Identification of secondary metabolites extracted from *Leptographium* spp. in search of novel biochemical compounds with probable antimicrobial/therapeutic potential

By

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Abstract

The World Health Organization (WHO) highlights on the antimicrobial resistance (AMR) in bacterial pathogens as a leading cause fatalities and health risks associated with bacterial infections in humans. As a result, new and effective treatment options are urgently needed. Secondary metabolites (SMs) from microorganisms such as bacteria and fungi are the major source for the currently used antibiotics. Fungi belonging to the Ophiostomatales have not been studied much with regards to the production of SMs. In this study, around 100 different strains of *Leptographium spp.,* belonging to the order Ophiostomatales, were screened for bioactivity via agar plug assays. From the bioactivity screen, several strains of *Leptographium wingfieldii* showed an indication of antimicrobial activity especially against an efflux deficient strain of *Pseudomonas aeruginosa* PAO750. SMs were recovered from spent media (solid state fermentation) using the ethyl-acetate based extraction protocol; the "crude extracts" were prepared by evaporation of the solvent. Chemical compounds produced by these fungi were characterized using liquid chromatography-mass spectrometry (LC-MS). Antibase 2012 was used to identify novel compounds. Previously known compounds with antimicrobial activity such as Limazepine B1 and B2, and Pulcherriminic acid were identified. However, some compounds yielded signals that were "no hits" in the Antibase search. These molecules potentially could have novel chemical or biological significance. In conclusion, the detection of SMs from *Leptographium* spp. may lead to the discovery of new antibiotics and help in treatments against antibiotic resistant bacteria.

Dedication

To the one and only Raba'a Al habib, this is for you.

الى امي رابعة الحبيب اليك وحدك أهدي هذا العمل

To Mawia Ibrahim, your prayers have borne fruit.

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To my better half Elwasila Hussein, I don't think the words to describe how grateful I am for you, exist yet

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To my brothers Mohamed, Ahmad, Mahmoud, and Ibrahim you have always been, supportive, protective, and caring.

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Rationale and Objectives

Since 2016 approximately 1200 different fungi belonging to the Order Ophiostomatales were screened by the Hausner/Kumar research groups including about 200 strains of *Leptographium.* These fungi were tested for their potential antibacterial bioactivity using the agar plug assay. From this initial screen several strains of *Leptographium wingfieldii* showed some evidence of antimicrobial activity against various bacterial pathogens with AMR or a hypersensitive strain that was efflux deficient.

In a follow up study by Engelberg *et al*. (2018) a drug sensitive reporter strain of *P. aeruginosa* PAAK106 that was designed to optimize screening strategies. This strain can be used in a luminescence-based assay to accelerate the process of screening fungal SMs with potential for antimicrobial activity (Engelberg *et al*., 2018). The aim of this work was to develop a sensitive and high throughput tool that can quickly screen a large number of fungal strains for numerous SMs that could be antimicrobial compounds.

SMs isolated from *Leptographium* species contain potential novel antimicrobial compounds. Species of *Leptgraphium* (Order Ophiostomatales) are only distantly related to members of *Penicillium* (Order Eurotiales) so one could assume that they have different SMs profiles.

The objective of this study was to examine *Leptographium* strains in more detail with regards to potentially identify novel antimicrobial compounds through phenotypic AST, complimented with a high-performance liquid chromatography (HPLC) and LC-MS. This group of fungi has not been previously explored extensively with regards to secondary metabolites, hence

this study may offer new insights as to the utility of these fungi in bioprospecting for compounds of economic value.

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List of abbreviations

6-MSA	6-methylsalicylic acid
AMEs	Aminoglycoside modifying enzymes
Na_2SO4	Anhydrous sodium sulfate
ARP	Antibiotic resistance platform
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
BGCs	Biosynthetic gene clusters
CTAB	Cetyltrimethylammonium bromide
DNA	Deoxyribonucleic acid
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DMATS	Dimethylallyl tryptophan synthetases
ESI	Electro Spray Ionisation
FQ	Fluoroquinone
HPLC	High-performance liquid chromatography
HCl	hydrochloric acid
ITS	Internal transcribed spacer
iChip	Isolation chip
LC-MS	Liquid chromatography-mass spectrometry
LAMP	Loop-mediated isothermal amplification
LB	lysogeny broth
MEA	Malt extract agar
m/z	Mass-to-charge ratios
MIC	minimum inhibitory concentration
MPB	Mountain pine beetle
MDR	Multi-drug resistance
NRPS	Non-ribosomal protein synthases
NMR	Nuclear magnetic resonance
PKS	Polyketide synthases

PCR	Polymerase chain reaction
PEI	Prince Edward Island
RCF	Relative Centrifugal Force
Rf	Retention factor
RNA	Ribonucleic acid
rDNA	Ribosomal
SMs	Secondary metabolites
FT	The flow-through
FDA	The Food and Drug Administration
TLC	Thin layer chromatography
TS	Tryptophan synthetases
TB	Tuberculosis
WHO	World Health Organization

Introduction

Fungi have recently been investigated as a source of SMs of economic importance. Among the SMs, one of the beneficial classes consist of antimicrobial compounds that can be used as a therapeutic option, especially in the current times when multi-drug resistance (MDR) has limited the use of traditionally popular antibiotics. In this study, a hundred *Leptographium* spp. were screened in order to detect novel biochemical compounds with potential antimicrobial and/or therapeutic application. *Leptographium* spp. that belong to the Ophiostomatales, have been explored as a source of SMs before in the Hausner / Kumar labs. In a previous study, a luminescence-based screening method was employed to accelerate the detection of SMs and this study aimed at facilitating high throughput and fast screening of the fungal strains for novel bioactive compounds Engelberg *et al*. (2018).

In the current study, phenotypic antimicrobial susceptibility testing (AST) techniques were used such as the bioactivity assays based on the principle of formation of zone of inhibition on the test bacterial lawn by the antimicrobial compound secreted by the cultured fungal strains. An efflux-deficient strain of *Pseudomonas aeruginosa* PAO750 was used as the reporter strain for the detection of compounds with antibacterial activity. This is a suitable strain for detecting molecules that have been secreted in low concentrations as it is hyper-susceptible to antibiotics due to the deletion of a crucial efflux pump operons. The bioactivity assays helped in the preliminary screening of the *Leptographium* spp. strains that can be a potential source of antimicrobial compounds. From the initial number of fungal strains screened by the bioactivity assays, some which showed bioactivity were sent for LC-MS based investigation, along with other strains that did not show antimicrobial activity for the purpose of comparison. LC-MS facilitated the detection of bioactive compounds in several *Leptographium* spp. strains that have potential uses in various

therapeutic contexts and not just limited to antimicrobial activities. This study paves the way for using culture-based screening to detect novel metabolites in fungi, which can be complemented with co-culture techniques or stress induction to optimize culture conditions and maximize the production of SMs.

A phylogenetic tree was constructed using the internal transcribed spacer region (ITS sequences) from *Leptographium* strains which helped in confirming the identity of the strains by grouping them in clades with sequences of annotated strains of the genera *Ophiostoma* and using a known outlier group. *Leptographium* spp. are distantly related to *Penicillium* spp. (Order Eurotiales) which have been established as a rich source of SMs, especially antibiotics. It was expected that *Leptographium* strains produce a variety of SMs and that they my produce different compounds compared to members of the genus *Penicillium.* Members of the genus Leptographium could offer a promising mining ground for potential bioactive compounds. However, this study is not conclusive as far as characterizing new antimicrobial compounds is concerned. To achieve this, future experiments can be specifically designed towards induction of SM producing biosynthetic gene clusters (BGCs) in the fungi through various chemical/environmental stress cue-based stimulation. The production of SMs is dependent upon the expression of a variety of genes that can be induced by various environmental factors. Phenotypic AST methods can be complemented with genotypic and computational methods that might increase the chance of detection of novel antimicrobial compounds. Overall, the discovery of new antibiotics from fungi can provide alternative therapeutic options and bypass the concern of MDR that is rampant in the global population.

Chapter 1: Literature Review

1. An introduction to Antibiotics

1.1. A brief history of antimicrobial therapy

The use of antibiotics preceded the advent of modern medicine, and this has been exemplified by ancient documentations from different ages and different parts of the world. One such example is the use of bread with filamentous fungal growth for treatment of wounds in ancient Egypt (Pećanac *et al.*, 2013). Some interesting instances date back to the 19th century, with Sir John Scott Burden-Sanderson, of the absence of bacteria from a liquid culture with mold growth. In 1871, the potential inhibitory effect of *Penicillium glaucum* on bacterial growth was exploited by Joseph Lister to treat injuries. In 1877, Louis Pasteur and Jules Francois Joubert noted the bactericidal effect exhibited by some species of aerobic bacteria on *Bacillus anthracis* (Newsom, 2003). The term 'antibiosis' was coined in 1889 by Jean Paul Vuilleman who defined it as a biological relationship in which "one living organism kills another to ensure its own existence" (Amino, 2010). Thesis works of Ernest Duschene published in 1897 mentioned inhibition of *Escherichia coli* by *P. glaucum* (Lewis, 2013).

Incidentally, the first antimicrobial compounds discovered were synthetic chemical compounds. Paul Ehrlich (1897) hypothesized about therapy targeting structures exclusive to pathogens, and discovered arsephamine, an arsenic derivative active against the causative agent of syphilis, *Treponema pallidum*, which commercially came to be known as Salvarsan® in 1911, then as Mapharsen® and this continued to be the most prescribed drug till the discovery of penicillin. This discovery opened up the avenue of chemical modifications and screening to add to a library of antimicrobial compounds, laying the foundation of modern pharmaceutical research. (Strebhardt and Ullrich, 2008; Williams, 2009; Ferrie, 2014).

Salvarsan® was soon replaced with a more water-soluble derivative with less side-effects, called Neosalvarsan. Diverse structural variations in synthesis led to the synthesis of the first azo compounds. In 1930, the antibiotic effects of sulphanilamide was discovered by Gerhard Domagk, which facilitated the synthesis of Prontosil®, commercialized in 1935 and this paved the way for the sulpha drugs, which are still used today, a common one being sulfamethoxazole (Wainwright and Kristiansen, 2011). The greatest impact on antimicrobial discovery, came in the form of Alexander Flaming's 'accidental' detection of the growth inhibition of *Staphylococci* growth around mold colonies in Petri dishes. This led to the extensive study on the activity spectrum, potency, leukocyte interaction and toxicity of the active molecule from *Penicillium notatum* which was purified and called penicillin (Fleming, 1945; Ligon, 2004). However, clinical testing and industrial production of penicillin did not start till 1939, when Howard Florey, Norman Heatly and Ernst Chain developed a method for antibiotic production from *Penicillium chrysogenum* (Abraham and Chain, 1940; Bynum, 2018). In 1940, René Dubos isolated the oligopeptide gramicidin from *Bacillus brevis* which widely inhibited Gram-positive bacterial species, but it could only be used for local treatment due to highly toxic side-effects. Efforts continued for coming up with a fully chemical synthetic method for penicillin production, which was finally achieved in 1950 by John Sheehan, and the first synthetic natural penicillin V was made available in 1957 (Abraham and Chain, 1940; Bynum, 2018).

