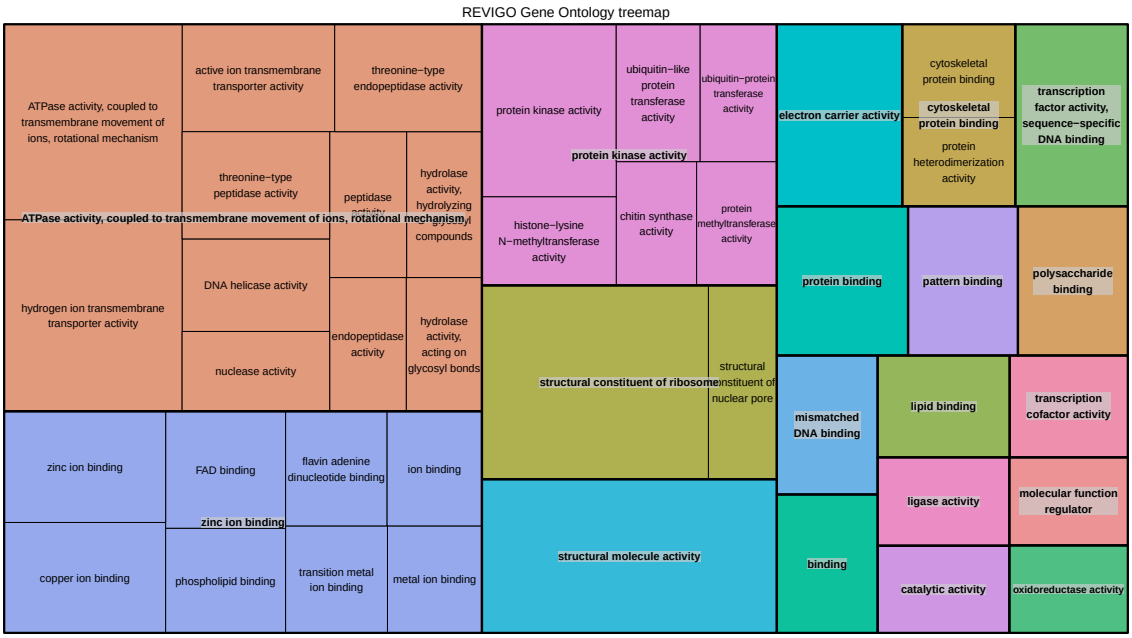
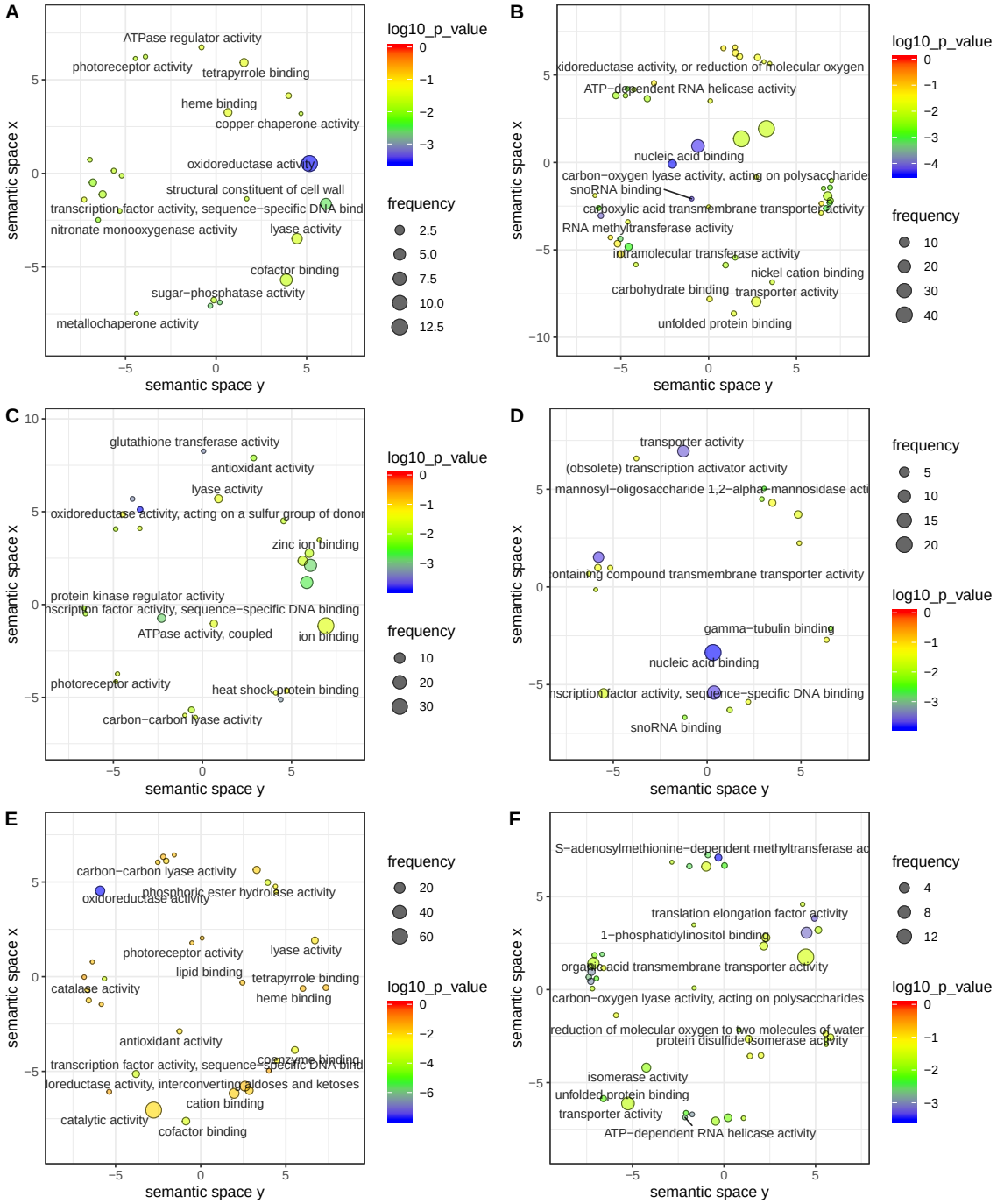


Figure 3.18: Analysis of Genes Ontology assignments for *N. crassa* DEGs A. DEGs Over 1 LFC in 2-Undecanone, B. Under -1 LFC in 2-Undecanone, C. DMTS Over, D. DMTS Under, E. Blend Over, F. Blend Down



Chapter 4

Conclusions

I have shown that the bacteria *Serratia marcescens* and *Serratia proteamaculans* produce volatile profiles that contain fungistatic compounds. These compounds are expressed high enough at biological levels to inhibit fungal growth. I have also characterized the volatile profiles from both *Serratia marcescens* and *Serratia proteamaculans* as well as a control bacteria *Bacillus subtilis*. Furthermore, I have shown that these microbes have their own unique volatile profile which each contains at least one powerful fungistatic compound, 2-undecanone, dimethyl trisulfide, and anisole. I explored the fungistatic potential effects of these volatiles on 2 phylogenetically diverse fungi, Neurospora, a genetic model and well-studied fungi and *Rhizopus stolonifer*, a model zygomycete and post-harvest pathogen. I have shown that the exposure of these pure volatiles up-regulates genes to break down the compounds and exhibit signs of stress and reduced growth and vacuolation.

I have sequenced over 100 zygomycete fungi and in analyzing those sequences I found that many of the genomes contain large bacterial contigs. This supports the current

literature in that these bacteria identified have been known to be associated with a few members of zygomycetes but until now an estimate of the number of zygomycetes with bacteria associated was unknown.

However many questions still remain unanswered.

Not all of the volatiles found in the bacterial profiles have been tested for antifungal potential. Butanoic acid would be an important compound to study for its antifungal potential and it is abundantly produced by *Bacillus subtilis*. After studying the volatiles produced by these organisms under lab condition work needs to be done looking at the production of volatiles in different environments such as heat or high pH. If these potential biocontrols will be used in cooler climates more work needs to be done to understand how volatile profiles change with temperature. Heat may change the rate at which a bacterium metabolizes or change the activation energy for a process and therefore create more metabolic waste products. pH typically has a greater effect on bacteria than their fungal counterparts, and fungi use this to their advantage to prevent bacteria from growing in the vicinity. Is it possible for bacteria to acidity their environment with volatiles similar to how they acidity media in the lab? Looking at volatile expression over time would show when volatiles are being produced and if they have different peak production timepoints. This work has already begun to take place and while still very preliminary there does seem to be a change of volatiles overtime from microbes [Misztal et al., 2018].

Pairing transcriptomic data and metabolic expression may help to tease out which gene pathways are active while volatiles levels increase or decrease, even more specifically, which types of volatiles increase with given genes. This will help to create a connection

between the genome, transcriptome and the metabolites produced by organisms. Currently, there is no way to connect what is in the volatile profile to what is in the genome. A popular thought in the community is that volatiles are the result of metabolic waste products from other biosynthetic pathways. Given this, different environments (root, skin, etc) would produce a different array of volatiles. Do the same bacteria have a different profile on different plant hosts?

Just like one might introduce new genes to make a bio-control a better partner to a host one might also consider bioengineering genes that create volatile products. Is it possible to extend the reach of these bacterial profiles or fill them with more powerful antifungals? Can the volatile profiles be improved? For example for deck known would it have greater fungal growth inhibitor inhibition or potential fungicidal effects if the fatty acid chain were extended? This underscores the need for being able to predict volatiles created by bacteria or fungi.

Do bacteria sense fungi and the fungal volatile profile? Differences in gene expression or volatile expression after exposure to fungal volatiles may lead to the discovery of secondary metabolites used in defense. Of course, they could also be by-products of fungal metabolism. So by choosing potential fungal elicitors like chitin, one could potentially elicit a response from bacteria without using fungal volatiles. This would allow for the collection of the bacterial volatiles without contamination.

Many studies have been devoted to looking at a single volatile produced by a single bacterial species in the lab. Some have studied multiple volatiles of a single bacteria, but there are no studies looking at communities of microbes in natural environments and their

volatile production. This is a crucial next step as a microbial members of a community will interact with each other, potentially leading to a completely different volatile profile than any microbe observed on its own. What volatile are expressed in the presence of an insect, nematode, or other single-celled organisms? How do volatile profiles change with applications of fungicide? Using a bacteria biocontrol may lose its effectiveness if the community is not healthy or normal.

Understanding how microbial communities contribute to a pan volatile profile may also help to recapitulate those associated with beer or cheese making. Potentially this work can be used to repopulate desert soils to ensure moisture and soil trapping and other functions of those crusts continue to be carried out as the climate continues to change. The core concept of volatile antifungal treatments is that the metabolites inhibiting growth are affecting the fungi in a multitude of ways for example attacking the cell walls, respiration, ROS, and transport. A common mechanism for preserving and antimicrobial is to treat infections with multiple antifungals at once, inherently making it difficult for fungi to develop resistance to all these offending compounds simultaneously.

As discoveries and applications for volatile profiles begin to emerge we must look at the downstream effects and regulation. How long do the volatile stay in the environment? What is their half-life? What is the range of lifespan of a volatile profile? Only after understanding what constitutes an effective antifungal volatile profile can we use volatiles to cure a fungal infection. The use of volatiles to treat patients with fungal infections could also help prevent fungal resistance from developing due to the fact that these profiles simultaneously treat with multiple antifungals.

One interesting observation I found was that spores/conidia wait for optimal conditions before germination. Exposing spores to a volatile that had a short half-life only paused their growth until the volatile levels subsided and germination can begin. What are the receptors or protein targets that might be sensing the volatiles in the environment and cueing the spores or conidia postpone germination? Identifying and exploiting these targets could be key to fighting fungal infections in agriculture. By using volatiles that prevent germination, fungi would be unable survive saprophytically or mate and gain resistance through sexual reproduction. Also by preventing growth from the start, there is no asexual reproduction that could give rise to resistant strains. In combination with volatiles, compounds that might "deafen" or prevent the fungi from responding to volatiles, would lead to germination in an unfavorable environment and making it unable to survive.

Chapter 5

Appendix: Zygomycetes and Bacteria Associations In Silico

5.1 Abstract

Filamentous fungi are important model organisms that have advanced genetics, cellular and molecular biology fields. Key experimental systems *Aspergillus* and *Neurospora* have improved understanding of genetic recombination, cellular trafficking, and development. Here I describe how we extracted nucleic acid data from hundreds of different species of early diverging fungi (mostly Mucoromycota and Zoopagomycota), and go on to explore the sequenced data. using the data from this project, I look into the abundant number of bacterial reads from the sequencing.

5.2 Introduction

The zygosporic fungi, comprised of the phyla Mucoromycota and Zoopagomycota, represent a transitional group given that the flagellum is lost with the expansion of the phyla, coinciding with a transition from an aquatic lifestyle, as in the earliest branching flagellated fungi, to a terrestrial lifestyle. Many of these fungi are sources of chemicals relevant to human and environmental health. The anticancer drug camptothecin is isolated from seeds and bark of two tree species, but a recent study described an endophytic Mucoromycete that also produces the drug [Uzma et al., 2018]. Another Mucoromycete with relevance to human health is *Blakeslea trispora* which produces lycopene and β-carotene, compounds with antioxidant properties [Rodrguez-Siz et al., 2004]. Additionally, *Cunninghamella elegans* is able to detoxify fluoranthene, a polycyclic hydrocarbon produced from the burning of fossil fuels [Pothuluri et al., 1990]. Although a wealth of knowledge exists with regard to isolation and culturing [Benny, 2008, Benny et al., 2014] understanding the molecular and genetic diversity of these enigmatic clades will require the development and adaptation of molecular techniques. One way to analyze the vast diversity of these fungi is to look at their genomes. Upon inspection of many of these fungal genomes, we found that there was a substantial amount of bacterial contamination despite our efforts to clean the genomes. We can see that these bacteria have been reported in the literature to be associated with many of the early diverging fungi and can sometimes play an important role in extending the fungal genome. For example, rhizoxcin, an important toxin used by *Rhizopus* for killing rice seedlings is actually produced by its bacterial symbiont, *Burkholderia* not the fungi [Partida-Martinez and Hertweck, 2005].

Mucoromycota fungi have numerous bacterial interactions as a result of their saprophytic lifestyle and the environments from which they come. Many Mucromycota fungi are "first colonizers" like *Rhizopus stolonifer* and grow quickly on sugar substrates. Being among the first of the decomposers these fungi would also have to compete with fast-growing bacteria. These initial observations have also been seen in other environments like between Mucors and bacteria growing on cheese. Interestingly, these relationships seem to be less antagonistic and may even be beneficial [Zhang et al., 2018]. In the cheese microbial community assembly, bacteria and open up niches and provide nutrients to fungi while the bacteria can utilize the hyphae as a superhighway for transportation. In another example transcriptomic analysis of the AMF Gigaspora margarita reveals that bioenergetic capacity increased with increased ATP production, detoxification of reactive oxygen species and detection of strigolactone was improved after reintroduction of the endobacterium Candidatus Glomeribacter gigasporarum [Salvioli et al., 2016]. Understanding these interactions is important for studying pathology as well as creating microbial communities for food production like beer and cheese.

5.3 Methods:

5.3.1 Growth of fungi:

Media:

Malt extract-yeast extract agar (MEYE): Malt Extract Yeast Extract Media: Peptic digest of animal tissue 5g, Yeast extract 3g, Malt extract 3g, Dextrose 10g, Agar 15g, and 1 Liter Water. Sterilize by autoclaving.

Cornmeal agar (CM): yellow cornmeal, 20 gboil 10 min in 700 mL distilled water, filter and add distilled water to make 1 L, dextrose, 10 g; agar, 15 g; adjust pH to 6.0 (Benjamin 1958, 1959). Or Corn Meal Agar (Sigma, USA, Catalog No: 42347-500G)

Emersons yeast-phosphate-soluble starch agar (YpSs): soluble starch, 15 g; yeast extract, 4 g; K2HPO4, 1.0 g; MgSO4N7H2O, 0.5 g; agar, 20 g [15 g used later; Benny and Benjamin 1975]; distilled water, 1 L (Benjamin 1959).

Potato dextrose agar (PDA): potatoes, peeled and cut, 200 gboil extract 10 min in 700 mL distilled water, filter, adjust final volume to 1 L; dextrose, 20 g; agar, 15 g (Schipper 1969pH 6.6; Benjamin 1958, 1959pH not mentioned). Or BD Difco Dehydrated Culture Media: Potato Dextrose Agar (Fisher, USA, Catalog No: DF0013)

5.3.2 Growth Protocols:

For most of the zygomycetes, growing on MEYE media was sufficient for fast and healthy growth. For high yield extractions young tissue or recently sporulating will provide the longest fragments and quantity of DNA.

5.3.3 Culturing

For cultures that were contaminated with other microbes, 1/4 CMA was used to purify. This poor media slows the growth and allows for easy-to-pick single colonies. These isolated colonies can then be transferred to rich-media once. Another technique is to add antibiotics if the fungi have bacterial contamination. I used Streptomycin sulfate and Gentamycin (Gold bio) in tandem. Benomyl is useful to select zygomycetes from ascomycete contamination when isolating cultures from environmental samples. Benomyl

acts to depolymerize microtubules but appears to have little affected on Mucoromycotina fungi so it can be used to limit contamination. However, Benomyl cannot be used for members of the Mortierella family as they are sensitive to the drug. Kanamycin has negative effects on *R. stolonifer* growth among other zygomycetes and its use should be avoided. All samples were grown at 25°C in 12-hour light/dark conditions.

To maximize material useful for DNA or RNA extractions, Mucoromycotina fungi are grown on rich media (MEYE) with antibiotics. This increases the growth rate and allows for the extraction of high biomass/high viability tissue. Older tissue can be more difficult to isolate long fragment DNA and undegraded RNA. Some members of Kickxellomycotina are slow growers, to increase the biomass I collected the spores of mature colonies with a sterile toothpick then suspended them in autoclaved water. This spore suspension was then spread on MEYE at a concentration of 100-500 spore/μl and a volume of 20 μl.

5.3.4 Nucleic Acid Extraction Protocols:

For all DNA extractions, we used the following DNA extraction protocol revised from a high molecular weight DNA extraction for plant tissue with CTAB extraction (Murray and Thompson 1980). Because it can be difficult to get biomass from zygomycetes we scaled down the protocol to take less input, faster spins, and a phenol clean-up step. DNA was extracted from young tissue 1 week old.

Reagents required: BUFFER A (0.35 M sorbitol 0.1 M Tris-HCl, pH 9 5 mM EDTA, pH 8), BUFFER B (0.2 M Tris-HCl, pH 9 50 mM EDTA, pH 8 2 M NaCl 2% CTAB), BUFFER C (5% Sarkosyl (N-lauroylsarcosine sodium salt SIGMA L5125)), Potassium Acetate 5M (KAc precipitate polysaccharides) pH 7.5, RNAse A (10 mg/ml), Proteinase K

(20 mg/ml), 0.1% PVP (Polyvinylpyrrolidone), (PCI) Phenol:Chloroform:Isoamyl alcohol (25:24:1), (CI) Chloroform:Isoamyl alcohol (24:1), Sodium Acetate (NaAc) 3M, 100% Isopropanol, and freshly prepared 70% Ethanol. First, the Lysis Buffer (650 μl Buffer A, 650 μl Buffer B, 260 μl Buffer C, 175 μl .1% PVP, 10 μl Proteinase K) is added to 2 mL microcentrifuge tube, heated and mixed, then split equally into two 2 mL tubes. Lysis Buffer is incubated on a 65°C hot plate while proceeding to tissue processing. Young fungal tissue is then ground in liquid nitrogen, add 50-100 mg of powdered tissue to each tube containing Lysis Buffer. Tubes are then incubated for at least 30 minutes at 65°C with occasional mixing by inversion (once every 2-5 minutes). 288 μl KAc is added to each tube, mixed, then the tubes are incubated on ice for 5 min. Next 500 μl PCI is added to each tube and mixed by inversion (¿5 minutes) or vortex briefly then incubate for 2 minutes. The tubes were then spun in a centrifuge at 10,000 g for 10 min. The supernatant was aliquoted into a fresh tube and an equal volume CI was mixed in by inversion (¿5 minutes) or vortexed briefly then incubated for 2 minutes at room temperature. The tubes were spun at 10,000 g for 10 minutes. The supernatant can be treated with an optional RNase treatment (2.5 μl RNase, 37°C, 90-120 min), followed by another CI wash as described above. DNA was precipitated by the addition of 1/10 volume NaAc, mixed, then 1 volume Isopropanol is added. The samples were incubated at room temperature for 5 minutes, then spun at 3,000 g for 2 minutes. Then each tube was washed with 1 mL freshly prepared, cold 70% ethanol, spun at 3,000 g for 2 minutes. The resulting pellet was dried at 65°C for less than 2 minutes, then resuspended in 100 μl TE at 65°C.

Low coverage genomes were checked for quality by nanodrop and sent to the Joint Genome Institute for Illumina sequencing (10-15x coverage for 40mb genomes, 100bp PE reads). For reference genomes, the DNA was purified by Genomic Tip 100 (Qiagen) according to the manufacturer's protocol with some modifications. The Genomic Tip 100 (Qiagen) was primed with QBT buffer then loaded with at least 12μg of DNA. It was then flushed twice with QC buffer, before being eluted with QF heated to 65°C. Then the samples were split between 4 1.5ml microcentrifuge tubes, spun at room temp for 2 minutes at 3,000 RMP then the DNA was washed with fresh ice-cold 75% ethanol and spun at the same speed for the same duration. These were coupled with RNA and submitted for PacBio sequencing at JGI as a part of the ZyGoLife Community Sequencing Project.

RNA extractions were conducted using TRIzol manufacturer protocols. Briefly, 100 mg of liquid nitrogen ground tissue was added to 2 mL centrifuge tubes containing 1 mL of TRIzol, then the samples were incubated at room temperature for five minutes. Then 0.2 mL of chloroform was added to each tube. After a 2 minute incubation, the samples were centrifuged at 4 degrees C for 15 minutes at 12,000 x g. The supernatant was pipetted into a new 2 mL tube and 500 mL of Isopropanol was added and mixed into solution by inversion. The samples were incubated at room temperature for 10 minutes, then spun at 12,000 x g for 10 minutes at 4 degrees C. The resulting pellet was washed with cold 75% ethanol, dried on a hot plate at 65 degrees C for 5 minutes, then resuspended in DEPC-treated water.

5.3.5 Genome Sequencing

Samples arrayed on plates with at least 1 μg of DNA and 260/280 less than 2, and 260/230 greater than 1.3 as analyzed by Nanodrop (Nanodrop, USA) were submitted to

JGI for sequencing. Other teams which contributed samples for sequencing include the lab of Greg Bonito (Michigan State University, USA) with all of the Mortierella strains, Joey Spatafora's lab (Oregon State University, USA) which contributed many reference genome samples, Tim James's lab (University of Michigan, USA) which contributed Zoopagomycota samples and single genome sequencing [James et al., 2013], and Matt Smith (University of Florida, Gainsville USA) with many Coemansia strains and others. In all over 600 samples were submitted and sequenced by the Joint Genome Institute.

5.3.6 Draft Genome Assembly

The program AAFTF (Stajich, Palmer, unpublished) was used to assemble short-read Illumina sequences into a genome assembly of contigs. The contigs were evaluated by Kaiju to assess the contigs organism of origin. `https://github.com/stajichlab/AAFTF` The single-copy orthologous proteins from the genomes were aligned to build the tree as previously described [Spatafora et al., 2016]

5.3.7 Kaiju Analysis

Kaiju analyzed reads and contigs from the ZyGoLife community sequencing project. To begin all contigs and reads were translated and searched against the NCBI nonredundant prokaryote database. The reads that passed the minimum score (65) and e-value (.05) were then taxified to genus based on the closest match to the sequence. These reads were then filtered to remove unclassified reads and sorted by bacteria and counted for each fungus. The nonredundant prokaryote NCBI database was used to match reads to known genomes.

For the contig analysis reads were required to meet a kaiju score of 200.

The scripts for Kaiju (pipeline/01_kaiju_makedb.sh, 02_kaiju.sh and 03_kaiju_tax.sh) and sorting and counting (scripts/sorting.sh) can be found at

`https://github.com/stajichlab/DCH_Zygo_Scripts/tree/master/pipepline`

5.3.8 R Analysis

R analysis of the kaiju results showed all the fungal genomes with their associated bacteria reads. These were then plotted to find a cutoff value for noise. A cut off of 10,000 bacterial read hits/genome was used to filter out over 85% of the hits.

The R script can be found at `https://github.com/stajichlab/DCH_Zygo_Scripts/tree/master/pipepline`

5.3.9 iTOL

`https://itol.embl.de/` iTOL [Letunic and Bork, 2019] was used to visualize the heatmap of Kaiju scores combined with the zygomycete phylogeny. As well as the Burkholderia tree.

5.4 Results:

Young and viable fungal tissue yielded the best DNA. (Note biomass does not necessarily equate to DNA.) Cultures that were one to two weeks in age at the time of extraction were considered young. By spreading spores on rich media (MEYE, PDA) in an even lawn we were able to increase biomass and decrease the age of mycelium at the time of extraction. Sporulating fungi were usually a good source of DNA although melanin

can inhibit some downstream procedures. Overall, 642 fungal genomes were sequenced and assessed for bacteria reads.

In all about 2% of the reads, 120,311,504, were classified as bacteria out the approximately 5 billion reads (8 million reads/genome). The number of reads per genomes ranged from hundreds to hundreds of thousands. 5.1 Acinetobacter and Staphylococcus had matching sequences to many reads in many fungal genomes. Additionally, the following bacteria had at least half a million reads detected in a genome: Achromobacter, Actinomadura, Alloscardovia, Bacillus, Bacteroides, Bordetella, Burkholderia, Candidatus Glomeribacter, Cellulomonas, Clostridioides, Cupriavidus, Enterococcus, Flavobacterium, Klebsiella, Komagataeibacter, Lactobacillus, Mycetohabitans, Paenibacillus, Paraburkholderia, Paraclostridium, Pseudomonas, Serratia, Staphylococcus, Streptococcus, Streptomyces, Xanthomonas. Many of these bacteria are Proteobacteria, a major phylum of Gram-negative bacteria. These short reads were sufficient for detecting the bacteria but long reads may help to identify bacteria with a high genomic DNA abundance.

After the reads were assembled into contigs, Kaiju analysis showed that some bacteria were detected more often than others, such as *Parakburkholderia* and *Burkholderia*. Many phylogenetic clades of Mucoromycetes had these two types of bacteria. The best contig score for each fungi bacteria combination is shown in the following figure. 5.2 The contigs in relationship to the total amount of reads sequenced were examined using blobplot (data not shown). Kaiju scores are calculated based on the number of nucleotide matches

to the query sequence. So more matches yield a higher score. In general, many of the Kaiju scores for the bacteria were between 2000-4000.

Many of the *Mortierella* and *R. microsporus* clades showed the prescence of *Burkholderia* and *Paraburkholderia*. This was visualized by combining the Kaiju scores with the zygomycete phylogeny using iTOL. 5.3, 5.4, 5.5, 5.6, 5.7, 5.8 Additional bacteria genera identified in this analysis included Photorhabdus, Pseudomonas, Stenotrophomonas. However, Stenotrophomonas, is a common contaminate in sequencing in general, and may not be associated with the fungi naturally.

Finally, preliminary data shows that many of these Burkholderia strains are very similar based on nucleotide identity. 14 Burkholderia from Mortierella species, 16 Burkholderia from Rhizopus strains, and 2 from *Apophysomyces*.

These preliminary results show that bacteria are associated with many Mucoromycota lineages and can be detected through whole-genome sequencing of the fungi. Assembly of the genomes of these bacteria is also possible in some cases allowing for the potential future studies on how these species are adapting to life with their fungal host. It establishes a need to address the functional role of these Bacteria. Do their genomes show patterns of reduction as is seen in obligate symbionts?

On the host side, it would be useful to understand fungal transcriptional responses to bacterial infection. Are there genes expressed which relate to putative components of fungal innate immunity, like presence or absence of Leucine-Rich Repeats (LRR) which

are used to detect MAMPS. However previous work shows LRRs have a severely diminished presence in fungal genomes [Soanes and Talbot, 2010]. Fungal response to MAMPS [Ipcho et al., 2016] demonstrates that there is a transcriptional response to bacteria that involves down-regulation of growth and upregulation of xenobiotic pumps and detoxification. Other research on bacteria-fungal interactions showed a link to a phenotype of hyphae branching and thickening, defense responses like environment manipulation, antioxidant production, and offense responses like toxin production [Gkarmiri et al., 2015]. Chitin synthase inhibitors are seen upregulated after the Mucoromycota interacts with macrophages [Sephton-Clark et al., 2019]; Chitinase introduction caused plasma membrane instability which led to an induction of ROS in *Neurospora crassa* leads to up-regulation of oxidoreductases and plasma member repair [Lopez-Moya et al., 2016]. Other work has shown that *Serratia* can detect and respond with volatiles to the presence of *Aspergillus* [Schmidt et al., 2017]. In a clever assay to detect potential fungal defense mechanism, Mathioni et al., introduced an antagonistic bacteria Lysobacter enzymogenes and a mutant strain that could not secrete, to *Magnaporthe oryzae* [Mathioni et al., 2013]. They found that *M. oryzae* had many repressed genes when exposed to the virulent strain but they were expressed when exposed to the non-virulent strain as it was unable to produce effectors. The genes in the fungal defense response that were repressed had activities such as transport, oxidoreductase.

Despite the fact that these two Kingdoms have been interacting and fighting for millions of years we know very little about the defense or offense mechanism or other more nuanced interactions. Microbes must fight for resources and space, and chemical warfare success in important. These compounds and secretions are sources for novel antibiotic

discovery, as their exudates have the potential to be used in medicine and agriculture. While microbes compete for carbon sources or highly limited resources like iron (Fe2+) they also compete for physical space. Which makes rhizobacteria an important partner to plants as they commonly colonize the roots of plants creating a physical barrier from pathogens. Additionally, bacteria like *Serratia* are able to phagocytize various fungi, including many zygomycetes [Hover et al., 2016].