1.2. A timeline of the development of different platforms for antibiotic discovery

A systematic strategy was developed for research on antimicrobial activities of various organisms. A set of culture techniques using systemic agar overlay process was developed by Selman Waksman in the beginning of the 20th century (see timeline in Figure 1), called the 'Waksman platform' which led to the study of competitive growth allowing complex soil bacteria, actinomycetes to inhibit other bacteria (Woodruff, 2014). During this 'golden age', several major antibiotics and antifungals emerged from this platform (Table 1), including actinomycin (from *Streptomyces* spp.), streptomycin (from *Streptomyces griseus*), neomycin (from *Streptomyces fradiae*), fumigacin (from *Aspergillus fumigatus*) and clavacin (from *Aspergillus clavatus*). Streptomycin, for which Merck obtained The Food and Drug Administration (FDA) approval in 1946 revolutionized the treatment of tuberculosis (TB), tuberculosis meningitis and later, diseases caused by pathogens outside penicillin's spectrum of activity (Wainwright, 1991).

Table 1. Different classes of antibiotics discovered during the 'Golden Age'

Antibiotic class	Example of Antibiotic	Source	Principle of antimicrobial activity	Reference
Aminoglycoside	Streptomycin	*Streptomyces griseus*	Inhibit protein synthesis by binding to bacterial 30S ribosomal subunit	(Woodruff, 2014)
Amphenicol	Chloramphenicol	*Streptomyces venezuelae*	Inhibit protein synthesis by reversible binding to bacterial 50S ribosomal subunit	(Wiest *et al*., 2012)
Tetracycline	Chlorotetracycline	*Streptomyces aureofaciens*	Inhibit protein synthesis by binding to bacterial 30S ribosomal subunit	(Liu and Myers, 2016)
Macrolide	Erythromycin	*Saccharopolyspora erythrea*	Inhibit protein synthesis by reversible binding to bacterial 50S ribosomal subunit	(Cyphert *et al*., 2017)
Streptogramin	Virginiamycin	*Streptomyces virginiae*	Polyunsaturated macrolactone and cyclic hexadepsipeptide groups of antibiotic bind to 50S ribosomal subunit	(Mast and Wollheben, 2014)
Glycopeptide	Vancomycin	*Amycolatopsis orientalis*	Interfere with transpeptidation and transglycosylation steps of cell wall synthesis	(James *et al*., 2012)
Ansamycin	Rifamycin	*Amycolatopsis mediterranei*	Inhibit DNA-dependent RNA polymerase	(Floss and Yu, 2005)
Lipopeptide	Daptomycin	*Streptomyces roseosporus*	Interfere with cell wall synthesis and disrupt cell cycle	(Taylor and Palmer, 2016)

Then, semi-synthetic antibiotics started to be produced through the modification of existing scaffolds through a fermentative method which was crucial for commercial production, as it improved chemical stability and reduced toxic side-effects. This led to the development of dihydrostreptomycin through catalytic hydrogenation of streptomycin, enhancing chemical stability (Adeyemo *et al.,*2016). Semi-synthesis expanded penicillin from a single drug to a range of semi-synthetic derivatives such as penicillin G, forming the beta-lactams. A group of semi-synthetic cephalosporins, which forms another class of beta-lactams, was developed, giving rise to five generations of drugs, with the last generation coming out in 2013 (example: Ceftobiprole) (Adeyemo *et al.,* 2016; Gröger *et al*., 2017).

This led to the age of fully synthetic antibiotics, though the first completely synthetic antibiotic chloramphenicol, was already introduced back in 1949 (see timeline in Figure 1). The quinolone class, discovered as a by-product of the synthesis of antimalarial compound chloroquine, was an important scaffold in the synthesis of nalidixic acid, followed by three more generations, with fluoroquinolones being made by chemical modifications. The development of fully synthetic beta-lactams was crucial, as they led to carbapenems, with a similar core structure to penicillin, but with improved potency, spectrum of activity and better resistance to beta-lactamases (Bisacchi, 2015; El-Gamal *et al*., 2017).

Next generation sequencing in part lead to the modern era of antibiotic discovery which relies on target-based screening through omics approach. During the genomics era (1995-2004), over 200 microbial genomes were discovered. The basic principle for identification involved using a pipeline for sequencing genomes of relevant organisms, compiling target list and predicting druggability, identifying the active molecule and optimization. This led to improvement of activity, spectrum, pharmacokinetics, toxicology and pharmacodynamics (Mills, 2006; Land et al., 2015;

Ribeiro de Cunha *et al.,* 2019). On the other hand, reverse genomics use a strategy of first screening microbial cells by conducting phenotypic and cytotoxic screening of lead compounds with antimicrobial activity. This was followed by applying genomic, biochemical or molecular biology tools to identify the target and finally optimization (Foulkes, 2002). The post-genomics era implemented information from diverse origins into target identification, such as from transcripts (transcriptomics), proteins (proteomics) and lipids (lipidomics). Initial transcriptomic technologies were hybridization-based, such as Northern blot and microarrays, followed by RNA-seq and whole-genome expression profiling, which elucidated molecular and cellular processes to antibiotic stresses (Croucher and Thomson, 2010; McGettigan, 2013). Proteomic studies initially utilized 2D gel-based assays which were soon replaced by chromatography and mass spectrometry, which opened the avenue for discovery of novel antimicrobial targets (Carneiro et al., 2016). Lipids take part in a variety of biological events, such as signaling, trafficking and metabolic processes and application of lipidomics to characterization of pathogenic microbe's cell wall, revealing essential enzymes against which inhibitors can be developed (Sandra and Sandra, 2013; Vihervaara et al., 2014).

Most recently, meta-omics have facilitated natural product discovery, including antibiotics. Metabolomics provide a view of microbial metabolism, through complex analytical methods such as NMR and chromatography/mass spectrometry, alongside data analysis algorithms. Metagenomics identifies sequences of interest from microorganisms 'uncultivable' in laboratory conditions, for cloning and expression in laboratory-friendly microbes (Milshteyn *et al.,* 2014). Human-associated metagenomic studies reveal biosynthetic gene clusters with antibiotic potential. These approaches can contribute to the design of novel therapeutics, which is crucial in current

times with a pressing demand of new antibiotics (Lindon and Nicholson, 2006; Gao and Xu, 2015; Nagana Gowda and Raftery, 2015).

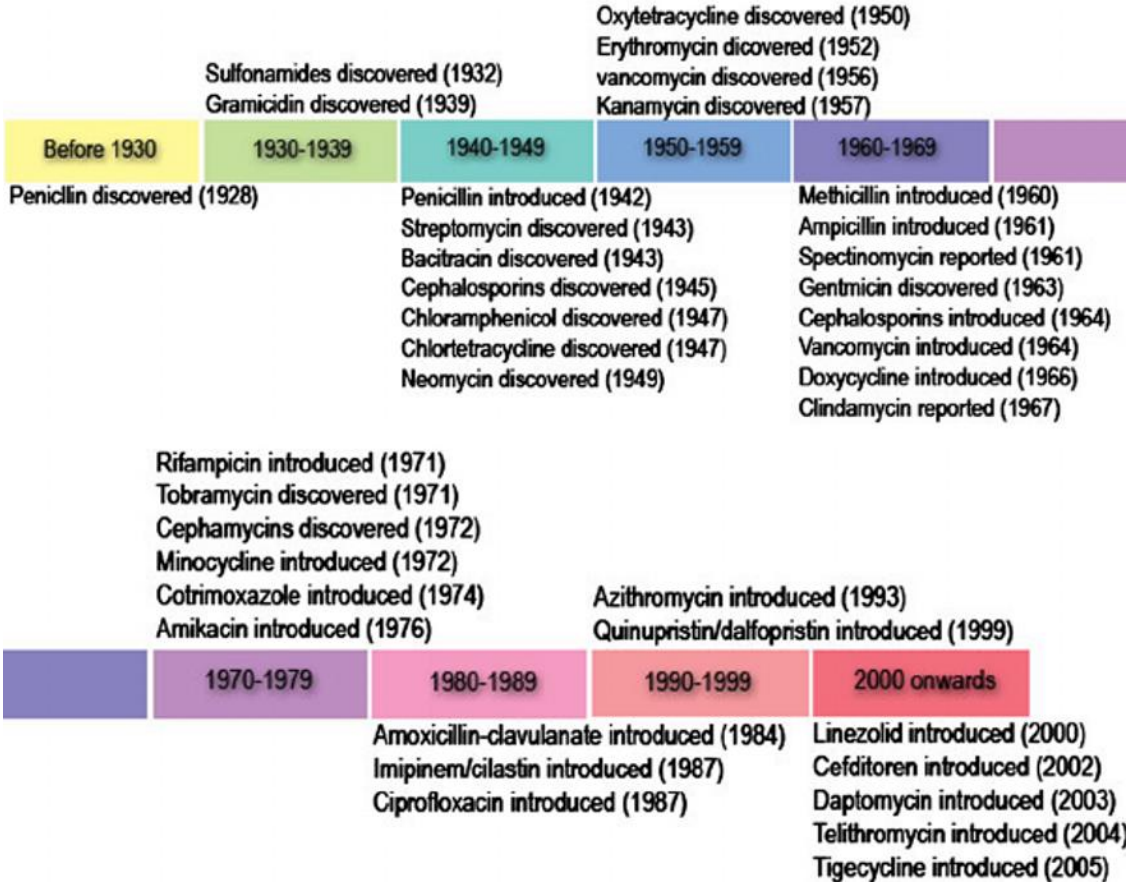

Figure1: Golden age of antibiotic/antibacterial agent discovery (Adopted from cvm.msu.edu, 2011). (Bbosa, *et al.*, 2014). By author permission.

2. Antimicrobial Resistance (AMR) Crisis

The development of antibiotic resistance was observed from the very early days of antibiotic discovery, dating back to the discovery of penicillin (Ventola, 2015; Michael *et al.*, 2014). The emergence and spread of MDR bacteria on a global scale, continues to threaten the effectiveness of the currently used antibiotics, and this in turn, poses danger to public health (Rossolini *et al.*, 2014). The World Health Organization (WHO) launched the Global Action Plan in 2015 to reduce the misuse of antimicrobials by using effective, fast, and affordable diagnostic strategies to optimize the use of antimicrobial compounds (WHO, 2014) (Table 2).

There are five main mechanisms by which bacteria can resist the effects of antibiotics: i) modification of target site, modification or destruction of the antibiotic, ii) antibiotic efflux (efflux transporters), iii) reduced antibiotic influx through decreased membrane permeability, iv) prevention to reach the antibiotic target (by decreasing penetration or actively extruding the antimicrobial compound), and v) changes and/or bypass target sites.