To further understand the role of the bacteria it will be important to generate metagenome-assembled genomes of the bacteria. These metagenomes can help inform what functions if any the bacteria are performing while associated with the fungi when compared to other strains and species not found associated with fungi. Burkholderia may have a long relationship with zygomycetes. Obigate parasites like microsporidia shed much of there genome and instead utilize host cytoplasm to live [Cuomo et al., 2012, James et al., 2013]. Phylogeny and comparative genomics of the Bacteria associated with fungi to see if they have any co-evolutionary patterns. Unfortunately, the assembled contigs did not provide all the mark genes to build the tree, but with metagenomes more branch support could help validate that these strains have a long-term relationship with their host fungi.

The role of these bacteria in the fungal genomes could be evaluated by investigating the the LRR and fungal immunity genes within Mucoromycotina. If these domains/protein counts differ greatly from closely related uninhabited fungi there may be some type of genomic change to facilitate the colonization by bacteria. Another underlying question might be "Why does Mucoromycotina has so many fungi with bacterial associates?" Future work could be done into the role of coenocytic cells to understand if this characteristic

might be important for colonization. Other factors like similar environments or differences in innate immunity might also be important factors. Understanding how these fungi have evolved, and how they interact with bacteria may help to shed light on their deadly ability to resist antifungals in hospital settings. Additionally, by learning how they benefit each other, advances might lead to biocontrol methods that select for these beneficial fungi and their partners, and what impacts fungal treatments might have on the plant hosts.

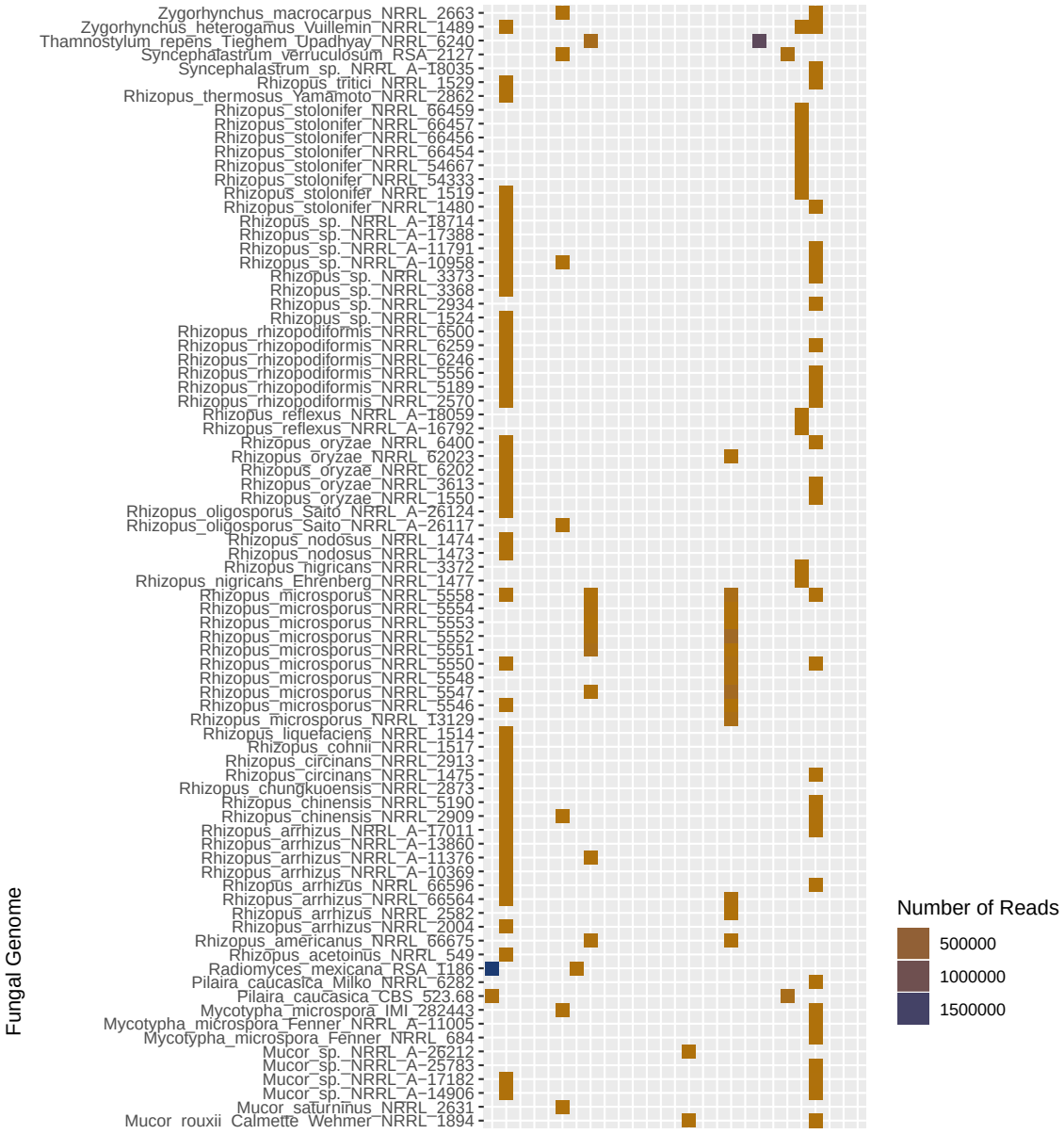
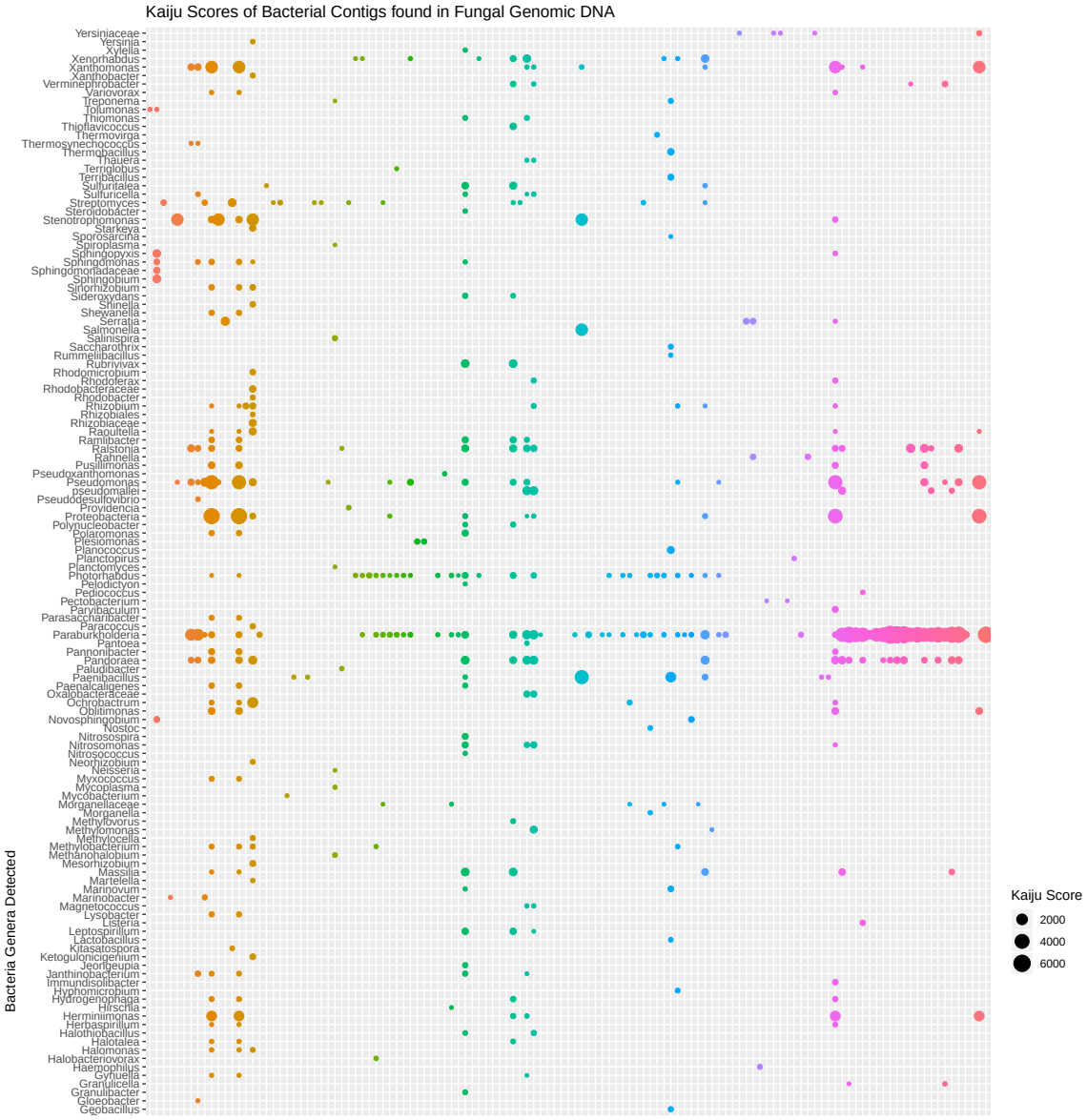

Fungal Genome
Zygorhynchus_macrocarpus_NRRL_2663
Zygorhynchus_heterogamus_Vuillemin_NRRL_1489
Thamnostylum_repens_Tieghem_Upadhyay_NRRL_6240
Syncephalastrum_verruculosum_RSA_2127
Syncephalastrum_sp._NRRL_A−18035
Rhizopus_tritici_NRRL_1529
Rhizopus_thermosus_Yamamoto_NRRL_2862
Rhizopus_stolonifer_NRRL_66459
Rhizopus_stolonifer_NRRL_66457
Rhizopus_stolonifer_NRRL_66456
Rhizopus_stolonifer_NRRL_66454
Rhizopus_stolonifer_NRRL_54667
Rhizopus_stolonifer_NRRL_54333
Rhizopus_stolonifer_NRRL_1519
Rhizopus_stolonifer_NRRL_1480
Rhizopus_sp._NRRL_A−18714
Rhizopus_sp._NRRL_A−17388
Rhizopus_sp._NRRL_A−11791
Rhizopus_sp._NRRL_A−10958
Rhizopus_sp._NRRL_3373
Rhizopus_sp._NRRL_3368
Rhizopus_sp._NRRL_2934
Rhizopus_sp._NRRL_1524
Rhizopus_rhizopodiformis_NRRL_6500
Rhizopus_rhizopodiformis_NRRL_6259
Rhizopus_rhizopodiformis_NRRL_6246
Rhizopus_rhizopodiformis_NRRL_5556
Rhizopus_rhizopodiformis_NRRL_5189
Rhizopus_rhizopodiformis_NRRL_2570
Rhizopus_reflexus_NRRL_A−18059
Rhizopus_reflexus_NRRL_A−16792
Rhizopus_oryzae_NRRL_6400
Rhizopus_oryzae_NRRL_62023
Rhizopus_oryzae_NRRL_6202
Rhizopus_oryzae_NRRL_3613
Rhizopus_oryzae_NRRL_1550
Rhizopus_oligosporus_Saito_NRRL_A−26124
Rhizopus_oligosporus_Saito_NRRL_A−26117
Rhizopus_nodosus_NRRL_1474
Rhizopus_nodosus_NRRL_1473
Rhizopus_nigricans_NRRL_3372
Rhizopus_nigricans_Ehrenberg_NRRL_1477
Rhizopus_microsporus_NRRL_5558
Rhizopus_microsporus_NRRL_5554
Rhizopus_microsporus_NRRL_5553
Rhizopus_microsporus_NRRL_5552
Rhizopus_microsporus_NRRL_5551
Rhizopus_microsporus_NRRL_5550
Rhizopus_microsporus_NRRL_5548
Rhizopus_microsporus_NRRL_5547
Rhizopus_microsporus_NRRL_5546
Rhizopus_microsporus_NRRL_13129
Rhizopus_liquefaciens_NRRL_1514
Rhizopus_cohnii_NRRL_1517
Rhizopus_circinans_NRRL_2913
Rhizopus_circinans_NRRL_1475
Rhizopus_chungkuoensis_NRRL_2873
Rhizopus_chinensis_NRRL_5190
Rhizopus_chinensis_NRRL_2909
Rhizopus_arrhizus_NRRL_A−17011
Rhizopus_arrhizus_NRRL_A−13860
Rhizopus_arrhizus_NRRL_A−11376
Rhizopus_arrhizus_NRRL_A−10369
Rhizopus_arrhizus_NRRL_66596
Rhizopus_arrhizus_NRRL_66564
Rhizopus_arrhizus_NRRL_2582
Rhizopus_arrhizus_NRRL_2004
Rhizopus_americanus_NRRL_66675
Rhizopus_acetoinus_NRRL_549
Radiomyces_mexicana_RSA_1186
Pilaira_caucasica_Milko_NRRL_6282
Pilaira_caucasica_CBS_523.68
Mycotypha_microspora_IMI_282443
Mycotypha_microspora_Fenner_NRRL_A−11005
Mycotypha_microspora_Fenner_NRRL_684
Mucor_sp._NRRL_A−26212
Mucor_sp._NRRL_A−25783
Mucor_sp._NRRL_A−17182
Mucor_sp._NRRL_A−14906
Mucor_saturninus_NRRL_2631
Mucor_rouxii_Calmette_Wehmer_NRRL_1894
Number of Reads
500000
1000000
1500000

Figure 5.1: Top bacteria genera detected in fungal genome reads


Kaiju Scores of Bacterial Contigs found in Fungal Genomic DNA
Bacteria Genera Detected
Yersiniaceae
Yersinia
Xylella
Xenorhabdus
Xanthomonas
Xanthobacter
Verminephrobacter
Variovorax
Treponema
Tolumonas
Thiomonas
Thioflavicoccus
Thermovirga
Thermosynechococcus
Thermobacillus
Thauera
Terriglobus
Terribacillus
Sulfuritalea
Sulfuricella
Streptomyces
Sterolibacter
Stenotrophomonas
Starkeya
Sporosarcina
Spiroplasma
Sphingopyxis
Sphingomonas
Sphingomonadaceae
Sphingobium
Sinorhizobium
Sideroxydans
Shinella
Shewanella
Serratia
Salmonella
Salinispira
Saccharothrix
Rummeliibacillus
Rubrivivax
Rhodomicrobium
Rhodoferax
Rhodobacteraceae
Rhodobacter
Rhizobium
Rhizobiales
Rhizobiaceae
Raoultella
Ramlibacter
Ralstonia
Rahnella
Pusillimonas
Pseudoxanthomonas
Pseudomonas
pseudomallei
Pseudodesulfovibrio
Providencia
Proteobacteria
Polynucleobacter
Polaromonas
Plesiomonas
Planococcus
Planctopirus
Planctomyces
Photorhabdus
Pelodictyon
Pediococcus
Pectobacterium
Parvibaculum
Parasaccharibacter
Paracoccus
Paraburkholderia
Pantoea
Pannonibacter
Pandoraea
Paludibacter
Paenibacillus
Paenalcaligenes
Oxalobacteraceae
Ochrobactrum
Oblitimonas
Novosphingobium
Nostoc
Nitrosospira
Nitrosomonas
Nitrosococcus
Neorhizobium
Neisseria
Myxococcus
Mycoplasma
Mycobacterium
Morganellaceae
Morganella
Methylovorus
Methylomonas
Methylocella
Methylobacterium
Methanohalobium
Mesorhizobium
Massilia
Martelella
Marinovum
Marinobacter
Magnetococcus
Lysobacter
Listeria
Leptospirillum
Lactobacillus
Kitasatospora
Ketogulonicigenium
Jeongeupia
Janthinobacterium
Immundisolibacter
Hyphomicrobium
Hydrogenophaga
Hirschia
Herminiimonas
Herbaspirillum
Halothiobacillus
Halotalea
Halomonas
Halobacteriovorax
Haemophilus
Gynuella
Granulicella
Granulibacter
Gloeobacter
Geobacillus
Kaiju Score
2000
4000
6000

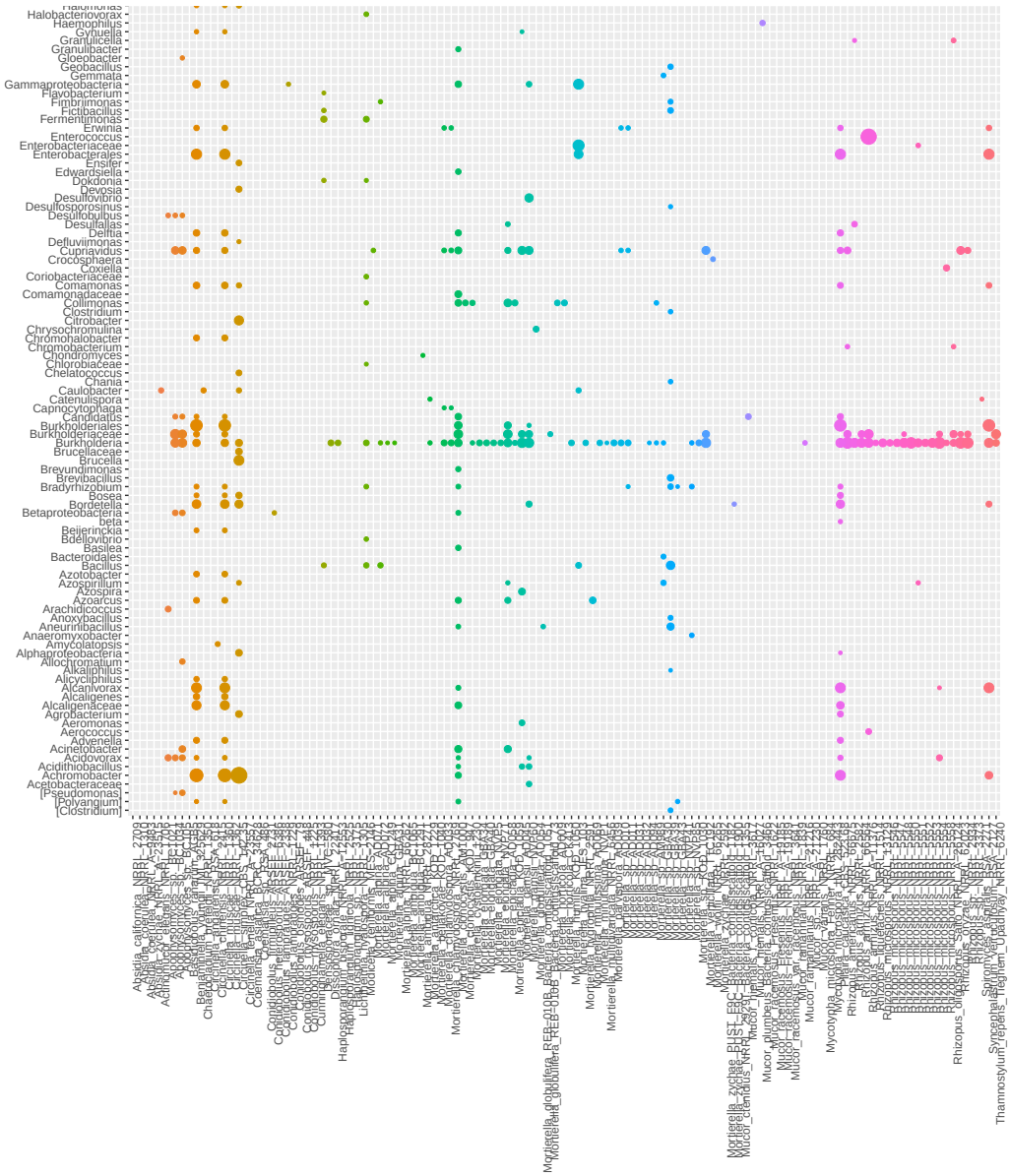


Figure 5.2: Top bacteria genera detected in assembled contigs

Figure 5.3: Nearly 20% of Zygos have bacterial reads, section 1 of tree

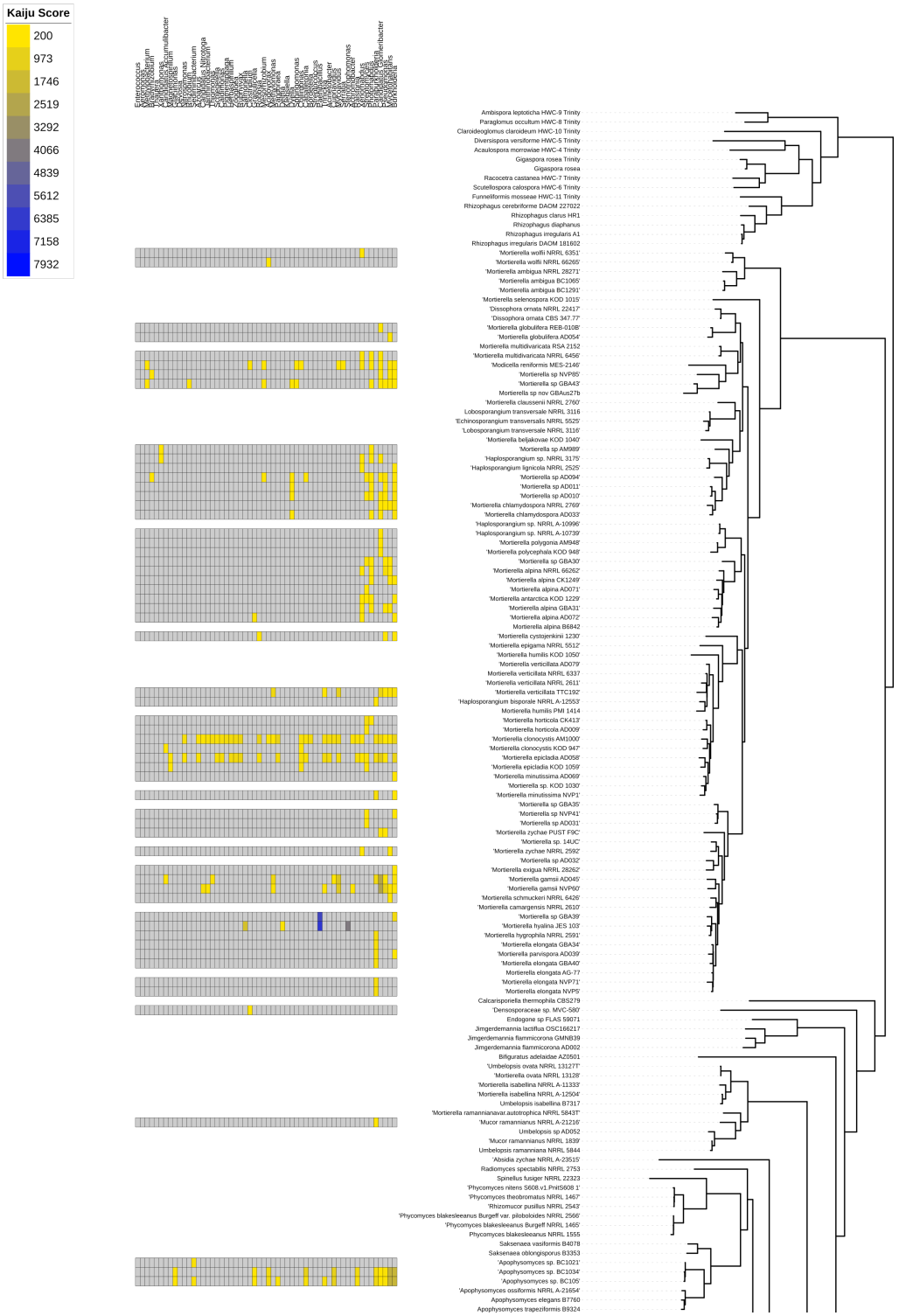


Figure 5.4: Nearly 20% of Zygos have bacterial reads, section 2 of tree

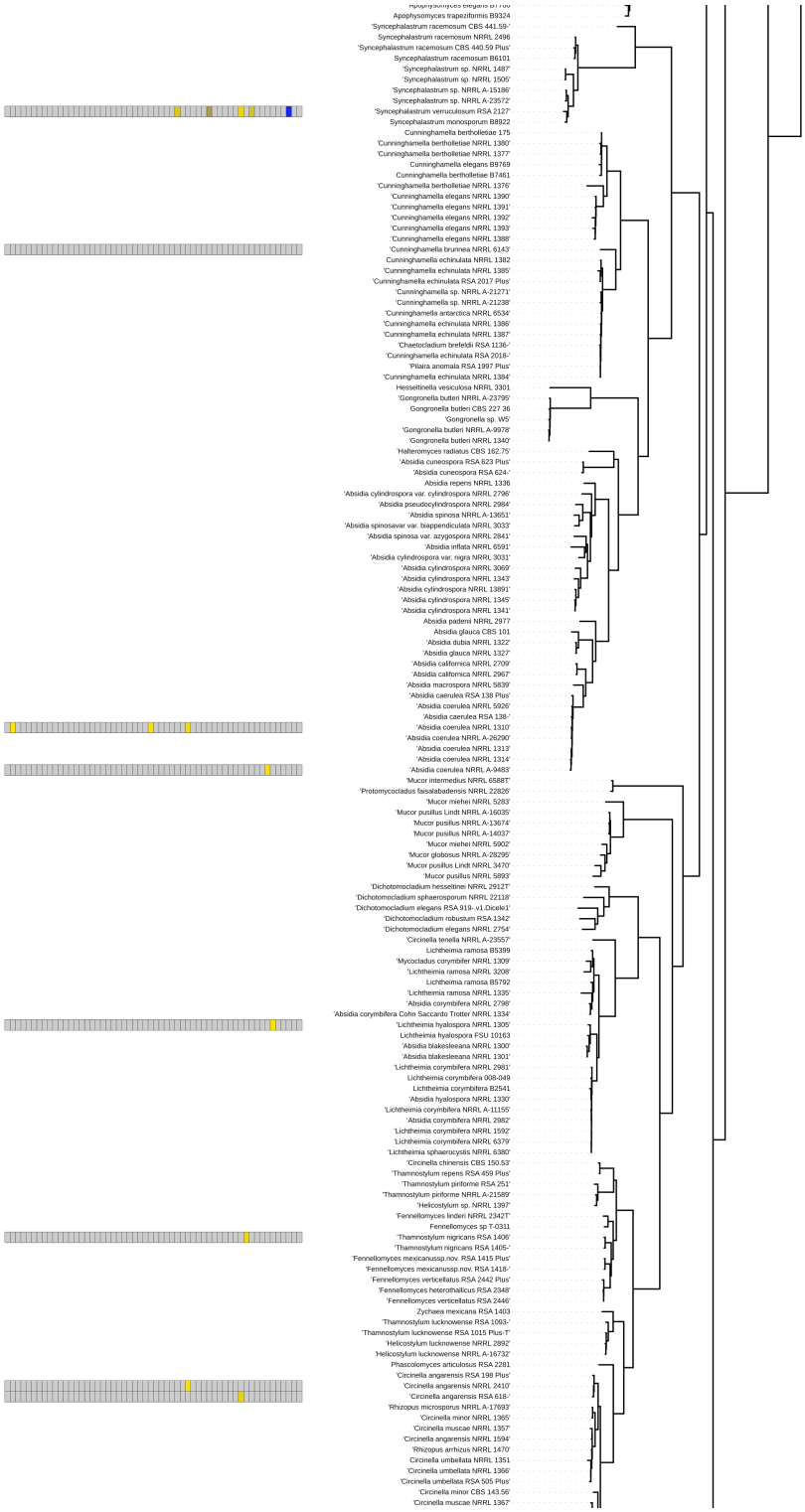


Figure 5.5: Nearly 20% of Zygos have bacterial reads, section 3 of tree

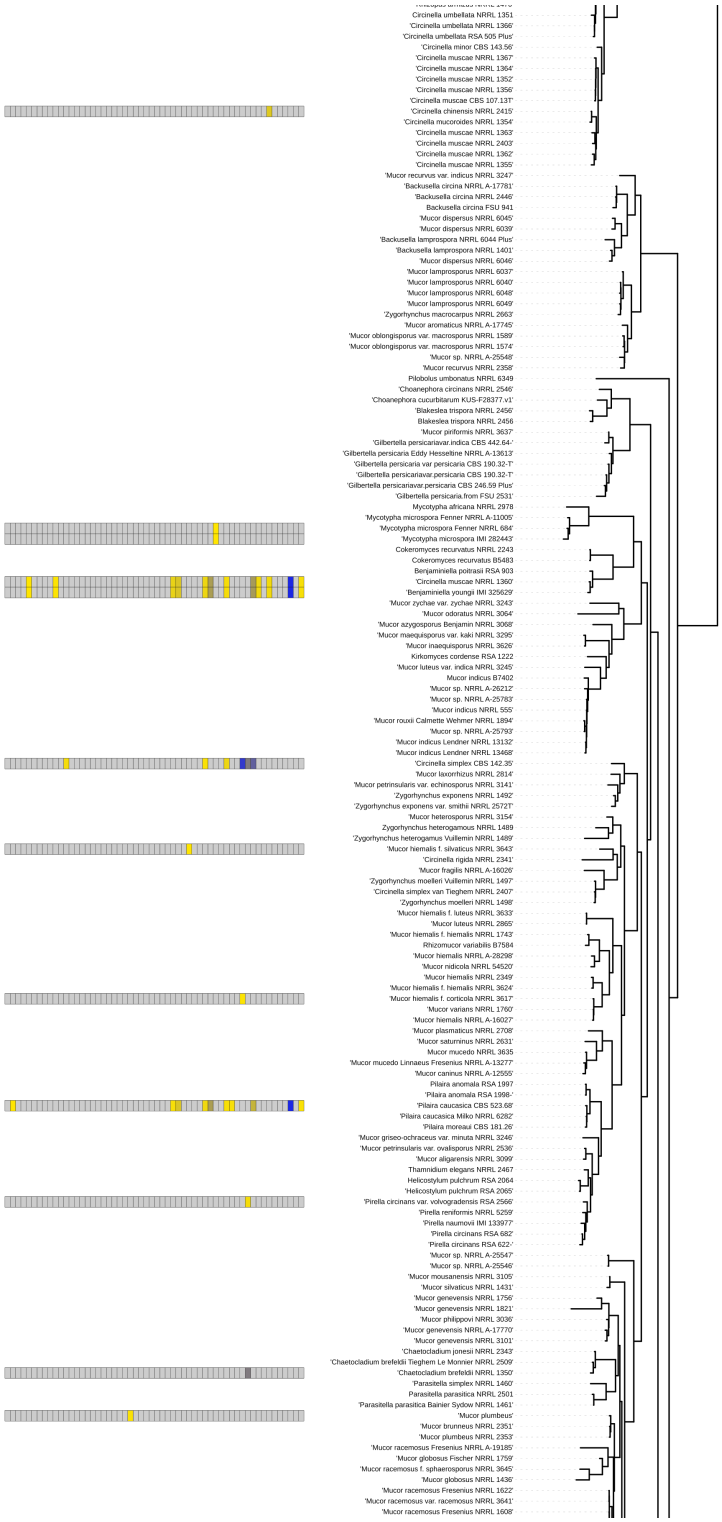


Figure 5.6: Nearly 20% of Zygos have bacterial reads, section 4 of tree

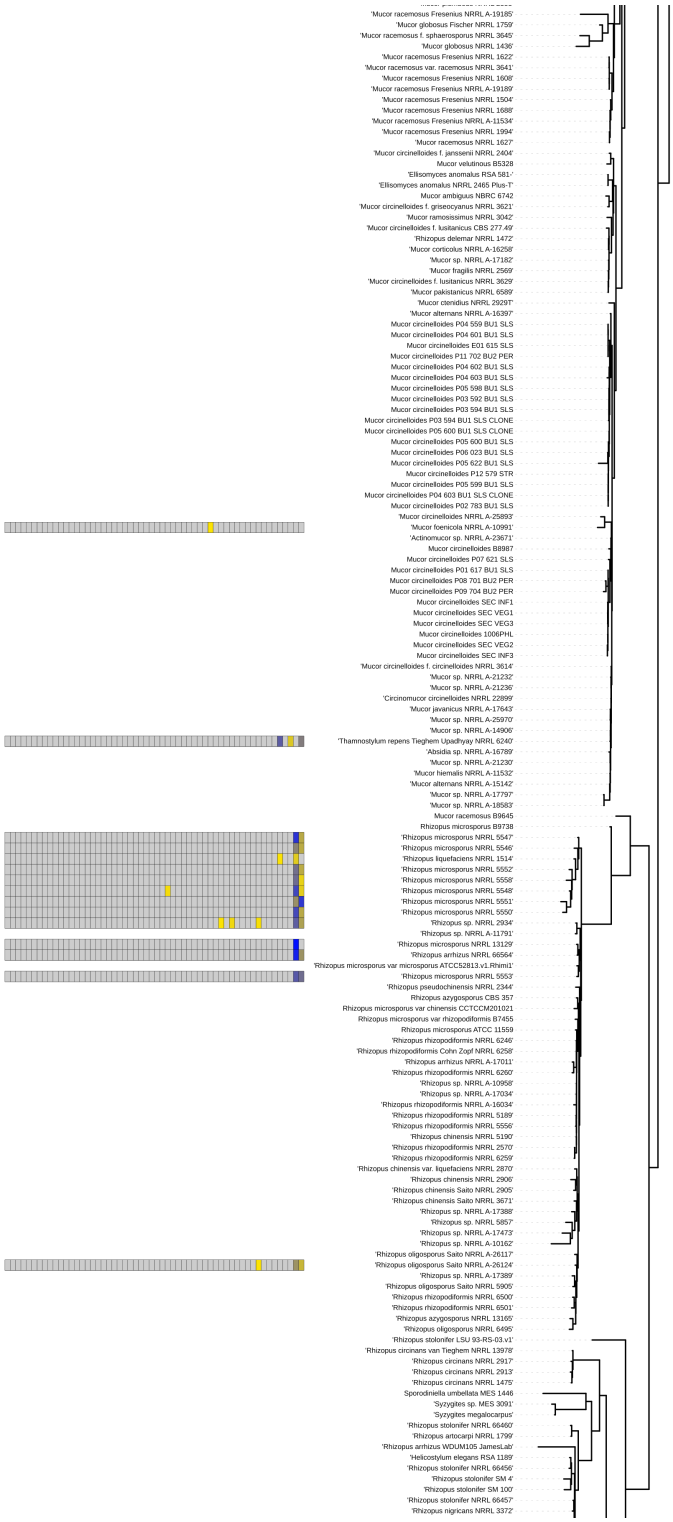


Figure 5.7: Nearly 20% of Zygos have bacterial reads, section 5 of tree

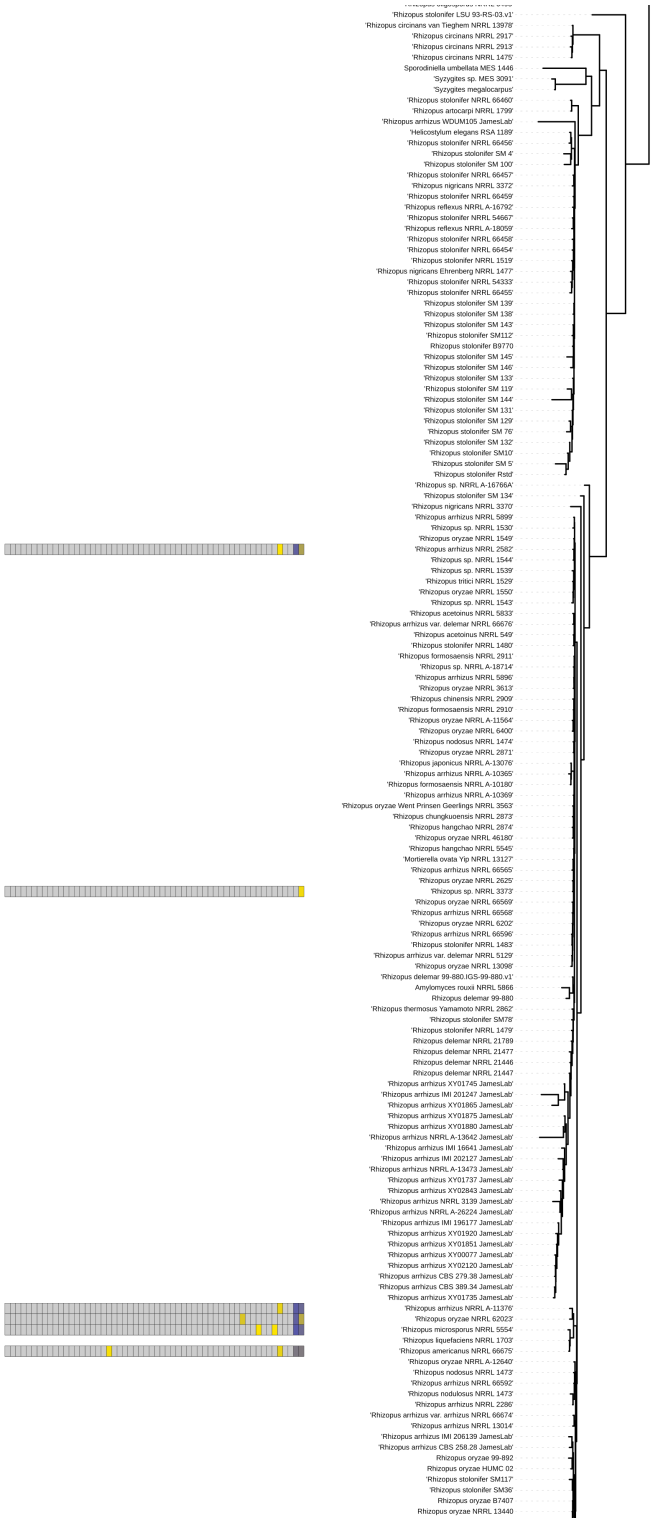
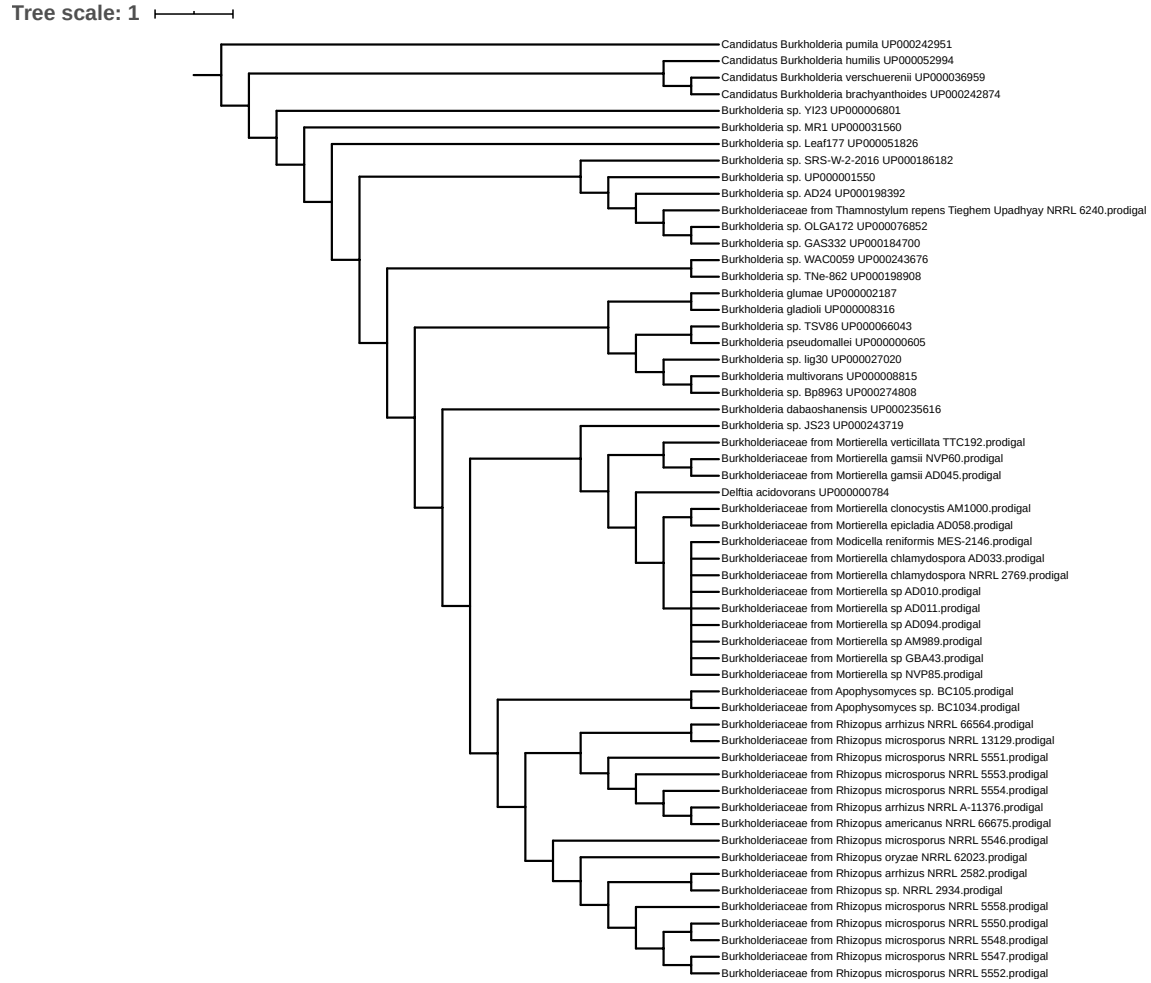


Figure 5.8: Nearly 20% of Zygos have bacterial reads, section 6 of tree

Figure 5.9: Burkholderia strains sequenced from fungal genomes cluster together when compared to other strains deposited in NCBI



Bibliography

[Allocati et al., 2018] Allocati, N., Masulli, M., Di Ilio, C., and Federici, L. (2018). Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. *Oncogenesis*, 7:8.

[Almeida et al., 2019] Almeida, F., Rodrigues, M. L., and Coelho, C. (2019). The still underestimated problem of fungal diseases worldwide. *Front. Microbiol.*, 10:214.

[Angerer et al., 1992] Angerer, A., Klupp, B., and Braun, V. (1992). Iron transport systems of serratia marcescens. *J. Bacteriol.*, 174(4):1378–1387.

[Aryal et al., 2017] Aryal, S. K., Carter-House, D., Stajich, J. E., and Dillman, A. R. (2017). Microbial associates of the southern mole cricket (scapteriscus borellii) are highly pathogenic. *J. Invertebr. Pathol.*, 150:54–62.

[Atkinson and Williams, 2009] Atkinson, S. and Williams, P. (2009). Quorum sensing and social networking in the microbial world. *J. R. Soc. Interface*, 6(40):959–978.

[Avenot and Michailides, 2010] Avenot, H. F. and Michailides, T. J. (2010). Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Protection*, 29(7):643–651.

[Baasiri et al., 1997] Baasiri, R. A., Lu, X., Rowley, P. S., Turner, G. E., and Borkovich, K. A. (1997). Overlapping functions for two g protein alpha subunits in neurospora crassa. *Genetics*, 147:137–145.