2.1. Intrinsic resistance

Intrinsic resistance refers to the existence of genes in bacterial genomes that result in a resistance phenotype, i.e., proto- or quasi-resistance (Hollenbeck and Rice, 2012). A comprehensive survey of Keio *E. coli* mutant library identified 140 distinct isolates that were hypersensitive to a wide range of antibiotic classes (Tamae, 2008). Some antimicrobial compounds are unable to cross the outer membrane, which is an example of intrinsic resistance of Gram-negative bacteria antibiotics. For example, in Gram-positive bacteria, vancomycin inhibits peptidoglycan cross-linkage, whereas, in Gram-negative bacteria, it cannot pass the outer membrane. Mutations can cause changes in crucial metabolic pathways by targeting of regulatory networks. An example is, fluoroquinolone (FQ) resistance can occur through mutation of genes

encoding their target site (DNA gyrase, topoisomerase) (Munita and Arias, 2016). Modifications of the antibiotic molecule can occur due to chemical alteration, such as acetylation, phosphorylation and adenylation (example, in aminoglycosides). Aminoglycoside modifying enzymes (AMEs) can lead to the covalent modification of hydroxyl groups of aminoglycoside which is the chief mechanism of aminoglycoside resistance (Ramirez and Tolmasky, 2010). Beta-lactam resistance mechanism relies on the destruction of these compounds by the enzymes that break the amide bond of the beta-lactam ring.

2.2. Extrinsic or acquired resistance

In case of extrinsic resistance mechanism, bacteria acquire resistance genes from other bacteria which already have resistance to harsh environmental conditions, through mobile gene transfer, horizontal gene transfer and recombination. An example of such genetic exchange facilitated by a mobile genetic element, is seen in methicillin-resistance which is governed by the *mcr-1* gene identified on a transmissible plasmid. Thereafter, *mcr-1* variants have been identified as the backbone of multiple plasmids found in various host strains (Liu XQ, 2016).

Acquired multi-drug resistance (MDR) infections have increased exponentially due to production of extended spectrum of beta-lactamases (CTX-M), enzymes, carbapenemases and metallo-beta-lactamases, leading to a third-generation cephalosporin and carbapenem resistance (Blair JM, 2015). The beta-lactamase genes are ancient and have been found in remote and desolate environments, which implies that novel beta-lactamases with altered substrate ranges occur in the environment (Allen, 2009). The CTX-M genes are highly successful at transmission (via mobile elements and conjugation) and rapid mutational radiation, which are hard to control. Resistance to macrolides, which are widely used against Gram-positive infections, can occur by modification of

the RNA or protein components of the peptide exit tunnel of the 50S ribosomal subunit, to which they are known to bind (Long, 2006).

Many antibiotics used clinically have intracellular targets, or in case of Gram-negative bacteria, targets located in the inner membrane. Hydrophilic molecules like beta-lactams and FQs are affected by changes in membrane permeability, as they use porins to cross the barrier (Hooper, 2002). Gram-negative bacteria have developed mechanisms to prevent antibiotic passage, as exemplified by alteration of porins in *E. coli* by changing expression level of porin genes such as *ompF*, *ompC* and *phoE*. Efflux-mediated resistance has been noted for tetracycline, where the Tet efflux pumps (of major facilitator superfamily) extrude tetracyclines using proton exchange as source of energy (Nikaido, 2003). Changes in target sites can occur through enzymatic modification, as seen for macrolide resistance, caused by the methylation of the ribosome catalyzed by an enzyme encoded by the *erm* genes (Weisblum, 1995; Roberts, 2008). Complete replacement or bypass of the target site has been seen in MRSA which evolves new targets for biochemical functions (Chambers, 2009; Moellering, 2012). Lastly, global cell adaptation has been achieved by some pathogens such as VRSA, to prevent attack by the host immune system or rival organisms (Sievert *et al.*, 2008).

Table 2: Resistance mechanisms of commonly used antibiotics and resistance threat

Antibiotic	Antibiotic class	Resistance mechanism	Resistance threat (CDC assessment, 2019)	References
Carbapenem	Beta-lactam	Hydrolysis, efflux, altered target	Urgent threat; example, Carbapenem-resistant Enterobacteriaceae (CRE)	(Queenan and Bush, 2007)
Methicillin	Beta-lactam	Hydrolysis, efflux, altered target	Serious threat; example, Methicillin-resistant *Staphylococcus aureus* (MRSA)	(Chambers and Deleo, 2009)
Vancomycin	Glycopeptide	Reprogramming peptidoglycan biosynthesis	Serious threat; example, Vancomycin-resistant Enterococci (VRE)	(Courvalin, 2006)
			Concerning threat; example, Vancomycin-resistant *Staphylococcus aureus* (VRSA)	
Erythromycin	Macrolide	Nucleotidylation, efflux, altered target	Concerning threat; example, Erythromycin-resistant Group A *Streptococcus*	(Weisblum, 1995)
Cefotaxime	Extended spectrum beta-lactamase	Hydrolysis, efflux, altered target	Serious threat; example, Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL)	(Fani *et al.*, 2013)

3. Strategies for novel antibiotic discovery and susceptibility testing

As stated previously a traditional strategy for detection of antibiotics comes from the Waksman platform which is a culture-based approach (Waksman, 1947). Several bioassays such as disc-diffusion, well diffusion and broth/agar dilution are still commonly used. AST can be used for drug discovery, epidemiology and prediction of therapeutic outcome (Jorgensen and Ferraro, 2009). Conventional bioassays can be complemented with flow cytofluorometry, thin-layer chromatography and mass spectrometry, which allow quantitative detection of antimicrobials (Balouiri et al., 2016). In current times, automated systems have been developed which include robust and quantitative high-throughput screening methods for antibiotic production in bacterial libraries (Khan *et al*., 2019). Machine learning approach can also lead to discovery of novel antibiotics, besides identifying antimicrobial peptides in available protein databases using pattern recognition-like systems (Fuente-Nunez, 2019).

Whole genome analyses show that many genes participating in synthesis of "unknown" SMs are not easily detected since they are not often expressed under conventional culture conditions. So, the key is to optimize the culture conditions such that the silent genes can be activated in these microbes. In their natural habitats, microorganisms form various associations with other microorganisms cohabiting with them. Interactions between these microorganisms are not replicated in laboratory-based pure cultures and hence co-cultivation of such microbes can help in discovering new antimicrobial compounds (Ueda and Beppu, 2017).

To identify novel antibiotics, the first critical step is to eliminate known antibiotics in natural product extracts through dereplication. With no new antibiotics on the horizon, efforts to preserve existing ones by blocking resistance with antibiotic adjuvants have emerged as a viable

strategy. In this scenario, antibiotics are co-formulated with an inhibitor of resistance. The inhibitor blocks the resistance element, freeing the antibiotic to target the bacterium. This strategy offers an orthogonal mechanism to new antibiotic discovery in preserving our existing drug arsenal (El-Elimat *et al.*, 2013).

3.1. Culture-based discovery and Phenotypic AST methods

Culture-based screening for antibiotic discovery can be carried out in solid or liquid culture. The former involves mainly three approaches: cross-streak, spot-on-the-lawn and well diffusion, based on the method of inoculation of the test bacteria on the media containing the indicator strain. In liquid broth co-culture, simultaneous culture of test species and indicator strain is allowed, separated by a filter, allowing diffusion of nutrients, but not cells (Durand et al., 2019).

Agar disc-diffusion testing, developed in the 1940s, continues to be the routine method used in many clinical microbiology laboratories for routine AST (Waksman, 1947; Williston *et al.*, 1947). For this, agar plates are inoculated with standardized inoculum of test organisms and filter paper discs containing test compound at a desired concentration are placed on agar surface. Upon incubation, antimicrobial agent diffuses into agar and inhibits the germination and growth of test microorganism and then diameters of inhibition growth zones are measured (Caron, 2012). In agar well-diffusion, a hole is punched into the agar surface with microbial inoculum, and an antimicrobial agent is introduced into the well and the inhibitory effect is measured as before (Magaldi *et al.*, 2004). A variation of this method is the agar plug diffusion, in which an agar-plot is cut from a plate having a culture of the strain of interest and deposited on the surface of another plate, already inoculated by test organism. Zone of inhibition around the agar plug is then correlated to antimicrobial activity, as before (Jiménez-Esquilín and Roane, 2005).

Screening of organic extracts can be carried out by TLC-bioautography, which allows localization of active constituents of a complex mixture on a TLC plate. This is a rapid technique for screening a large number of samples for bioactivity and in bioactivity-guided fractionation. Minimum inhibitory concentration (MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of an organism) of the antibiotic can be measured using dilution methods, such as broth dilution and agar dilution. Flow cytofluorometric method relies on the principle of detection and quantification of damaged cells when treated with antimicrobial compounds, using a DNA stain, propidium iodide. Mass-spectrometry is a sensitive method for evaluation of susceptible and resistant bacteria through spectral peak analysis.

3.2. Genome mining and genotypic AST

Secondary metabolites are encoded by biosynthetic gene clusters (BGC), which potentially encode unknown molecules, many of which may have antimicrobial activity. The most commonly used mining approaches are sequence-based, ecology-based, mode-of-action-based and function-based. Putative bacteriocins are encoded by the BGCs, an example being lichenicidin, from *Bacillus licheniformis* (Begley *et al.,* 2009). Molecular or genotypic AST are effective direct technique that overcome the issue of tedious bacterial cultures, long incubation, of contamination, and transmission of diseases. Some genotypic techniques used for detection of antibiotic resistance are PCR, DNA microarray, DNA chips and loop-mediated isothermal amplification (LAMP) (Li *et al.,* 2017).

3.3. Co-cultivation as a strategy for mining of novel antimicrobials

Sonnenbichler *et al*. (1994) researched the interactions between fungal strains and observed that the growth of two Basidiomycetes, *Heterobasidion annosum* and *Gloeophyllum abietinum*, proceeds in an antagonistic manner, the antimicrobial substances specifically produced in the coculture of the two fungal organisms were investigated. The result showed that the concentrations of oosponol and oospoglycol, the metabolites produced by *G. abietinum*, were remarkably elevated in coculture and induced the synthesis of fomannosin in *H. annosum*. This effect represents the interactive mode in which the production of a secondary metabolite in one organism takes place after sensing of a secondary metabolite produced by another organism (Sonnenbichler *et al.*, 1994). Another analysis of the interactions involving *Streptomyces* strains was performed by Onaka *et al*. (2011) who discovered that cocultivation with mycolic-acid-containing bacteria represented by Tsukamurella causes marked stimulation of antibiotic production in various *Streptomyces* strains. Burgess *et al*. (1999) studied the antagonistic response of marine bacteria to the challenge with terrestrial bacteria. Epibiotic bacterial strains isolated from the surface of seaweed were tested for their ability to produce antibiotics in response to the presence of living cells of pathogenic bacteria such as *Staphylococcus aureus* or *Pseudomonas aeruginosa*. They found that a high proportion of marine bacterial isolates produces a higher amount of antibiotics if they are cultured in the presence of living cells of pathogenic bacteria (Burgess *et al.,* 1999). The evidence implied that the cocultivation is a promising strategy for drug mining.