[Bahram et al., 2018] Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., and Bork, P. (2018). Structure and function of the global topsoil microbiome. *Nature*, 560(7717):233–237.

[Ballou, 2017] Ballou, E. R. (2017). Fungal diseases deserve our attention. https://naturemicrobiologycommunity.nature.com/users/30340-elizabeth-r-ballou/posts/14558-fungal-diseases-deserve-our-attention. Accessed: 2019-5-27.

[Bassler, 1999] Bassler, B. L. (1999). How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.*, 2(6):582–587.

[Beardsley et al., 2018] Beardsley, J., Halliday, C. L., Chen, S. C.-A., and Sorrell, T. C. (2018). Responding to the emergence of antifungal drug resistance: perspectives from the bench and the bedside. *Future Microbiol.*, 13:1175–1191.

[Bencurova et al., 2018] Bencurova, E., Gupta, S. K., Sarukhanyan, E., and Dandekar, T. (2018). Identification of antifungal targets based on computer modeling. *Journal of fungi (Basel, Switzerland)*, 4.

[Benedict et al., 2018] Benedict, K., Jackson, B. R., Chiller, T., and Beer, K. D. (2018). Estimation of direct healthcare costs of fungal diseases in the united states. *Clinical Infectious Diseases*, 68(11):1791–1797.

[Benny, 2008] Benny, G. L. (2008). Methods used by dr. r. k. benjamin, and other mycologists, to isolate zygomycetes. *Aliso: A Journal of Systematic and Evolutionary Botany*, 26(1):37–61.

[Benny et al., 2014] Benny, G. L., Humber, R. A., and Voigt, K. (2014). 8 zygomycetous fungi: Phylum entomophthoromycota and subphyla kickxellomycotina, mortierellomycotina, mucoromycotina, and zoopagomycotina. In *Systematics and Evolution*, pages 209–250. Springer Berlin Heidelberg.

[Berbee et al., 2017] Berbee, M. L., James, T. Y., and Strullu-Derrien, C. (2017). Early diverging fungi: Diversity and impact at the dawn of terrestrial life. *Annu. Rev. Microbiol.*, 71:41–60.

[Blehert et al., 2009] Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., Coleman, J. T. H., Darling, S. R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R. J., and Stone, W. B. (2009). Bat white-nose syndrome: An emerging fungal pathogen? *Science*, 323(5911):227–227.

[Brauer et al., 2019] Brauer, V. S., Rezende, C. P., Pessoni, A. M., Paula, R. G. D., Rangappa, K. S., Nayaka, S. C., Gupta, V. K., and Almeida, F. (2019). Antifungal agents in agriculture: Friends and foes of public health. *Biomolecules*, 9(10):521.

[Bray et al., 2016] Bray, N. L., Pimentel, H., Melsted, P., and Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*, 34(5):525–527.

[Briard et al., 2016] Briard, B., Heddergott, C., and Latgé, J.-P. (2016). Volatile compounds emitted by pseudomonas aeruginosa stimulate growth of the fungal pathogen aspergillus fumigatus. *mBio*, 7(2).

[Brown et al., 2018] Brown, N. A., Schrevens, S., van Dijck, P., and Goldman, G. H. (2018). Fungal g-protein-coupled receptors: mediators of pathogenesis and targets for disease control. *Nature Microbiology*, 3(4):402–414.

[Cabrera et al., 2015] Cabrera, I. E., Pacentine, I. V., Lim, A., Guerrero, N., Krystofova, S., Li, L., Michkov, A. V., Servin, J. A., Ahrendt, S. R., Carrillo, A. J., Davidson, L. M., Barsoum, A. H., Cao, J., Castillo, R., Chen, W.-C., Dinkchian, A., Kim, S., Kitada, S. M., Lai, T. H., Mach, A., Malekyan, C., Moua, T. R., Torres, C. R., Yamamoto, A., and Borkovich, K. A. (2015). Global analysis of predicted G Protein-Coupled receptor genes in the filamentous fungus, neurospora crassa. *G3*, 5(12):2729–2743.

[Cameron et al., 2011] Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., and Griswold, T. L. (2011). Patterns of widespread decline in north american bumble bees. *Proceedings of the National Academy of Sciences*, 108(2):662–667.

[Campoy and Adrio, 2017] Campoy, S. and Adrio, J. L. (2017). Antifungals. *Biochemical Pharmacology*, 133:86–96.

[Capobianco et al., 1993] Capobianco, J., Zakula, D., Coen, M., and Goldman, R. (1993). Anti-candida activity of cispentacin: The active transport by amino acid permeases and possible mechanisms of action. *Biochemical and Biophysical Research Communications*, 190(3):1037–1044.

[Caramalho et al., 2017] Caramalho, R., Tyndall, J. D. A., Monk, B. C., Larentis, T., Lass-Fl
"orl, C., and Lackner, M. (2017). Intrinsic short-tailed azole resistance in mucormycetes is due to an evolutionary conserved aminoacid substitution of the lanosterol 14α-demethylase. *Sci. Rep.*, 7(1):15898.

[Carter-House, 2019] Carter-House, D. (2019). Will we soon be living in a post-fungicide era? *The Journal of Science Policy and Governance*.

[Casadevall, 2018] Casadevall, A. (2018). Fungal diseases in the 21st century: The near and far horizons. *Pathog Immun*, 3(2):183–196.

[Chapeland et al., 1999] Chapeland, F., Fritz, R., Lanen, C., Gredt, M., and Leroux, P. (1999). Inheritance and mechanisms of resistance to anilinopyrimidine fungicides in botrytis cinerea (botryotinia fuckeliana). *Pesticide Biochemistry and Physiology*, 64(2):85–100.

[Chen and Boutros, 2011] Chen, H. and Boutros, P. C. (2011). VennDiagram: a package for the generation of highly-customizable venn and euler diagrams in r. *BMC Bioinformatics*, 12(1).

[Chen et al., 2017] Chen, S., Blom, J., and Walker, E. D. (2017). Genomic, physiologic, and symbiotic characterization of serratia marcescens strains isolated from the mosquito anopheles stephensi. *Front. Microbiol.*, 8:1483.

[Chong and Xia, 2018] Chong, J. and Xia, J. (2018). MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. *Bioinformatics*, 34(24):4313–4314.

[Chong et al., 2019] Chong, J., Yamamoto, M., and Xia, J. (2019). MetaboAnalystR 2.0: From raw spectra to biological insights. *Metabolites*, 9(3).

[Choudoir et al., 2019] Choudoir, M., Rossabi, S., Gebert, M., Helmig, D., and Fierer, N. (2019). A phylogenetic and functional perspective on volatile organic compound production by actinobacteria. *mSystems*, 4(2):e00295–18.

[Chow et al., 2019] Chow, N. A., Toda, M., Pennington, A. F., Anassi, E., Atmar, R. L., Cox-Ganser, J. M., Da Silva, J., Garcia, B., Kontoyiannis, D. P., Ostrosky-Zeichner, L., Leining, L. M., McCarty, J., Al Mohajer, M., Murthy, B. P., Park, J.-H., Schulte, J., Shuford, J. A., Skrobarcek, K. A., Solomon, S., Strysko, J., Chiller, T. M., Jackson, B. R., Chew, G. L., and Beer, K. D. (2019). Hurricane-Associated mold exposures among patients at risk for invasive mold infections after hurricane harvey - houston, texas, 2017. *MMWR Morb. Mortal. Wkly. Rep.*, 68(21):469–473.

[Coates and Johnson, 1997] Coates, L. and Johnson, G. (1997). Postharvest diseases of fruit and vegetable. *APSnet*.

[Cuomo et al., 2012] Cuomo, C. A., Desjardins, C. A., Bakowski, M. A., Goldberg, J., Ma, A. T., Becnel, J. J., Didier, E. S., Fan, L., Heiman, D. I., Levin, J. Z., Young, S., Zeng, Q., and Troemel, E. R. (2012). Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome research*, 22:2478–2488.

[Darshan and Manonmani, 2015] Darshan, N. and Manonmani, H. K. (2015). Prodigiosin and its potential applications. *J. Food Sci. Technol.*, 52(9):5393–5407.

[Davidse, 1986] Davidse, L. C. (1986). Benzimidazole fungicides: Mechanism of action and biological impact. *Annual Review of Phytopathology*, 24(1):43–65.

[de Souza Pereira and Geibel, 1999] de Souza Pereira, R. and Geibel, J. (1999). Direct observation of oxidative stress on the cell wall of saccharomyces cerevisiae strains with atomic force microscopy. *Mol. Cell. Biochem.*, 201(1-2):17–24.

[Desirò et al., 2018] Desirò, A., Hao, Z., Liber, J. A., Benucci, G. M. N., Lowry, D., Roberson, R., and Bonito, G. (2018). Mycoplasma-related endobacteria within mortierellomycotina fungi: diversity, distribution and functional insights into their lifestyle. *ISME J.*, 12(7):1743–1757.

[Deveau et al., 2018] Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Hervé, V., Labbé, J., Lastovetsky, O. A., Mieszkin, S., Millet, L. J., Vajna, B., Junier, P., Bonfante, P., Krom, B. P., Olsson, S., Elsas, J. D. v., and Wick, L. Y. (2018). Bacterial - fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiol. Rev.*

[Dhar Purkayastha et al., 2018] Dhar Purkayastha, G., Mangar, P., Saha, A., and Saha, D. (2018). Evaluation of the biocontrol efficacy of a serratia marcescens strain indigenous to tea rhizosphere for the management of root rot disease in tea. *PLoS One*, 13(2):e0191761.

[Dixon and Hall, 2015] Dixon, E. F. and Hall, R. A. (2015). Noisy neighbourhoods: quorum sensing in fungal-polymicrobial infections. *Cellular Microbiology*, 17(10):1431–1441.

[dos Santos et al., 2012] dos Santos, N. S. T., Athayde Aguiar, A. J. A., de Oliveira, C. E. V., Veríssimo de Sales, C., de Melo E Silva, S., Sousa da Silva, R., Stamford, T. C. M., and de Souza, E. L. (2012). Efficacy of the application of a coating composed of chitosan and origanum vulgare l. essential oil to control rhizopus stolonifer and aspergillus niger in grapes (vitis labrusca l.). *Food Microbiol.*, 32(2):345–353.

[Downing et al., 2000] Downing, K. J., Leslie, G., and Thomson, J. A. (2000). Biocontrol of the sugarcane borer *Eldana saccharina* by expression of the *Bacillus thuringiensis* cry1ac7 and *Serratia marcescens* chia genes in sugarcane-associated bacteria. *Appl. Environ. Microbiol.*, 66(7):2804–2810.

[Duzhak et al., 2012] Duzhak, A. B., Panfilova, Z. I., Duzhak, T. G., Vasyunina, E. A., and Shternshis, M. V. (2012). Role of prodigiosin and chitinases in antagonistic activity of the bacterium serratia marcescens against the fungus didymella applanata. *Biochemistry*, 77(8):910–916.

[Emms and Kelly, 2015] Emms, D. M. and Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*, 16(1).

[Etienne et al., 2012] Etienne, K. A., Gillece, J., Hilsabeck, R., Schupp, J. M., Colman, R., Lockhart, S. R., Gade, L., Thompson, E. H., Sutton, D. A., Neblett-Fanfair, R., Park, B. J., Turabelidze, G., Keim, P., Brandt, M. E., Deak, E., and Engelthaler, D. M. (2012). Whole genome sequence typing to investigate the apophysomyces outbreak following a tornado in joplin, missouri, 2011. *PloS one*, 7:e49989.

[Fiddaman and Rossall, 1994] Fiddaman, P. and Rossall, S. (1994). Effect of substrate on the production of antifungal volatiles fromBacillus subtilis. *Journal of Applied Bacteriology*, 76(4):395–405.

[Fira et al., 2018] Fira, D., Dimkić, I., Berić, T., Lozo, J., and Stanković, S. (2018). Biological control of plant pathogens by bacillus species. *J. Biotechnol.*, 285:44–55.

[Fisher et al., 2009] Fisher, M. C., Garner, T. W., and Walker, S. F. (2009). Global emergence ofBatrachochytrium dendrobatidisand amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology*, 63(1):291–310.

[Fisher et al., 2018] Fisher, M. C., Hawkins, N. J., Sanglard, D., and Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, 360(6390):739–742.

[Fisher et al., 2012] Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., and Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature*, 484(7393):186–194.

[Fleming Alexander and Wright Almroth Edward, 1922] Fleming Alexander and Wright Almroth Edward (1922). On a remarkable bacteriolytic element found in tissues and secretions. *Proc. R. Soc. Lond. B Biol. Sci.*, 93(653):306–317.

[Frey-Klett et al., 2011] Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. (2011). Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiol. Mol. Biol. Rev.*, 75(4):583–609.

[Fu et al., 2014] Fu, L.-H., Hu, K.-D., Hu, L.-Y., Li, Y.-H., Hu, L.-B., Yan, H., Liu, Y.-S., and Zhang, H. (2014). An antifungal role of hydrogen sulfide on the postharvest pathogens aspergillus niger and penicillium italicum. *PloS one*, 9:e104206.

[Gaelle F. Hollandbeck and Todd, 2019] Gaelle F. Hollandbeck, E. D. and Todd, T. (2019). Kansas cooperative plant disease survey 2019. *Kansas Department of Agriculture*.

[Gallis et al., 1990] Gallis, H. A., Drew, R. H., and Pickard, W. W. (1990). Amphotericin b: 30 years of clinical experience. *Reviews of infectious diseases*, 12:308–329.

[Garbeva et al., 2014] Garbeva, P., Hordijk, C., Gerards, S., and de Boer, W. (2014). Volatile-mediated interactions between phylogenetically different soil bacteria. *Front. Microbiol.*, 5:289.

[Giorgio et al., 2015] Giorgio, A., De Stradis, A., Lo Cantore, P., and Iacobellis, N. S. (2015). Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on sclerotinia sclerotiorum. *Front. Microbiol.*, 6:1056.

[Gkarmiri et al., 2015] Gkarmiri, K., Finlay, R. D., Alstr
"om, S., Thomas, E., Cubeta, M. A., and H
"ogberg, N. (2015). Transcriptomic changes in the plant pathogenic fungus rhizoctonia solani AG-3 in response to the antagonistic bacteria serratia proteamaculans and serratia plymuthica. *BMC Genomics*, 16:630.

[Godfray et al., 2016] Godfray, H. C. J., Mason-D'Croz, D., and Robinson, S. (2016). Food system consequences of a fungal disease epidemic in a major crop. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 371.

[Gonzalez et al., 2018] Gonzalez, E., Pitre, F. E., Pagé, A. P., Marleau, J., Guidi Nissim, W., St-Arnaud, M., Labrecque, M., Joly, S., Yergeau, E., and Brereton, N. J. B. (2018). Trees, fungi and bacteria: tripartite metatranscriptomics of a root microbiome responding to soil contamination. *Microbiome*, 6(1):53.

[Griffin et al., 2004] Griffin, A. S., West, S. A., and Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, 430(7003):1024–1027.

[Groenhagen et al., 2013] Groenhagen, U., Baumgartner, R., Bailly, A., Gardiner, A., Eberl, L., Schulz, S., and Weisskopf, L. (2013). Production of bioactive volatiles by different burkholderia ambifaria strains. *J. Chem. Ecol.*, 39(7):892–906.

[Gutiérrez-Román et al., 2012] Gutiérrez-Román, M. I., Holguín-Meléndez, F., Bello-Mendoza, R., Guillén-Navarro, K., Dunn, M. F., and Huerta-Palacios, G. (2012). Production of prodigiosin and chitinases by tropical serratia marcescens strains with potential to control plant pathogens. *World J. Microbiol. Biotechnol.*, 28(1):145–153.

[Haddix et al., 2008] Haddix, P. L., Jones, S., Patel, P., Burnham, S., Knights, K., Powell, J. N., and LaForm, A. (2008). Kinetic analysis of growth rate, ATP, and pigmentation suggests an energy-spilling function for the pigment prodigiosin of serratia marcescens. *Journal of Bacteriology*, 190(22):7453–7463.

[Hauser et al., 2017] Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schith, H. B., and Gloriam, D. E. (2017). Trends in GPCR drug discovery: new agents, targets and indications. *Nature Reviews Drug Discovery*, 16(12):829–842.

[Havlickova et al., 2008] Havlickova, B., Czaika, V. A., and Friedrich, M. (2008). Epidemiological trends in skin mycoses worldwide. *Mycoses*, 51 Suppl 4:2–15.

[Heddergott et al., 2014] Heddergott, C., Calvo, A. M., and Latgé, J. P. (2014). The volatome of aspergillus fumigatus. *Eukaryot. Cell*, 13(8):1014–1025.

[Herrera-Estrella and Horwitz, 2007] Herrera-Estrella, A. and Horwitz, B. A. (2007). Looking through the eyes of fungi: molecular genetics of photoreception. *Molecular microbiology*, 64:5–15.

[Herv Pags, 2017] Herv Pags, M. C. (2017). Annotationdbi.

[Hoffman et al., 2013] Hoffman, M. T., Gunatilaka, M. K., Wijeratne, K., Gunatilaka, L., and Arnold, A. E. (2013). Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. *PLoS One*, 8(9):e73132.

[Horsburgh and Kirkpatrick, 1983] Horsburgh, C. R. and Kirkpatrick, C. H. (1983). Long-term therapy of chronic mucocutaneous candidiasis with ketoconazole: experience with twenty-one patients. *The American journal of medicine*, 74:23–29.