Some bacteria are not amenable to culture under laboratory conditions. So, their growth and isolation for experimental purposes becomes difficult. A device called Isolation chip (iChip) has been introduced that can overcome this problem. Bacteria that are usually not possible to grow

in laboratory-based cultures are instead grown in their natural habitat using a chip containing small pore-like chambers in which they are trapped and incubated. The natural growth conditions are suitable for growth of these organisms and the bacteria grown initially in chambers can be isolated for further laboratory-based experiments. This allows the possibility of exploring these almost unculturable bacteria for production of any novel antimicrobial compounds. Using these kinds of devices, a new class of antibiotics has been discovered called Teixobactin from soil bacteria; these were found to be effective against gram-positive bacteria and mycobacteria and their mechanism of action involves inhibition of bacterial peptidoglycan biosynthesis (Piddock, 2015). A variation of the iChip has also been designed with modified pore size (0.03 μ), which facilitates the process of co-culture of microbes residing in adjacent chambers in the device (Lodhi *et al.,* 2018). Combining iChip and co-culture techniques is a promising approach to discover microbial diversity and study SM production in these organisms that can prove to be of therapeutic use to counter pathogens (Lodhi *et al.,* 2018).

3.4. Computational analysis for antibiotic discovery and other emerging methods to overcome dereplication

Some recent methods for the detection of antibiotic resistance and antimicrobial activity include microfluidics, optical imaging, fluorescence tagging and bioluminescence assays. Recently, a method for screening antibiotics in bacteria libraries has been described, which screened over 260 bacterial species in monoculture, of which 38% and 34% were found to produce antibiotics capable of inhibition of *S. aureus* or *E. coli*. This method involved plating bacteria in 96-well plates, centrifugation and filtration, treating the filtrate with pathogenic bacteria (*S. aureus* or *E. coli*) and assessing pathogenic inhibition by optical density (Murray *et al.,* 2019). Chemical

and (bio)sensors for detection of antibiotics and assays coupling detection with screen-printed electrodes with immunomagnetic separation, have been described (Munteanu *et al.*, 2018).

A common antibiotic resistance platform (ARP) has been developed that can be used for both adjuvant discovery and antibiotic dereplication. The ARP is a rigorously curated library of mechanistically distinct antibiotic resistance genes present in an identical genetic background. The ARP can be utilized in cell-based screens of chemical libraries for the discovery of antibiotic adjuvants. The advantage of the varying MICs associated with the ARP is critical in selecting a strain with an appropriate screening window, where resistance is not overwhelming and thereby obscuring low abundance or weak inhibitors. Using a strain within the ARP a natural product that has been recently discovered, is capable of restoring carbapenem activity against multidrug-resistant (MDR) strains overexpressing metallo-b-lactamases. This approach can be easily applied to other classes of antibiotic; the ARP has been constructed to include numerous candidate resistance determinants that will be utilized in future screening efforts (Cox *et al.*, 2017). This approach can be easily applied to other classes of antibiotic; the ARP has been constructed to include numerous candidate resistance determinants that will be utilized in future screening efforts (King *et al.*, 2014).

4. Biosynthetic gene clusters for production of fungal secondary metabolites

The diversity of fungal species, particularly in the Ascomycota and Basidiomycota, and the accompanying diversification of biosynthetic genes and gene clusters indicates a huge potential for metabolic variation (Keller *et al.,* 2005; Bills and Gloer, 2016). Filamentous fungi produce a plethora of SMs which are low molecular weight compounds with a wide spectrum of biological activities, which are dichotomous in the sense that some are beneficial and some have detrimental effects (Watve *et al.,* 2001). Biosynthetic gene clusters (BGCs) are genomic segments that encode a set of proteins collectively responsible for the biosynthesis of antibiotics (Medema *et al.,* 2015). Not all of these SMs are produced under standard cultivation conditions, hence, to explore the potential of the strains producing these compounds often require the induction of BGCs which may be achieved by stress or manipulation of culture conditions and composition of the growth media. Altering conditions for culture of the fungal strains can trigger SM pathways (Ziemert *et al.,* 2016). While not directly involved in fundamental metabolic processes of growth and energy production, SMs exhibit bioactivities that contribute to the survival of the organism in an occupied ecological niche. Many of these SMs with bioactivities are considered for drug development (Chiang, 2011; Brakhage and Schroeckh, 211) (Table 3).

Formation of a SM proceeds under specific conditions, hence it is typical for a fungal strain to reveal only a fraction of its chemical diversity under a particular set of environmental cues (Brakhage, 2013). Both the biochemical potential of the producer itself and the encountered environmental cues determine whether a certain SM will be produced or not. Many BGCs remain silent under standard laboratory conditions and, as a consequence, the corresponding SMs may not be formed. Thus, induction of the BGCs may be required to achieve SM yield, which is challenging

since the search for potential SM producing fungal strains is complicated by search for suitable methods for such induction (Chavez *et al.,* 2015; Brakhage, 2013).

The two main strategies for triggering SM production are genetic manipulations to SM pathways and cultivation-based methods that recreate specific environmental cues. Genetic manipulation maybe in the form of heterologous expression of the BGC of interest in different compatible hosts (example, in the model organism yeast) (Houbraken *et al.*, 2011). Mimicking environmental cues, on the other hand, involve manipulation of growth media composition, physical parameters or cultivation strategies, as these signals influence fungal cells on multiple levels, including regulatory, signaling and metabolic pathways, developmental processes, morphology, adaptation and stress response. One common approach to induce secondary metabolism is to subject the cells to stress, like oxidative or osmotic which can trigger a plethora of molecular defensive mechanisms that involve SM production. Genetic engineering and cultivation-based methods can be used in combination to activate BGCs (Medema *et al.*,2015).

Table3: Examples of fungal SMs with potential biological activities

Secondary metabolite	Fungal species	Application	Reference
Penicillin G	*Penicillium rubens*	Antibiotic	(Pohl *et al.*, 2020)
Cephalosporin C	*Acremonium chrysogenum*	Antibiotic	(DeModena *et al.*, 1993)
Griseofulvin	*Penicillium griseofulvum*	Antifungal agent	(Zhang, 2017)
Taxol	*Taxomyces andreanae*	Anticancer drug	(Stierle *et al.*, 1993)
Cyclosporine A	*Tolypocladium inflatum*	Immunosuppresant	(Bushley *et al.*, 2013)
Mycophenolic acid	*Penicillium* sp.	Immunosuppresant	(Patel *et al.*, 2016)
Lovostatin	*Aspergillus terreus*	Cholesterol-lowering drug	(Boruta and Bizukogc, 2017)
Astaxanthin	*Phaffia rhodozyma*	Pigment	(Sedmak *et al.*, 1990)

5. Fungi as an emerging source of antibiotics

Fungi contain diverse biosynthetic pathways leading to the production of different metabolites, from beneficial antimicrobials to harmful mycotoxins. Surprisingly, the building blocks for the different pathways involved in synthesising fungal metabolites are quite limited as illustrated in (Figure 2) The three most commonly studied metabolic pathways are (1) the mevalonic acid pathway (synthesize terpenoids, steroids, etc), (2) the shikimic acid pathway (synthesize aromatic amino acids, alkaloids, etc), and (3) the acetate pathway (synthesize polyketides, fatty acids, etc) (Goyal *et al.,* 2016).

Antibiotics are widely distributed in nature and the most common sources include microorganisms (including fungi) belonging to the fungal genera *Penicillium* and *Acremonium*, and bacteria belonging to *Streptomyces*, *Micromonospora* and *Bacillus* (Walsh, 2003). The Gram-positive bacteria, *Streptomyces* which superficially resemble filamentous fungi are a chief source of many commercially important antibiotics such as erythromycin, streptomycin, tetracycline and vancomycin (Watve *et al*., 2001). Even though the earliest antibiotics which were successfully used to treat infections were penicillin drugs produced from molds, most of the naturally synthesized antibiotics come from bacteria. Cephalosporin produced by *Acremonium chrysogenum* (synonym = *Cephalosporium chrysogenum*) Thirum. & Sukapure, Mycologia 55: 565 (1963) [MB#327576] is an example of a fungal-produced antibiotic (DeModena *et al.,*1993). However, recently fungi have been established as an important “mining ground” for antibiotics, especially, with the discovery of bacteria and fungi from extreme habitats like the deep sea or the hot spring (Fair *et al.,* 2014).

Filamentous fungi produce a wide range of bioactive compounds with important pharmaceutical applications, such as antibiotic penicillins and cholesterol-lowering statins.

Recently in a study, nine *Penicillium* species, together with fifteen published genomes were sequenced, which led to the identification of 1,317 BGCs with immense potential for producing secondary metabolites. The grouping of the BGCs allowed the study of evolutionary trajectory of pathways based on 6-methylsalicylic acid (6-MSA) synthases and enabled the prediction of yanuthone (a polyeketide with antimicrobial potential) production in *P. rubens* and *P. flavigenum* (Nielsen *et al.*, 2017). These genomic studies show that species of *Penicillium* have the potential to produce far more compounds compared to what has so far been chemically detected; suggesting that many genes might be cryptic and are expressed only under certain conditions. With the advent of synthetic biology, the long-term strategy is to move components of BGC (genes or gene clusters) into other microorganism more amendable to genetic manipulation. This may reveal a whole range of novel compounds that could have pharmaceutical applications.

Currently there is great interest to examine fungi previously ignored as sources for antimicrobials. For examples fungal strains from marine habitats have been reported to have potential antimicrobial effects (Imhoff *et al.*, 2018). An example is *Engyodontium album*, produced polyketides engyodontochones A-F, which exhibited effects against MRSA and *S. epidermis*. *Talaromyces* sp. isolated from the Mediterranean Sea is the producer of the two oxaphenalenone dimers, talaromycesone A and B, which are also active against MRSA (Imhoff *et al.*, 2018). Antifungal and antibacterial prenylxanthones were identified from deep-sea fungus *Emericella* sp. isolated in South China sea and these were able to inhibit *E. coli*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Aeromonas hydrophila*. (Tortorella *et al.*, 2018). A tetramic acid derivative Ophiosetin was isolated from the mycopathogenic fungus *Elaphocordyceps ophioglossoides* HF272 from a soil specimen collected at the Tsuchiyu Hot Spring in Fukushima, Japan, which was found to have weak antibacterial activity (Mahajan and

Balachandran, 2017). All these studies show that bioprospecting for fungi from extreme habitats or environments previously ignored can lead to the discovery of useful secondary metabolites.