[Hover et al., 2016] Hover, T., Maya, T., Ron, S., Sandovsky, H., Shadkchan, Y., Kijner, N., Mitiagin, Y., Fichtman, B., Harel, A., Shanks, R. M. Q., Bruna, R. E., García-Véscovi, E., and Osherov, N. (2016). Mechanisms of bacterial (serratia marcescens) attachment to, migration along, and killing of fungal hyphae. *Appl. Environ. Microbiol.*, 82(9):2585–2594.

[Hu et al., 2018] Hu, W., Kuang, F., Lu, Z., Zhang, N., and Chen, T. (2018). Killing effects of an isolated serratia marcescens KH-001 on diaphorina citri via lowering the endosymbiont numbers. *Front. Microbiol.*, 9:860.

[Hunziker et al., 2015] Hunziker, L., B
"onisch, D., Groenhagen, U., Bailly, A., Schulz, S., and Weisskopf, L. (2015). Pseudomonas strains naturally associated with potato plants produce volatiles with high potential for inhibition of phytophthora infestans. *Appl. Environ. Microbiol.*, 81(3):821–830.

[Ipcho et al., 2016] Ipcho, S., Sundelin, T., Erbs, G., Kistler, H. C., Newman, M.-A., and Olsson, S. (2016). Fungal innate immunity induced by bacterial Microbe-Associated molecular patterns (MAMPs). *G3*, 6(6):1585–1595.

[Ivey et al., 1999] Ivey, F. D., Yang, Q., and Borkovich, K. A. (1999). Positive regulation of adenylyl cyclase activity by a galphai homolog in neurospora crassa. *Fungal genetics and biology : FG & B*, 26:48–61.

[James et al., 2013] James, T. Y., Pelin, A., Bonen, L., Ahrendt, S., Sain, D., Corradi, N., and Stajich, J. E. (2013). Shared signatures of parasitism and phylogenomics unite cryptomycota and microsporidia. *Current Biology*, 23(16):1548–1553.

[Kai et al., 2010] Kai, M., Crespo, E., Cristescu, S. M., Harren, F. J. M., Francke, W., and Piechulla, B. (2010). Serratia odorifera: analysis of volatile emission and biological impact of volatile compounds on arabidopsis thaliana. *Appl. Microbiol. Biotechnol.*, 88(4):965–976.

[Kanchiswamy et al., 2015] Kanchiswamy, C. N., Malnoy, M., and Maffei, M. E. (2015). Chemical diversity of microbial volatiles and their potential for plant growth and productivity. *Front. Plant Sci.*, 6:151.

[Khan et al., 2001] Khan, Z. U., Khoursheed, M., Makar, R., Al-Waheeb, S., Al-Bader, I., Al-Muzaini, A., Chandy, R., and Mustafa, A. S. (2001). Basidiobolus ranarum as an etiologic agent of gastrointestinal zygomycosis. *Journal of clinical microbiology*, 39:2360–2363.

[Kim and Harvell, 2004] Kim, K. and Harvell, C. D. (2004). The rise and fall of a six-year coral-fungal epizootic. *The American naturalist*, 164 Suppl 5:S52–S63.

[Kurze et al., 2001] Kurze, S., Bahl, H., Dahl, R., and Berg, G. (2001). Biological control of fungal strawberry diseases by serratia plymuthica HRO-C48. *Plant Dis.*, 85(5):529–534.

[Lemfack et al., 2018] Lemfack, M. C., Gohlke, B.-O., Toguem, S. M. T., Preissner, S., Piechulla, B., and Preissner, R. (2018). mVOC 2.0: a database of microbial volatiles. *Nucleic Acids Res.*, 46(D1):D1261–D1265.

[Lemfack et al., 2014] Lemfack, M. C., Nickel, J., Dunkel, M., Preissner, R., and Piechulla, B. (2014). mVOC: a database of microbial volatiles. *Nucleic Acids Res.*, 42(Database issue):D744–8.

[Lenardon et al., 2010] Lenardon, M. D., Munro, C. A., and Gow, N. A. R. (2010). Chitin synthesis and fungal pathogenesis. *Curr. Opin. Microbiol.*, 13(4):416–423.

[Leonard and Szabo, 2005] Leonard, K. J. and Szabo, L. J. (2005). Stem rust of small grains and grasses caused by puccinia graminis. *Molecular plant pathology*, 6:99–111.

[Letscher-Bru and Herbrecht, 2003] Letscher-Bru, V. and Herbrecht, R. (2003). Caspofungin: the first representative of a new antifungal class. *J. Antimicrob. Chemother.*, 51(3):513–521.

[Letunic and Bork, 2019] Letunic, I. and Bork, P. (2019). Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research*, 47(W1):W256–W259.

[Li et al., 2017] Li, Z., Yao, Q., Dearth, S. P., Entler, M. R., Castro Gonzalez, H. F., Uehling, J. K., Vilgalys, R. J., Hurst, G. B., Campagna, S. R., Labbé, J. L., and Pan, C. (2017). Integrated proteomics and metabolomics suggests symbiotic metabolism and multimodal regulation in a fungal-endobacterial system. *Environ. Microbiol.*, 19(3):1041–1053.

[Lindsey Pedroncelli, 2019] Lindsey Pedroncelli, Derreck Carter-House, N. G. H. A. C. D. B. D. (2019). The consequences of drought on plant pathology. *The Journal of Science Policy and Governance*.

[Liu et al., 2014] Liu, P., Cheng, Y., Yang, M., Liu, Y., Chen, K., Long, C.-A., and Deng, X. (2014). Mechanisms of action for 2-phenylethanol isolated from kloeckera apiculata in control of penicillium molds of citrus fruits. *BMC Microbiol.*, 14:242.

[Lopes et al., 2013] Lopes, L., Borges-Costa, J., Soares-Almeida, L., Filipe, P., Neves, F., Santana, A., Guerra, J., and Kutzner, H. (2013). Cutaneous alternariosis caused by alternaria infectoria: Three cases in kidney transplant patients. *Healthcare (Basel, Switzerland)*, 1:100–106.

[Lopez-Moya et al., 2016] Lopez-Moya, F., Kowbel, D., Nueda, M. J., Palma-Guerrero, J., Glass, N. L., and Lopez-Llorca, L. V. (2016). Neurospora crassa transcriptomics reveals oxidative stress and plasma membrane homeostasis biology genes as key targets in response to chitosan. *Mol. Biosyst.*, 12(2):391–403.

[Lorch et al., 2011] Lorch, J. M., Meteyer, C. U., Behr, M. J., Boyles, J. G., Cryan, P. M., Hicks, A. C., Ballmann, A. E., Coleman, J. T. H., Redell, D. N., Reeder, D. M., and Blehert, D. S. (2011). Experimental infection of bats with geomyces destructans causes white-nose syndrome. *Nature*, 480(7377):376–378.

[Machado et al., 2010] Machado, A. P., Anzai, M., and Fischman, O. (2010). Bacillus subtilis induces morphological changes in fonsecaea pedrosoi in vitro resulting in more resistant fungal forms in vivo. *J. Venom. Anim. Toxins Incl. Trop. Dis.*, 16(4):592–598.

[Mannaa et al., 2017] Mannaa, M., Oh, J. Y., and Kim, K. D. (2017). Biocontrol activity of Volatile-Producing bacillus megaterium and pseudomonas protegens against aspergillus flavus and aflatoxin production on stored rice grains. *Mycobiology*, 45(3):213–219.

[Martin Morgan, 2017] Martin Morgan, S. F. (2017). Gseabase.

[Mathioni et al., 2013] Mathioni, S. M., Patel, N., Riddick, B., Sweigard, J. A., Czymmek, K. J., Caplan, J. L., Kunjeti, S. G., Kunjeti, S., Raman, V., Hillman, B. I., Kobayashi, D. Y., and Donofrio, N. M. (2013). Transcriptomics of the rice blast fungus magnaporthe oryzae in response to the bacterial antagonist lysobacter enzymogenes reveals candidate fungal defense response genes. *PLoS ONE*, 8(10):e76487.

[Mazzola et al., 1992] Mazzola, M., Cook, R. J., Thomashow, L. S., Weller, D. M., and Pierson, 3rd, L. S. (1992). Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.*, 58(8):2616–2624.

[McCormack and Perry, 2005] McCormack, P. L. and Perry, C. M. (2005). Caspofungin: a review of its use in the treatment of fungal infections. *Drugs*, 65(14):2049–2068.

[Meiser et al., 2017] Meiser, A., Otte, J., Schmitt, I., and Grande, F. D. (2017). Sequencing genomes from mixed DNA samples - evaluating the metagenome skimming approach in lichenized fungi. *Sci. Rep.*, 7(1):14881.

[Mendes et al., 2018] Mendes, N. S., Bitencourt, T. A., Sanches, P. R., Silva-Rocha, R., Martinez-Rossi, N. M., and Rossi, A. (2018). Transcriptome-wide survey of gene expression changes and alternative splicing in trichophyton rubrum in response to undecanoic acid. *Sci. Rep.*, 8(1):2520.

[Meziane et al., 2006] Meziane, H., Gavriel, S., Ismailov, Z., Chet, I., Chernin, L., and H "ofte, M. (2006). Control of green and blue mould on orange fruit by serratia plymuthica strains IC14 and IC1270 and putative modes of action. *Postharvest Biol. Technol.*, 39(2):125–133.

[Miethke and Marahiel, 2007] Miethke, M. and Marahiel, M. A. (2007). Siderophore-based iron acquisition and pathogen control. *Microbiol. Mol. Biol. Rev.*, 71(3):413–451.

[Minerdi et al., 2009] Minerdi, D., Bossi, S., Gullino, M. L., and Garibaldi, A. (2009). Volatile organic compounds: a potential direct long-distance mechanism for antagonistic action of fusarium oxysporum strain MSA 35. *Environ. Microbiol.*, 11(4):844–854.

[Minerdi et al., 2008] Minerdi, D., Moretti, M., Gilardi, G., Barberio, C., Gullino, M. L., and Garibaldi, A. (2008). Bacterial ectosymbionts and virulence silencing in a fusarium oxysporum strain. *Environ. Microbiol.*, 10(7):1725–1741.

[Misztal et al., 2018] Misztal, P. K., Lymperopoulou, D. S., Adams, R. I., Scott, R. A., Lindow, S. E., Bruns, T., Taylor, J. W., Uehling, J., Bonito, G., Vilgalys, R., and Goldstein, A. H. (2018). Emission factors of microbial volatile organic compounds from environmental bacteria and fungi. *Environ. Sci. Technol.*, 52(15):8272–8282.

[Mowat et al., 2010] Mowat, E., Rajendran, R., Williams, C., McCulloch, E., Jones, B., Lang, S., and Ramage, G. (2010). Pseudomonas aeruginosa and their small diffusible extracellular molecules inhibit aspergillus fumigatus biofilm formation. *FEMS microbiology letters*, 313:96–102.

[Na, 2016] Na, F. (2016). *Identification of Two Novel Fungal Species Associated with Kuroshio Shot Hole Borer (Euwallacea sp.) and Evaluation of Novel Biological Control Method to Inhibit the Fungal Associates of the Invasive Ambrosia Beetle Species in California*. PhD thesis, UC Riverside.

[Neblett Fanfair et al., 2012] Neblett Fanfair, R., Benedict, K., Bos, J., Bennett, S. D., Lo, Y.-C., Adebanjo, T., Etienne, K., Deak, E., Derado, G., Shieh, W.-J., Drew, C., Zaki, S., Sugerman, D., Gade, L., Thompson, E. H., Sutton, D. A., Engelthaler, D. M., Schupp, J. M., Brandt, M. E., Harris, J. R., Lockhart, S. R., Turabelidze, G., and Park, B. J. (2012). Necrotizing cutaneous mucormycosis after a tornado in joplin, missouri, in 2011. *The New England journal of medicine*, 367:2214–2225.

[Neupane et al., 2015] Neupane, S., Finlay, R. D., Alstr
"om, S., Elfstrand, M., and H
"ogberg, N. (2015). Transcriptional responses of the bacterial antagonist serratia plymuthica to the fungal phytopathogen rhizoctonia solani. *Environ. Microbiol. Rep.*, 7(1):123–127.

[Ordentlich et al., 1987] Ordentlich, A., Elad, Y., and Chet, I. (1987). Rhizosphere colonization by serratia marcescens for the control of sclerotium rolfsii. *Soil Biology and Biochemistry*, 19(6):747–751.

[Ossowicki et al., 2017] Ossowicki, A., Jafra, S., and Garbeva, P. (2017). The antimicrobial volatile power of the rhizospheric isolate pseudomonas donghuensis P482. *PLoS One*, 12(3):e0174362.

[Parani et al., 2011] Parani, K., Shetty, G. P., and Saha, B. K. (2011). Isolation of serratia marcescens SR1 as a source of chitinase having potentiality of using as a biocontrol agent. *Indian J. Microbiol.*, 51(3):247–250.

[Partida-Martinez and Hertweck, 2005] Partida-Martinez, L. P. and Hertweck, C. (2005). Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature*, 437(7060):884–888.

[Partida-Martinez et al., 2007] Partida-Martinez, L. P., Monajembashi, S., Greulich, K.-O., and Hertweck, C. (2007). Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. *Curr. Biol.*, 17(9):773–777.

[Pawlowska et al., 2018] Pawlowska, T. E., Gaspar, M. L., Lastovetsky, O. A., Mondo, S. J., Real-Ramirez, I., Shakya, E., and Bonfante, P. (2018). Biology of fungi and their bacterial endosymbionts. *Annu. Rev. Phytopathol.*, 56:289–309.

[Popova et al., 2014] Popova, A. A., Koksharova, O. A., Lipasova, V. A., Zaitseva, J. V., Katkova-Zhukotskaya, O. A., Eremina, S. I., Mironov, A. S., Chernin, L. S., and Khmel, I. A. (2014). Inhibitory and toxic effects of volatiles emitted by strains of pseudomonas and serratia on growth and survival of selected microorganisms, caenorhabditis elegans, and drosophila melanogaster. *Biomed Res. Int.*, 2014.

[Pothuluri et al., 1990] Pothuluri, J. V., Freeman, J. P., Evans, F. E., and Cerniglia, C. E. (1990). Fungal transformation of fluoranthene. *Applied and environmental microbiology*, 56:2974–2983.

[Powell et al., 2019] Powell, D., Milton, M., Perry, A., and Santos, K. (2019). drpowell/degust 4.1.1.

[Price et al., 2015] Price, C. L., Parker, J. E., Warrilow, A. G. S., Kelly, D. E., and Kelly, S. L. (2015). Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens. *Pest Manag. Sci.*, 71(8):1054–1058.

[Randal M. Hauptmann, 1985] Randal M. Hauptmann, Jack M. Widholm, J. D. P. (1985). "benomyl: A broad spectrum fungicide for use in plant cell and protoplast culture",. *Plant Cell Reports*.

[Robert Gentleman, 2017] Robert Gentleman, Seth Falcon, R. C. B. P. M. (2017). Gostats.

[Robinson et al., 2010] Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edger: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics (Oxford, England)*, 26:139–140.

[Roden et al., 2005] Roden, M. M., Zaoutis, T. E., Buchanan, W. L., Knudsen, T. A., Sarkisova, T. A., Schaufele, R. L., Sein, M., Sein, T., Chiou, C. C., Chu, J. H., Kontoyiannis, D. P., and Walsh, T. J. (2005). Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 41:634–653.