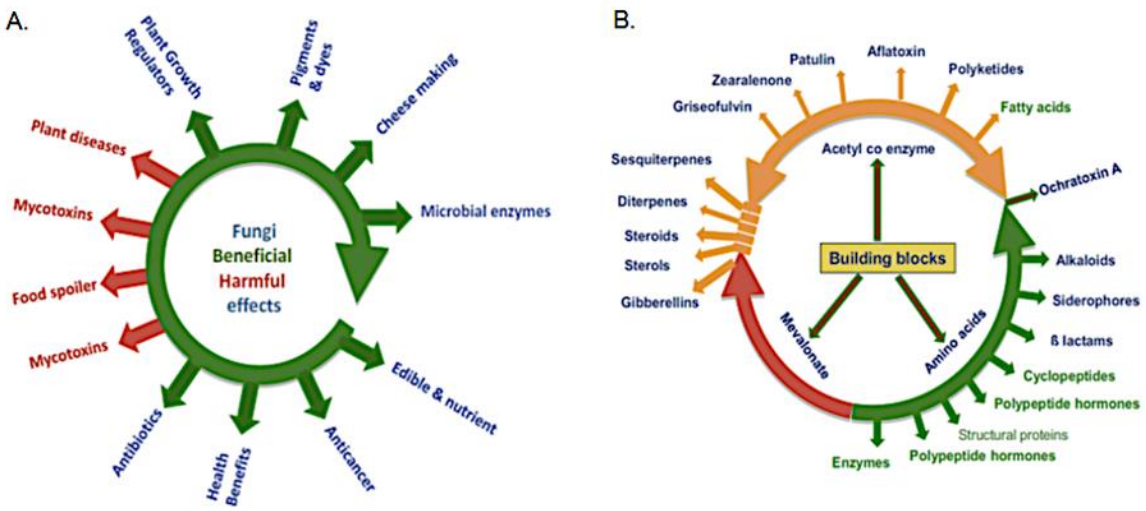

Figure 2: Beneficial and deleterious effects of fungi due to production of various primary and secondary metabolites (A) *Blue* represents the beneficial compounds/effects and *red* represents harmful compounds/effects produced by fungi, (B) *Green* represents the primary metabolites and *blue* represents the secondary metabolites produced from the building blocks/photosynthates from chief metabolic pathways in fungi. *Adapted from* (Goyal *et al.*, 2016). By author permission.

6. Fungal species belonging to the Ophiostomatales: a focus on *Leptographium* spp.

Phytopathogenic fungi belonging to Ophiostomatales, are frequently associates of bark beetles and in some instances contribute to beetle-associated mortality of trees. Mountain pine beetle (MPB) outbreaks in Canada are associated with MPB vectored fungi such as *Grosmannia clavigera, Leptographium longiclavatum* and *Ophiostoma montium.* (Rice *et al.*, 2008). Many species of the Ophiostomatales cause blue stain in timber, and some are serious tree pathogens, such as the causative agents of Dutch elm disease (members of the *Ophiostoma ulmi* species complex). The blue stain or sapstain is a result of excessive melanin secretion by fungi, such as *Ophiostoma, Ceratocystis, Leptographium* or *Sphaeropsis* species that utilize simple carbohydrates, fatty acids, triglycerides and other components of the sapwood (Blanchette *et al*., 1992). Sapstain fungi are not thought to affect the strength properties of wood in early stages of colonization, although discoloration decreases the economic value of wood used for lumber or paper production (Wang *et al*., 1995). Stained lumber is also difficult to export due to concerns of exporting/importing fungal pathogens.

The Order Ophiostomatales includes two Families, Kathistaceae and the Ophiostomataceae. The latter includes the following genera: *Aureovirgo, Ceratocystiopsis, Fragosphaeria, Graphilbum, Hawksworthiomyces, Raffaelea sensu stricto, Ophiostoma sensu stricto* and *Sporothrix* (Hyde *et al.*, 2020); in addition, the genera *Ophiostoma sensu lato, Esteya*, *Leptographium* and *Grosmannia* are also treated as members of the Ophiostomataceae (De Beer *et al.*, 2013a and 2013 b; 2016). Overall, the taxonomy of this order is complex and has been controversial, with some lineages (e.g., *Leptographium*, *Ophiostoma sensu lato*) still unresolved with regards to their monophyly and status within this order. Previously *Grosmannia* has been

used synonymously with the genus *Ophiostoma* but was later established as an independent genus depending on data analysis from DNA sequences from multiple loci in their genomes (Zipfel *et al*., 2006). *Leptographium* initially consisted of asexual forms of *Grosmannia* species, whose salient phenotypic features included dematiaceous, erect conidiophores (synematous or mononematous) that ended in penicillated branches leading to conidiogenous (holoblastic) cells. These cells produce conidia that are single celled and are found in drops of mucilage (Wingfield, 2000; Marin-Felix *et al.,* 2017). A more modern nomenclature system now allows for the inclusion of sexual forms within *Leptographium* genus. Hence, some *Leptographium* species are now described with a sexual state recognized by their globose ascomycota with elongated necks. These fungi produce sticky spores on long stalks that allows their dispersal by insects. Species of *Leptographium* have been known to be mainly associated with conifer-infesting bark beetles but there are also a few species known to be associated with non-conifer hosts (Robert *et al.*, 2018).

In studies conducted to determine which pathogenic fungal species were associated with *Tomicus* beetles, *L. wingfieldii*, a then presumed new introduction into North America, *L. procerum*, and *L. lundbergii* were found to be the dominant ones, in the samples collected from Ontario. Since variable morphological features could not help in distinguishing between *L. truncatum* and *L. lundbergii*, which were previously considered to be synonymous, ITS rDNA results were taken into consideration. However, there were conflicting results obtained on comparing ITS sequence data of *L. lundbergii* from Ontario versus that from Sweden and Norway, with results from ten isolates grouping them in the same clade, while seven other isolates showing that they belonged to separate clades. Moreover, high morphological variability was also encountered among the *L. wingfieldii* strains which indicated that they may not have been a newly introduced exotic pathogen, as previously assumed, but were simply a more virulent strain of a

previously introduced *L. wingfieldii* which was synonymous with *L. terebrantis*. Overall, though these studies established these three *Leptographium* species as the chief fungi associated with *Tomicus sp.*, the ITS profiling and morphological studies did not clearly define the relationships between these species, (Hausner *et al.*, 2003; Jacobs *et al.*, 2004; Hausner *et al.*, 2005).

More recent studies have shown that the genus *Leptographium* is probably quite large and accommodates many species found in Europe, Asia and North America. *Leptgraphium* spp. And Tomicus beetles are found all over Europe where they were described first – more recently they have been studied all over Asia. *Leptographium* species can be associates of various insect species and they can be recovered from both hard and soft wood species. They were described first in Europe, in association with various bark beetles such as members of genus *Tomicus*, however, only recently, they have been discovered through some studies in Asia. For example, *Tomicus* beetles have been noted to aggressively infest the Yunnan pine in South West China, aided by their virulent fungal associates. For example, two species of *Leptographium* (*Leptographium wushanense* and *L. sinense*) were observed to induce long necrotic lesions in the pinewood, eliciting strong defense responses in the form of antioxidant enzyme activities and monoterpene production (Pan, 2018). Recently, morphological studies and phylogenetic approaches on multilocus DNA sequence data (ITS2- partial r28S, partial β-tubulin, and EF-1α gene regions) revealed three *Leptographium* species (*Leptographium taigense*, *L. innermongolicum* sp. nov. and *L. zhangii* sp. nov.) occur in association with the larch bark beetle, *Ips subelongatus* that infest *Larix* spp. in Northern China (Liu, 2017).

Compared to investigations on coniferous trees, the occurrence of *Leptographium* species on hardwood trees has been poorly studied in Europe (and other parts of the world). During a

survey on fungi belonging to the Ophiostomatales, on various hardwood tree species in Norway and Poland, three unusual species, which fit in the broader morphological description of *Leptographium* spp., were found in association with *Trypodendron domesticum*, *Trypodendron signatum* and *Dryocoetes alni*, and from wounds on a variety of hardwoods. Phylogenetic analyses of sequence data for six different loci (ITS1–5.8 S–ITS2, ITS2-LSU, ACT, β-tubulin, CAL, and TEF-1α) showed that these *Leptographium* species are phylogenetically closely related to the species of the *Grosmannia olivacea* complex. Based on novel morphological characters and distinct DNA sequences, these fungi were recognised as new taxa (*Leptographium tardum* sp. nov., *Leptographium vulnerum* sp. nov., and *Leptographium flavum* sp. nov.). All the new species produce perithecia with elongated necks terminating in ostiolar hyphae and orange-section shaped ascospores with cucullate, gelatinous sticky sheaths. These species also produce dark olivaceous mononematous asexual states in culture (Jankowiak *et al.*, 2018). These recent studies showed that many *Leptographium* species are yet to be described from non-conifer hosts (i.e. soft wood species) and these studies also demonstrated that several loci have to be investigated in order to resolve species of *Leptographium*.

The root and stump feeding beetle *Hylurgus ligniperda*, which is native to Europe, was discovered in the United States (Los Angeles, California, 2003) and was found to be associated with *Leptographium tereforme sp. nov.* along with other Ophiostomatales. These were isolated from the logs of *Pinus halepensis* and *P. pinea* at two sites in California (Kim S, 2011). This study illustrates that the movement of bark beetles (and their fungal associates) introduces fungi into new geographic regions, exposing plant species to potential new pathogens. In other cases, the introduced beetles may provide native fungi with more efficient vectors thus facilitation the spread of fungi to new plant hosts. The taxonomy, diversity and pathogenicity, fungus/beetle interactions

of both conifer and hard wood infesting *Leptographium* species will have to be further explored through extensive surveys to cover larger geographic areas including more habitats globally. Currently over 100 species of *Leptographium* have been described with many more to come in the future (Jankowiak *et al.*, 2018).

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Chapter 2: Materials and Methods

2.1. Fungal strains and maintenance of cultures

Fungal strains belonging to the order of the *Ophiostomatales* (Genera: *Ceratocystis, Ophiostoma, Grosmannia, Ceratocystiopsis,* and *Leptographium*) were stored at + 4°C on malt extract agar slants (MEA, 30 g malt extract (VWR life science, Ohio, USA), 1 g yeast extract (Bacto™, Dickinson & Co., Maryland, USA) and 20 g bacteriological agar (Bacto™ BD) per liter) slants. The collection is housed at the University of Manitoba (Winnipeg, MB, Canada). Strains of *Leptographium* spp. (strain numbers: WIN(M) 1200 to WIN(M) 1375) were selected for analysis (Table 4). From the selected stock cultures agar pieces containing fungal growth were removed with wooden sterile inoculation sticks (VWR) and used to inoculated MEA plates. The MEA cultures were incubated at 22ºC for 7 to 10 days. From these MEA plates five to six agar pieces with fungal growth were used to inoculate100 mL ME broth (30 g malt extract and 1 g of yeast extract (Bacto™ BD) per liter. Fungal strains used in this study were maintained on MEA slants and by sub-culturing on MEA plates.

Table 4: Fungal strains use in this study

Winnipeg, University of Manitoba [WIN(M)] accession number	Strain identification	Other Culture collection numbers
1202	*Leptographium sp.*	DAOM[1] 62744
1204	*Ophiostoma sagamatospora*	DAOM 212704
1205	*Leptographium sp.*	TOM[2] 58.10
1206	*Leptographium sp.*	TOM 56.27
1207	*Leptographium sp.*	TOM 56.15
1208	*Leptographium sp.*	TOM 62.53
1209	*Leptographium sp.*	TOM 59.21
1210	*Leptographium procerum*	TOM 62.30
1211	*Leptographium procerum*	TOM 55.35
1212	*Leptographium sp.*	TOM 64.19
1214	*Leptographium serpens*	DAOM 173 660
1215	*Leptographium sp.*	TOM 59. 38
1216	*Leptographium sp.*	TOM 59.40
1217	*Leptographium sp.*	TOM 64.39
1218	*Leptographium sp.*	TOM 64.40
1227	*Leptographium sp.*	TOM 18.25
1228	*Leptographium sp.*	TOM 66.19
1229	*Leptographium. wingfieldii*	TOM 66.24
1230	*Leptographium sp.*	TOM 66.31
1231	*Leptographium sp.*	TOM 67.31
1232	*Leptographium sp.*	TOM 67.34
1234	*Leptographium sp.*	TOM 68.11
1235	*Leptographium sp.*	TOM 68.21
1236	*Leptographium sp.*	TOM 68.29
1237	*Leptographium wingfieldii*	TOM 69.26
1238	*Leptographium sp.*	TOM 69.28
1239	*Leptographium wingfieldii*	TOM 70.18
1240	*Leptographium wingfieldii*	T0M 70.25
1241	*Leptographium sp.*	T0M 70.34

1242	*Leptographium sp.*	TOM 72.19
1243	*Leptographium sp.*	TOM 72.24
1244	*Leptographium procerum*	TOM 73.12
1245	*Leptographium procerum*	TOM 74.14
1246	*Leptographium wingfieldii*	TOM 74.29
1247	*Leptographium sp.*	TOM 75.3
1248	*Leptographium wingfieldii*	T0M 75.11
1249	*Leptographium sp.*	TOM 76.6
1250	*Leptographium procerum*	TOM 76.8
1253	*Leptographium procerum*	TOM 76.32
1254	*Leptographium procerum*	TOM 76.36
1255	*Leptographium sp.*	TOM 76.41.02
1256	*Leptographium sp.*	TOM 76.54
1257	*Leptographium sp.*	TOM 76.67.01
1258	*Leptographium sp.*	TOM 77.5
1259	*Leptographium wingfieldii*	TOM 77.7
1260	*Leptographium wingfieldii*	TOM 77.11
1261	*Leptographium sp.*	TOM 77.23
1262	*Leptographium sp.*	TOM 78.2
1263	*Leptographium procerum*	TOM 78.8
1264	*Leptographium procerum*	TOM 78.13
1265	*Leptographium sp.*	TOM 79 .17
1266	*Leptographium sp.*	TOM 78. 18.03
1268	*Leptographiuum wingfieldii*	TOM 78.22 04
1269	*Leptographium sp.*	TOM 78.29
1270	*Leptographiuum wingfieldii*	TOM 78.38
1271	*Leptographiuum wingfieldii*	TOM 79.34.1
1272	*Leptographium procerum*	TOM 86.19
1273	Unknown (putative *Leptographium* sp.)	TOM 86.23
1274	Unknown (putative *Leptographium* sp.)	TOM 86.30
1275	*Ophiostoma minus*	TOM 86.34
1276	*Leptographium wingfieldii*	TOM 80.12
1277	*Leptographium wingfieldii*	TOM 80.17

1278	*Leptographium wingfieldii*	TOM 80.21
1279	*Leptographium sp.*	TOM 80.27
1280	*Leptographium sp.*	TOM 80.30
1281	*Leptographium sp.*	TOM 80.2
1282	*Leptographium sp.*	TOM 80.4
1283	*Leptographium sp.*	TOM 80.8
1284	*Leptographium sp.*	TOM 80.16
1285	*Leptographium sp.*	TOM 80.23
1286	*Leptographium sp.*	TOM 81.1
1287	*Leptographium wingfieldii*	TOM 81.8
1288	*Leptographium sp.*	TOM 81.9
1290	*Leptographium sp.*	TOM 81.16
1291	*Leptographium sp.*	TOM 81.19
1293	*Leptographium sp.*	TOM 82.17
1294	*Leptographium sp.*	TOM 82.20
1295	*Leptographium sp.*	TOM 82.23
1296	*Leptographium sp.*	TOM 82.25
1297	*Leptographium sp.*	TOM 82.29
1298	*Leptographium sp.*	TOM 83.12
1300	*Leptographium sp.*	TOM 84.17
1301	*Leptographium sp.*	TOM 84.20
1302	*Leptographium wingfieldii*	TOM 84.24
1303	*Leptographium sp.*	TOM 84.22
1304	*Leptographium sp.*	TOM 84.23
1306	*Leptographium sp.*	TOM 84.29
1307	*Leptographium sp.*	TOM 84.33
1311	*Leptographium sp.*	TOM 85.25
1312	*Leptographium sp.*	TOM 85.12
1321	*Leptographium sp.*	TOM 88.23
1322	*Leptographium sp.*	TOM 88.23
1323	*Leptographium sp.*	TOM 90.9
1324	*Leptographium sp.*	TOM 90.11
1327	*Leptographium sp.*	TOM 90.20

1330	*Leptographium sp.*	TOM 90.27
1331	*Leptographium sp.*	TOM 91.18
1335	*Leptographium sp.*	TOM 93.9
1342	*Leptographium sp.*	TOM 95.2
1375	*Leptographium procerum*	JR[3] 88 - 490A

[1]DAOM = Canadian Collection of Fungal Cultures Ottawa Research and Development Centre K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ontario.

[2]TOM = *Tomicus piniperda* (common pine shoot beetle) collection (see Hausner *et al.*, 2005);

[3]JR = James Reid collection (incorporated into the WIN(M) collection);

2.2. Agar plug bioactivity assay

Six bacterial strains were used to test the presence of the antimicrobial activity in the fungal growth media/culture (Table 5). Bacterial strains were cultured on lysogeny broth (LB) agar (Bacto™ BD) plates (20 g/L) and incubated for 24 hours at 37°C. A single colony from the cultured bacteria was inoculated into 2 mL LB broth (Bacto™ BD) and incubated at 37ºC in a shaker incubator at 250 rpm overnight. Bacterial cells from the overnight culture were diluted using 0.85% sodium chloride (NaCl) (Fisher Scientific, Ottawa, ON, Canada) and 0.5 McFarland turbidity standard (Standard number 0.5, BioMerieux, Durham, NC, USA) was used to normalize the number of cells to a density of 10^8 CFU/mL. The normalized bacterial cells were spread on LB agar plates (35 g/L) (Bacto™ BD) using cotton swabs encouraging the formation of a bacterial lawn. A circular agar plug was cut from the MEA containing fungal growth using pipette tips (100-1250ul) (VWR, Philadelphia, USA) and placed on top of the bacterial lawn.

Table 5: Bacterial strains used in this study

Bacterial Strains	Strains ID	Description	References
Pseudomonas aeruginosa	PAO1	Wild type	(Holloway, 1955)
Pseudomonas aeruginosa	PA0750	Efflux sensitive strain containing deletions in *mexAB-oprM, mexCD-oprJ, mexEF-oprN, mexJK, mexXY,* and *opmH*	(Kumar *et al*., 2006)
Pseudomonas aeruginosa	PA082	MDR clinical isolate	(Singh *et al*., 2017)
Acinetobacter baummanii	AB030	MDR clinical isolate	(Fernando *et al*., 2013)
Acinetobacter baummanii	ATCC17978	Wild type	(Piechaud and Second, 1951)
Staphylococcus aureus	SA007	Methicillin-resistant	(Saravolatz *et al*., 1982)

MDR = multidrug resistant

ATCC = American type culture collection

2.3. Secondary metabolite extraction

Fungal strains that showed antimicrobial activity against the bacterial strains were inoculated in 100 mL ME broth and incubated for 7 days. After seven days of growth the media was filtered twice, first using vacuum filtration with 0.2 µm pore size Millipore filter (VWR, Edmonton, AB, Canada) followed by using a Nalgene Syringe Filter (0.45 µm nylon membrane) (Thermo Scientific). The spent media was acidified by adding 1 mL of hydrochloric acid (HCl) (Thermo Scientific) extracted twice using a 1:1 ratio of ethyl acetate to spent media in order to recover secondary metabolites. The mixture was passed through a 500 mL separator funnel Anhydrous sodium sulfate (Na_2SO4) (Fisher Scientific) was added to the organic phase (30 gm in 100 mL of organic phase). The organic phase was evaporated using a rotary evaporator (Rotavapor R-210, BUCHI, New Castle, USA) to obtain the crude extract. The crude extract was transferred into storage vial and stored at -4℃.

2.4. Crude extract bioactivity assay (Disc diffusion)

LB agar plate with a lawn of *Pseudomonas aeruginosa* strain PA0750 bacteria was prepared as described in the previous section on agar plug bioactivity assay. The crude extract was dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific) to a final concentration of 150 µg/mL; 10 µl (1.5 mg) of this suspension was added to a sterilized 10 mm filter disc (GE healthcare life sciences Whatman, USA) and incubated overnight at 37℃. After the incubation period, the zone of inhibition (if present) was measured (as diameter in mm).

2.5. Analyzing crude extracts using a high-performance liquid chromatography (HPLC) system

HPLC analysis was done at Dr. John Sorenson's laboratory at the University of Manitoba (Department of Chemistry) according to the protocol described by (Abdel-Hameed *et al*., 2016). Briefly, in a HPLC vial, 5 gm of crude extract was dissolved in 1 mL Methanol (Fisher Scientist) to achieve 5mg/mL concentration. The crude analysis was performed using a Waters HPLC Separations Module 2695 combined with a PDA Detector Model 2996. The HPLC consisted of a μBondapak® Waters C_{18} (3.9 × 300 mm) column, with a particle diameter of 15–20 μm with 125 Å pores.

2.6. Identifying potential compounds within the crude extract using Liquid chromatography–mass spectrometry (LC-MS)

From selected crude extracts 30 mg to 60 mg was allocated for LC-MS analysis which was conducted by Dr. Russell Kerr (Department of Chemistry, University of Prince Edward Island (PEI)) with the following protocol (as provided by Dr. Kerr): Thermo Accela UHPLC Pump, Thermo Velos HRMS fitted with an ESI source, and Thermo PDA. A Phenomenex Synergi Polar-RP 80 Å column (150 × 4.60 mm, 4 μm, was used with a mobile-phase flow rate of 0.5 mL/min and injection of 36 volume of 10 μL (all samples were prepared in CH_3OH). The following elution method was used [A = H_2O (0.1% formic acid), B = CH_3CN (0.1% formic acid)]: 5% B from 0.0 to 0.2 min, linear gradient from 5% B at 0.2 min to 99% B at 4.8 min, 99% B from 4.8 to 8.0 min, linear gradient from 99% B at 8.0 min to 5% B at 8.5 min and 5% B from 8.5 to 10.0 min. The following HRMS parameters were used: positive ionization mode, mass resolution of 30,000, mass range of m/z 190 to 2,000, spray voltage of 2.0 kV, capillary temperature of 320 °C, S-lens RF

voltage of 60.0%, maximum injection time of 10 ms, and 1 microscan. The system was controlled by Thermo Xcalibur software modules (Gill *et al*., 2014).