[Rodrguez-Siz et al., 2004] Rodrguez-Siz, M., Paz, B., De La Fuente, J. L., Lpez-Nieto, M. J., Cabri, W., and Barredo, J. L. (2004). Blakeslea trispora genes for carotene biosynthesis. *Applied and environmental microbiology*, 70:5589–5594.

[Saha et al., 2013] Saha, R., Saha, N., Donofrio, R. S., and Bestervelt, L. L. (2013). Microbial siderophores: a mini review. *J. Basic Microbiol.*, 53(4):303–317.

[Salvioli et al., 2016] Salvioli, A., Ghignone, S., Novero, M., Navazio, L., Venice, F., Bagnaresi, P., and Bonfante, P. (2016). Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. *The ISME journal*, 10:130–144.

[Sang et al., 2018] Sang, H., Hulvey, J. P., Green, R., Xu, H., Im, J., Chang, T., and Jung, G. (2018). A xenobiotic detoxification pathway through transcriptional regulation in filamentous fungi. *mBio*, 9.

[Sangamwar et al., 2008] Sangamwar, A. T., Deshpande, U. D., and Pekamwar, S. S. (2008). Antifungals: need to search for a new molecular target. *Indian journal of pharmaceutical sciences*, 70:423–430.

[Sasnauskas et al., 1992] Sasnauskas, K., Jomantiene, R., Lebediene, E., Lebedys, J., Januska, A., and Janulaitis, A. (1992). Cloning and sequence analysis of a candida maltosa gene which confers resistance to cycloheximide. *Gene*, 116:105–108.

[Schmidt et al., 2015] Schmidt, R., Cordovez, V., de Boer, W., Raaijmakers, J., and Garbeva, P. (2015). Volatile affairs in microbial interactions. *ISME J.*, 9(11):2329–2335.

[Schmidt et al., 2017] Schmidt, R., Jager, V. d., Z
"uhlke, D., Wolff, C., Bernhardt, J., Cankar, K., Beekwilder, J., van Ijcken, W., Sleutels, F., Boer, W. d., Riedel, K., and Garbeva, P. (2017). Fungal volatile compounds induce production of the secondary metabolite sodorifen in serratia plymuthica PRI-2C. *Sci. Rep.*, 7(1):862.

[Schmidt et al., 2019] Schmidt, R., Ulanova, D., Wick, L. Y., Bode, H. B., and Garbeva, P. (2019). Microbe-driven chemical ecology: past, present and future. *ISME J.*, page 1.

[Schroeckh et al., 2009] Schroeckh, V., Scherlach, K., N
"utzmann, H.-W., Shelest, E., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C., and Brakhage, A. A. (2009). Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in aspergillus nidulans. *Proc. Natl. Acad. Sci. U. S. A.*, 106(34):14558–14563.

[Schulz et al., 2010] Schulz, S., Dickschat, J. S., Kunze, B., Wagner-Dobler, I., Diestel, R., and Sasse, F. (2010). Biological activity of volatiles from marine and terrestrial bacteria. *Mar. Drugs*, 8(12):2976–2987.

[Schulz-Bohm et al., 2017] Schulz-Bohm, K., Martín-Sánchez, L., and Garbeva, P. (2017). Microbial volatiles: Small molecules with an important role in intra- and Inter-Kingdom interactions. *Front. Microbiol.*, 8:2484.

[Selvakumar et al., 2008] Selvakumar, G., Mohan, M., Kundu, S., Gupta, A. D., Joshi, P., Nazim, S., and Gupta, H. S. (2008). Cold tolerance and plant growth promotion potential of serratia marcescens strain SRM (MTCC 8708) isolated from flowers of summer squash (cucurbita pepo). *Lett. Appl. Microbiol.*, 46(2):171–175.

[Sephton-Clark et al., 2019] Sephton-Clark, P., Muñoz, J. F., Itabangi, H., Voelz, K., Cuomo, C. A., and Ballou, E. R. (2019). Host-pathogen transcriptomics of macrophages, mucorales and their endosymbionts: a polymicrobial pas de trois.

[Shaffer et al., 2017] Shaffer, J. P., U'Ren, J. M., Gallery, R. E., Baltrus, D. A., and Arnold, A. E. (2017). An endohyphal bacterium (chitinophaga, bacteroidetes) alters carbon source use by fusarium keratoplasticum (f. solani species complex, nectriaceae). *Front. Microbiol.*, 8:350.

[Shafi et al., 2017] Shafi, J., Tian, H., and Ji, M. (2017). Bacillus species as versatile weapons for plant pathogens: a review. *Biotechnol. Biotechnol. Equip.*, 31(3):446–459.

[Sharifi and Ryu, 2018] Sharifi, R. and Ryu, C.-M. (2018). Sniffing bacterial volatile compounds for healthier plants. *Curr. Opin. Plant Biol.*, 44:88–97.

[Singh et al., 2011] Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P. K., Singh, S., and Govindan, V. (2011). The emergence of ug99 races of the stem rust fungus is a threat to world wheat production. *Annual review of phytopathology*, 49:465–481.

[SMITH et al., 2006] SMITH, K. F., SAX, D. F., and LAFFERTY, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology*, 20(5):1349–1357.

[Soanes and Talbot, 2010] Soanes, D. M. and Talbot, N. J. (2010). Comparative genome analysis reveals an absence of Leucine-Rich repeat Pattern-Recognition receptor proteins in the kingdom fungi. *PLoS One*, 5(9):e12725.

[Someya et al., 2005] Someya, N., Nakajima, M., Watanabe, K., Hibi, T., and Akutsu, K. (2005). Potential of serratia marcescens strain B2 for biological control of rice sheath blight. *Biocontrol Sci. Technol.*, 15(1):105–109.

[Souza et al., 2015] Souza, R. d., Ambrosini, A., and Passaglia, L. M. P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.*, 38(4):401–419.

[Spatafora et al., 2016] Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T. Y., O'Donnell, K., Roberson, R. W., Taylor, T. N., Uehling, J., Vilgalys, R., White, M. M., and Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, 108(5):1028–1046.

[Srobel et al., 1999] Srobel, G., Li, J. Y., Sugawara, F., Koshino, H., Harper, J., and Hess, W. M. (1999). Oocydin a, a chlorinated macrocyclic lactone with potent anti-oomycete activity from serratia marcescens. *Microbiology*, 145 (Pt 12):3557–3564.

[Stein et al.,] Stein, J., CEO of Cidara Therapeutics, Chairman of the Antimicrobials Working Group (AWG), Pappas, P. G., Director of the Mycoses Study Group Education, Research Consortium (MSGERC), of Medicine, and Div. The emerging battle against fungal infections - what healthcare organizations can do to help. Accessed: 2019-5-23.

[Taghavi et al., 2018] Taghavi, T., Kim, C., and Rahemi, A. (2018). Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. *Microorganisms*, 6(4).

[Takahashi et al., 2008] Takahashi, S., Ogawa, T., Inoue, K., and Masuda, T. (2008). Characterization of cytosolic tetrapyrrole-binding proteins in arabidopsis thaliana. *Photochemical & photobiological sciences : Official journal of the European Photochemistry Association and the European Society for Photobiology*, 7:1216–1224.

[Talbot, 2003] Talbot, N. J. (2003). On the trail of a cereal killer: Exploring the biology of magnaporthe grisea. *Annual review of microbiology*, 57:177–202.

[Tang et al., 2019] Tang, L., Mo, J., Guo, T., Huang, S., Li, Q., Ning, P., and Hsiang, T. (2019). Antifungal effects of dimethyl trisulfide against colletotrichum gloeosporioides infection on mango. *J. Phytopathol.*, 84:600.

[Tenorio-Salgado et al., 2013] Tenorio-Salgado, S., Tinoco, R., Vazquez-Duhalt, R., Caballero-Mellado, J., and Perez-Rueda, E. (2013). Identification of volatile compounds produced by the bacterium burkholderia tropica that inhibit the growth of fungal pathogens. *Bioengineered*, 4(4):236–243.

[Tribble and Rodriguez, 2014] Tribble, D. R. and Rodriguez, C. J. (2014). Combat-related invasive fungal wound infections. *Current fungal infection reports*, 8:277–286.

[Trunk et al., 2018] Trunk, K., Peltier, J., Liu, Y.-C., Dill, B. D., Walker, L., Gow, N. A. R., Stark, M. J. R., Quinn, J., Strahl, H., Trost, M., and Coulthurst, S. J. (2018). The type VI secretion system deploys antifungal effectors against microbial competitors. *Nature Microbiology*, page 1.

[Tully et al., 2009] Tully, C. C., Romanelli, A. M., Sutton, D. A., Wickes, B. L., and Hospenthal, D. R. (2009). Fatal actinomucor elegans var. kuwaitiensis infection following combat trauma. *Journal of clinical microbiology*, 47:3394–3399.

[Tzortzakis and Economakis, 2007] Tzortzakis, N. G. and Economakis, C. D. (2007). Antifungal activity of lemongrass (cympopogon citratus l.) essential oil against key postharvest pathogens. *Innov. Food Sci. Emerg. Technol.*, 8(2):253–258.

[Uehling et al., 2017] Uehling, J., Gryganskyi, A., Hameed, K., Tschaplinski, T., Misztal, P. K., Wu, S., Desirò, A., Vande Pol, N., Du, Z., Zienkiewicz, A., Zienkiewicz, K., Morin, E., Tisserant, E., Splivallo, R., Hainaut, M., Henrissat, B., Ohm, R., Kuo, A., Yan, J., Lipzen, A., Nolan, M., LaButti, K., Barry, K., Goldstein, A. H., Labbé, J., Schadt, C., Tuskan, G., Grigoriev, I., Martin, F., Vilgalys, R., and Bonito, G. (2017). Comparative genomics of mortierella elongata and its bacterial endosymbiont mycoavidus cysteinexigens. *Environ. Microbiol.*

[Uzma et al., 2018] Uzma, F., Mohan, C. D., Hashem, A., Konappa, N. M., Rangappa, S., Kamath, P. V., Singh, B. P., Mudili, V., Gupta, V. K., Siddaiah, C. N., Chowdappa, S., Alqarawi, A. A., and Abd Allah, E. F. (2018). Endophytic fungi-alternative sources of cytotoxic compounds: A review. *Frontiers in pharmacology*, 9:309.

[Vallabhaneni et al., 2015] Vallabhaneni, S., Walker, T. A., Lockhart, S. R., Ng, D., Chiller, T., Melchreit, R., Brandt, M. E., Smith, R. M., and Centers for Disease Control and Prevention (CDC) (2015). Notes from the field: Fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated dietary supplement–connecticut, 2014. *MMWR Morb. Mortal. Wkly. Rep.*, 64(6):155–156.

[Vespermann et al., 2007] Vespermann, A., Kai, M., and Piechulla, B. (2007). Rhizobacterial volatiles affect the growth of fungi and arabidopsis thaliana. *Appl. Environ. Microbiol.*, 73(17):5639–5641.

[Vicente et al., 2003] Vicente, M. F., Basilio, A., Cabello, A., and Peláez, F. (2003). Microbial natural products as a source of antifungals. *Clin. Microbiol. Infect.*, 9(1):15–32.

[Vleesschauwer, 2007] Vleesschauwer, D. (2007). Using serratia plymuthica to control fungal pathogens of plants. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 2(046).

[Walter et al., 2001] Walter, M., Jaspers, M. V., Eade, K., Frampton, C. M., and Stewart, A. (2001). Control of botrytis cinerea in grape using thyme oil. *Australas. Plant Pathol.*, 30(1):21–25.

[Wang et al., 2013a] Wang, K., sheng Yan, P., xin Cao, L., long Ding, Q., Shao, C., and fei Zhao, T. (2013a). Potential of ChitinolyticSerratia marcescensStrain JPP1 for biological control ofAspergillus parasiticusand aflatoxin. *BioMed Research International*, 2013:1–7.

[Wang et al., 2013b] Wang, X., Wang, J., Jin, P., and Zheng, Y. (2013b). Investigating the efficacy of bacillus subtilis SM21 on controlling rhizopus rot in peach fruit. *International Journal of Food Microbiology*, 164(2-3):141–147.

[Weise et al., 2014] Weise, T., Th
"urmer, A., Brady, S., Kai, M., Daniel, R., Gottschalk, G., and Piechulla, B. (2014). VOC emission of various serratia species and isolates and genome analysis of serratia plymuthica 4rx13. *FEMS Microbiol. Lett.*, 352(1):45–53.

[Weller, 2007] Weller, D. M. (2007). Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*, 97(2):250–256.

[Wenke et al., 2019] Wenke, K., Kopka, J., Schwachtje, J., van Dongen, J. T., and Piechulla, B. (2019). Volatiles of rhizobacteria serratia and stenotrophomonas alter growth and metabolite composition of arabidopsis thaliana. *Plant Biol.*, 21 Suppl 1:109–119.

[Wheatley, 2002] Wheatley, R. E. (2002). The consequences of volatile organic compound mediated bacterial and fungal interactions. *Antonie Van Leeuwenhoek*, 81(1):357–364.

[Woodhams et al., 2017] Woodhams, D. C., LaBumbard, B. C., Barnhart, K. L., Becker, M. H., Bletz, M. C., Escobar, L. A., Flechas, S. V., Forman, M. E., Iannetta, A. A., Joyce, M. D., Rabemananjara, F., Gratwicke, B., Vences, M., and Minbiole, K. P. C. (2017). Prodigiosin, violacein, and volatile organic compounds produced by widespread cutaneous bacteria of amphibians can inhibit two batrachochytrium fungal pathogens. *Microb. Ecol.*

[Xie et al., 2016] Xie, F., Chang, W., Zhang, M., Li, Y., Li, W., Shi, H., Zheng, S., and Lou, H. (2016). Quinone derivatives isolated from the endolichenic fungus phialocephala fortinii are mdr1 modulators that combat azole resistance in candida albicans. *Sci. Rep.*, 6:33687.

[Yang and Borkovich, 1999] Yang, Q. and Borkovich, K. A. (1999). Mutational activation of a galphai causes uncontrolled proliferation of aerial hyphae and increased sensitivity to heat and oxidative stress in neurospora crassa. *Genetics*, 151:107–117.

[Yang et al., 2002] Yang, Q., Poole, S. I., and Borkovich, K. A. (2002). A g-protein beta subunit required for sexual and vegetative development and maintenance of normal g alpha protein levels in neurospora crassa. *Eukaryotic cell*, 1:378–390.

[Yu et al., 2018] Yu, J. M., Wang, D., Pierson, 3rd, L. S., and Pierson, E. A. (2018). Effect of producing different phenazines on bacterial fitness and biological control in pseudomonas chlororaphis 30-84. *Plant Pathol. J.*, 34(1):44–58.

[Yu et al., 2000] Yu, K., Hamilton-Kemp, T. R., Archbold, D. D., Collins, R. W., and Newman, M. C. (2000). Volatile compounds from escherichia coli O157:H7 and their absorption by strawberry fruit. *J. Agric. Food Chem.*, 48(2):413–417.

[Zhang et al., 2018] Zhang, Y., Kastman, E. K., Guasto, J. S., and Wolfe, B. E. (2018). Fungal networks shape dynamics of bacterial dispersal and community assembly in cheese rind microbiomes. *Nat. Commun.*, 9(1):336.