2.7. Fractionation of crude extracts and thin layer chromatography (TLC) analyses using biotage separation system for the crude extract fraction obtained from *Leptographium* sp. WIN(M)1238

Leptographium strain WIN(M)1238 was grown in 4 L of ME broth to increase the crude extract yield amount and to facilitate the separation of the crude extract components. Thin layer chromatography (TLC) was used to identify the proper solvent system. A drop of crude extract (in Methanol) was placed on a TLC plate using a capillary tube. Two different TLC systems were used: hexane:ethyl acetate (70:30) and dichloromethane:methanol (90:10). The most ideal system for this extraction was dichloromethane:methanol in a ratio of 90:10 (Figure 3). Based on the separation of the crude extract on the TLC plate and the PEI result (which showed that the 'no hit' compounds (potential novel compounds) were highly polar) the polar spots were chosen for further investigation. The selected polar spots were applied into the Biotage system and dichloromethane:methanol was selected as the solvent system. Based on the information programed to the Biotage system, we obtained the results that indicated the use of SNAP Ultra 50g column (Biotage, Inc., Charlotte, NC), flowrate of 50 mL/min, dichloromethane as solvent A (90 %) and methanol as solvent B (10%). The cutoff point of threshold was 40 mAU and any compounds eluted after this point will not be collected because the background noise is higher in this case. A total of 6.50 g of crude extract was reconstituted with methanol and mixed with 15 g of silica powder and dried using a rotavap evaporation system. The dried crude extract was then placed on the SNAP column and was eluted between two wavelengths (254 and 365 nm) as the

fractions were collected. A total of 117 fractions were collected and TLC plates were conducted to identify similar fractions that can be combined and dried. (Silva *et al*., 2018).

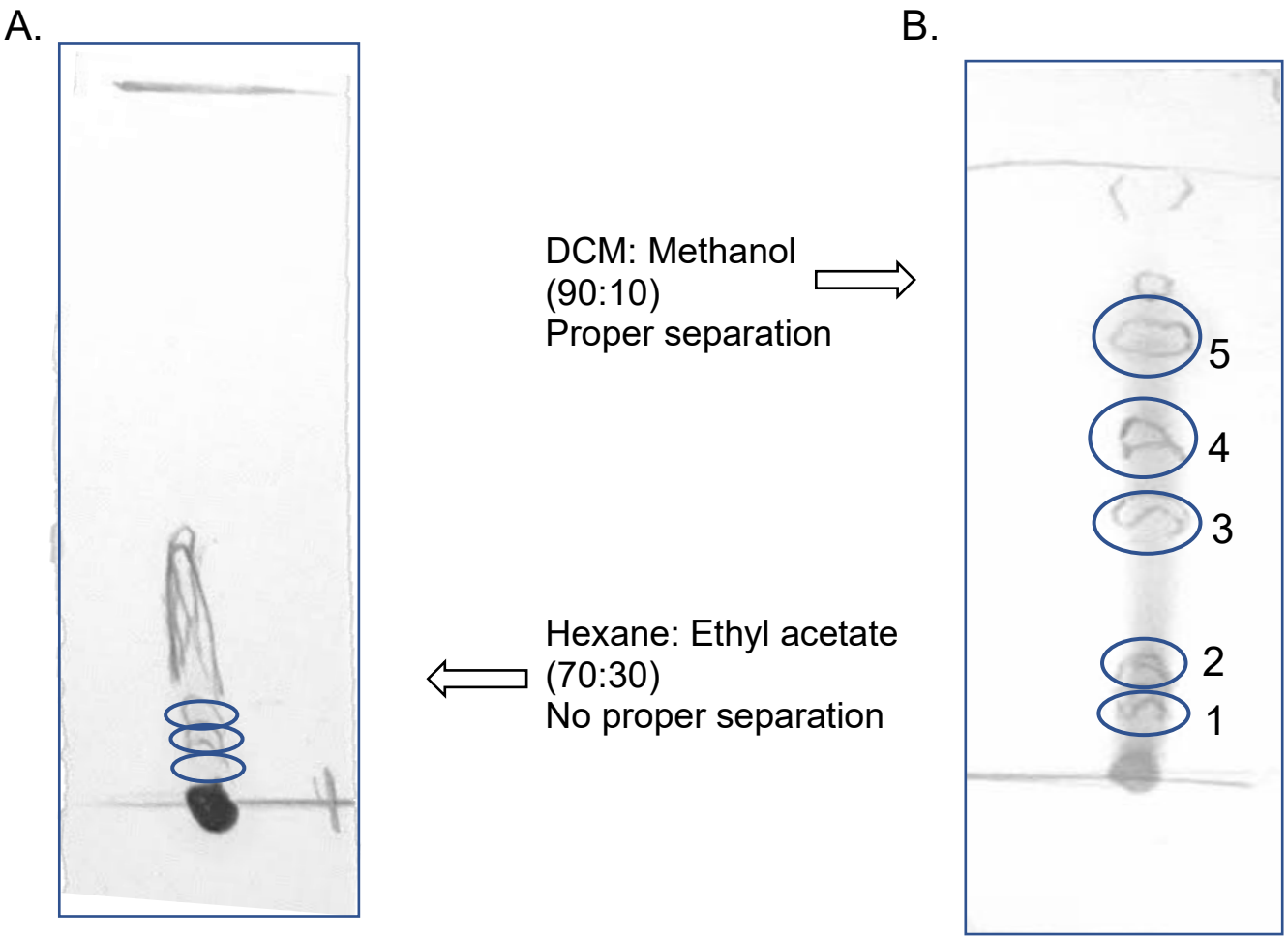


Figure 3: TLC plates showing crude extract separation into various components using two solvent systems; A. hexane:ethyl acetate (70:30), and B. dichloromethane:methanol (90:10); the calculated Rfs for the circled spots of interests for the optimum solvent system DCM: methanol are: Rf1= 0.09, Rf2= 0.21, Rf3= 0.29, Rf4= 0.43 and Rf5= 0.50.

2.8. Bioactivity assay of fractionated crude extract for *Leptographium sp.* WIN(M)1238

Bioactivity assay of fractionated crude extract was done as described before in section 2.4 for the crude extract bioactivity assay. Briefly, the fractionated crude extract was diluted to a final concentration of 150 µg/mL and 10 µl (1.5 mg) of it was added to a sterilized 10 mm filter disc, placed on a *Pseudomonas aeruginosa* strain PAO750 bacterial lawn.

2.9. Fungal nucleic acid (DNA) extraction

Selected *Leptographium* strains that showed antimicrobial activity against bacterial strains listed in (Table 6) were inoculated into 100 mL ME broth and incubated at 23 ℃ for 7 days. Six strains that did not show antimicrobial activity were also selected for DNA extraction. After sufficient growth was observed the inoculated media were filtered, and the mycelia were collected by vacuum filtration. DNA extraction was carried out using Cetyltrimethylammonium bromide (CTAB) buffer [2% CTAB (Fisher Scientific), 100 mM Tris-HCl, pH 8 (Fisher Scientific), 20 mM Ethylenediaminetetraacetic acid (EDTA) (Fisher Scientific), 1.4 M NaCl] followed by chloroform-extraction and ethanol precipitation (as previously described in Hausner *et al.,* 1992). In more detail the mycelium was weighed and ground up with acid washed sands and 2X CTAB buffer (1 g : 1.5 g : 2 mL) until a slurry was formed. This slurry was transferred into 50 mL conical centrifuge tubes and incubated for 1 hour at 65 ℃ in a water bath with gentle mixing every 20 minutes. Afterwards, it was cooled at 15 ℃ for 1 min. An equal volume of chloroform (Fisher Scientific) was added and mixed before the centrifugation at 3,500 Revolutions Per Minute **(**rpm) for 10 min at 1510 Relative Centrifugal Force (RCF). The aqueous layer was carefully transferred into new tube. RNase A (100 mg/mL) was added (1 µl per 1 mL) and the mixture was incubated at 65 ℃ for 1 h for removing the RNA. The chloroform extraction step was repeated twice until a clear

aqueous layer was obtained. The aqueous layer was transferred to a new tube and three volumes of 95% chilled ethanol were added to the aqueous solution and the mixture was mixed by inversion. The mixture was placed overnight at -20 ℃. The following day, the tubes were centrifuged at 3,500 rpm for 15 min. After discarding the supernatant, 1mL of 70 % ethanol was added to wash the DNA pellet and the tube was centrifuged at 3,500 rpm for 5 min. In some instances, the ethanol precipitation steps were repeated until a clear pellet was obtained. The pellet was allowed to dry to evaporate the ethanol completely and then resuspended in 300 µl of nuclease free water and the DNA solution was transferred to 1.5 mL microcentrifuge tubes and stored at -20 ℃ (Hausner *et al*., 1992).

Table 6: Selected fungal strains for DNA extraction

Fungal Strains	[WIN(M)]:	
showing antimicrobial activity	1278	1205
	1280	1206
	1282	1234
	1291	1236
	1327	1238
	1330	1240
		1268
Not showing antimicrobial activity	1293	1208
		1210
		1212
		1290

2.10. Polymerase chain reaction (PCR)

The marker selected for the verification of the fungal strains identities was the internal transcribed spacer (ITS) region of the rDNA. Primers SS3-F and LS2-R were the selected forward and reverse primers, respectively (Table 7). Standard PCR protocol was followed with the ingredients and conditions listed in (Table 8 and 9). PCR products were separated and visualised by agarose gel electrophoresis (AGE) on 1% agarose gels. Staining with ethidium bromide (0.5 µg/mL) allowed the visualization of the DNA bands.

Table 7: list of the selected primers

Locus	Primers	Direction	Primers Sequence 5'-3'
Internal Transcribed Spacer (ITS) region of rRNA [~ 450-800 bp]	SS3	Forward	GTC GTA ACA AGG TCT CCG
	LS2	Reverse	GAT ATG CTT AAG TTC AGC G

Table 8: Components for PCR

Component	Initial concentration	Final concentration	Amount (µL)
10X Taq Buffer (GeneDirX, Inc., Taiwan)	10X	1X	5 µl
dNTPs (Invitrogen, Canada)	10 mM	0.2 mM	1 µl
forward primer (Alpha DNA, Canada)	10 µM	0.2 µM	1 µl
reverse primer (Alpha DNA, Canada)	10 µM	0.2 µM	1 µl
Taq DNA (GeneDireX)	--	--	0.5 µl
DNA template	--	<00 ng	1 µl
Nuclease free water (Invitrogen)	--	--	40.5 µl

Table 9: Reaction conditions for PCR amplification of the ITS region using SS3/LS2 primers.

#	Steps	Temperature (°C)	Time (second)
1	Initial denaturation	95 ℃	300 sec
2	Denaturation	95 ℃	30 sec
3	Annealing	58 ℃	30 sec
4	Extension	72 ℃	45 sec
	Step 2-4 Repeat for 35 cycles		
5	Final extension	72 ℃	300 sec
6	Hold	4 ℃	--

2.11. PCR products purification

A PCR purification kit (QIAGEN) was used for cleaning up the PCR products (Table 10); the protocol supplied by the manufacturer was followed: briefly to 1 volume of the PCR sample, 5 volumes of Buffer PB were added and mixed. The mixture was applied to a QIAquick spin column that was placed into a 2 mL collection tube. For DNA binding to the column, centrifugation was carried out at 13,000 rpm for 60 s. The flow-through (FT) was discarded and 750 µl of Buffer PE (with 95 % ethanol added) was added to the column and centrifuged at 13,000 rpm for 60 s for washing the bound PCR products. The FT was discarded, and the column was placed back in the tube and an additional one minute of dry spin was carried out to ensure no residual ethanol remained on the column. The column was then placed into a new 1.5 mL microcentrifuge tube and DNA elution was carried out by adding 50 µl of Buffer EB to the center of the tube followed by centrifuging at 13,000 rpm for 60 s. The purified DNA was sent to the Manitoba Institute of Cell Biology at the University of Manitoba for Sanger sequencing utilizing the same primers used for the original PCR reactions.

Table 10: QIAquick PCR Purification Kit Catalog no. (50) 28104 components.

Component	Quantity
QIAquick Spin Columns	50
Collection Tubes (2 mL)	50
Binding Buffer (Buffer PB)	30 mL
Wash Buffer (Buffer PE)	2 x 6 mL
Elution Buffer (Buffer EB)	15 mL

2.12. Sequence analysis and phylogenetic tree construction

A total of 100 ITS region sequences were collected from the nucleotide database at the NCBI website https://www.ncbi.nlm.nih.gov/nucleotide/. These included sequences representing five *Leptographium* species (*Leptographium lundbergii, Leptographium procerum, Leptographium truncatum, Leptographium terebrantis, Leptographium wingfieldii*) in addition sequences for *Sporothrix schenckii* was also collected (Appedix Table S1). In addition to this, sequences from eighteen *Leptographium* strains from my study (Table 6), were selected, and for these the sequences were trimmed and error-corrected using the Sequence Scanner Software v2.0 (Applied Biosystems, Waltham, Massachusetts, USA). The MAFFT website (Katoh *et al*., 2019) was used for sequence alignments and the alignments were visualized and edited using the AliView program (Larsson, 2014). A phylogenetic tree for the ITS region sequences was generated based on Bayesian estimation (MrBayes, version 3.2.6; Ronquist and Huelsenbeck, 2003; Ronquist *et al*., 2012). In MrBayes, the following parameters were selected: MCMC chains were run for 8 000 000 generations and trees were sampled every 100 generations. Substitution models were sampled using lset nst = mixed command. The burn-in command discarded the first 25% of the sampled trees.

Chapter 3: Results

The current results reflect the work done before the lockdown due to COVID-19. The remaining samples will be duly analyzed in future and hence, only partial number of samples could be analyzed due to time constraint. Whenever - possible consider having Preliminary data presented in the Appendix section.

3.1. Bioactivity assay

3.1.1. Agar plug bioactivity assay

There were hundred strains of *Leptographium* screened for antimicrobial activity against six bacterial strains (Table 5). Agar plugs containing fungal growth were placed on the bacterial lawn growing on agar places. The zones of inhibition were determined (in cm) by subtracting the diameter of the agar plugs from the diameter of the zones of bacterial clearance. Eighteen fungal strains showed zones of inhibition that indicated antimicrobial activity against multiple strains of bacteria (Table 11). All of the fungal strains except for WIN (M) 1230 showed activity against *Pseudomonas aeruginosa* strain PA0750. Among the hundred fungal strains, two strains that showed relatively larger zones of inhibition. WIN (M) 1238 and 1291 (zones of 1.3 cm and 1.6 cm, respectively) were selected for further detection. Overall, thirteen fungal strains that showed zones of inhibition greater than or equal to 0.3 cm were selected for further investigation as these showed the potential for antimicrobial activity.

3.1.2. Crude extract bioactivity assay

The bioactivity of the selected thirteen fungal strains was confirmed by testing the prepared crude extracts against *Pseudomonas aeruginosa* strain PA0750. Filter paper discs with 150 µg / mL (1.5 mg on each disk) of crude extracts, dissolved in DMSO, were placed on the bacterial lawn

on agar plates. The zones of inhibition were obtained by subtracting the diameter of the diffusion discs 1.1cm from the diameter of the zones of bacterial clearance. The thirteen tested fungal strains showed antimicrobial activity indicated by variable zones of inhibitions as shown in (Table 12). Most fungal strains showed similar zones of inhibition except for WIN (M)1291 and WIN (M) 1330 which appeared to show larger zones of inhibition (0.9 cm, and 0.6 cm respectively) which might suggest that these have a higher potential for antimicrobial activity. For the thirteen fungal strains that showed antimicrobial activity in the agar plug assay crude extracts were prepared to be sent for analysis by LC-MS (Dr. Russell Kerr, Department of Chemistry, University of Prince Edward Island (PEI)). However, due to time limitation in the current situation, only four extracts from WIN (M), 1205, 1206, 1238 and 1291 are described herein with addition to three extracts derived from WIN (M) 1290, 1293 and 1281. These later three strains did not show antimicrobial activity in the agar plug assay. The extract for WIN (M) 1281 was selected as the whole genome sequence is currently being worked on in this laboratory. Extracts for the remaining samples that showed antibacterial activity will be sent to PEI, these have not been completely analyzed so data for these strains is not available.

Table 11: Fungal strains showing antimicrobial activity against selected bacterial strains (see table 5 in material and methods section) using the agar plug bioactivity assay. Zone of inhibition (diameter) measured in in cm

#	Fungal strains WIN (M)	Zone of inhibition (diameter in cm) of bacterial strains					
		PA0750	PAO1	PA82	ATCC17978	ABO30	MRSA
1	1205	1.0	-	-	-	-	-
2	1206	0.6	-	-	-	-	-
3	1230	-	-	-	-	-	0.2
4	1234	1.1	-	-	-	-	-
5	1236	0.4	-	-	-	-	-
6	1238	1.3	-	-	-	-	-
7	1240	0.6	-	-	-		-
8	1268	0.6	-	-	-	-	-
9	1278	1.1	-	-	-	-	0.2
10	1280	1.1	-	-	-	-	-
11	1282	0.8	-	-	-	-	-
12	1291	1.6	-	-	0.3	-	0.2
13	1301	0.1	-	-	-	-	-
14	1303	0.1	-	-	-	-	-
15	1304	0.1	-	-	-	-	-
16	1306	0.1	-	-	-	-	-
17	1327	0.3	-	-	-	-	-
18	1330	0.7	-	-	-	-	-

Table 12: Crude extracts isolated from fungal strains showing antimicrobial activity against *Pseudomonas aeruginosa* PAO750 caused by the extracts (150 µg/ml) from various fungal strains

Crude extract from fungal Strains WIN (M)	*Pseudomonas aeruginosa* PAO750(zone of inhibition in cm)
1205	0.2
1206	0.2
1234	0.1
1236	0.1
1238	0.2
1240	0.2
1268	0.1
1278	<0.1
1280	<0.1
1282	0.2
1291	0.9
1327	0.1
1330	0.6

3.2. Secondary metabolite extraction

Crude extracts, derived from 100 mL spent media, were obtained from one hundred strains of *Leptographium*. The net weights of the extracts ranged between 20 to 40 mg under normal lab conditions, with strains WIN (M) 1208 and WIN (M) 1312 producing 97.4 mg (highest) and 5.7 mg extracts (lowest), respectively (Appendix Table S 2)

3.3. Chromatographic analyses

3.3.1. Identifying potential compounds within the crude fungal extracts using Liquid chromatography–mass spectrometry (LC-MS)

The seven samples selected for LC-MS are extracts recovered from the following fungal strains WIN(M) 1205, 1206, 1238, 1281, 1290, 1291 and 1293. LC-MS is a suitable quantification strategy for detecting compounds with different biochemical activities in the crude extract, since it is highly selective and sensitive, allowing detection with very little noise level, and in trace amounts. The MS system measures the molecular masses of potential compounds indirectly, by measuring the mass-to-charge ratios (m/z) of the ions formed from the molecules. The main advantage of using Electro Spray Ionisation (ESI)-MS for quantitative LC-MS is the formation of protonated or deprotonated ions, ideal for selection of precursor ions and for maximizing sensitivity. The compounds were detected as “hits” against the Antibase 2012 database, which is a comprehensive database of more than 40,000 natural compounds from microorganisms and higher fungi. Three compounds were found to be present in all the samples, however, these are probably metabolites abundant across all domains of life. However, several compounds were identified with potential antimicrobial, antitumor and cytotoxic activities (Table 13). In addition to these compounds, there were two compounds that could not be detected when matched against

the database. This could suggest that the ‘no-hit’ compounds are unknown compounds that may have potential novel biochemical activities. These are listed in (Table 14).

Table 13: showing compounds with potential biochemical activities detected in the *Leptographium* derived crude extract samples

#	Potential compounds	Ions (row retention time _row m/z)	Functions	Fungal strains WIN (M)	Sources
1	2-(1H-Indol-3-yl) ethyl 2-hydroxypropanoate	5.86_234.1125	Indole derivative, potential anti-oxidative and antimicrobial	All	(Sugiyama *et al.*,2009; Kochanowska-Karamyan *et al.*,2010)
2	trans-Zeatin	5.03_242.1021	Plant Growth Regulator Antiaging effect on human fibroblast cells	All	PubChem CDI 449093, (Osugi *et al.*, 2017; Mooi *et al.*, 2015)
3	Citrinolactone B	6.02_233.0809	Plant Growth Regulator	All	(Masato *et al.*, 2007) (Zhang *et al.*, 2016)
4	Limazepine B1	5.28_291.1343	Antitumor, antibacterial	1205, 1206, 1238, 1291	(Fotso *et al.*, 2009) (Han *et al.*, 2013)
5	Limazepine B2	5.28_291.1343	Antitumor, antibacterial	1205, 1206, 1238, 1291	(Fotso *et al.*, 2009) (Han *et al.*, 2013)
6	1-(2-Hydroxy-6-methoxyphenyl)butan-1-one	6.55_195.1020	Antimicrobial	1205, 1291	(Dai *et al.*, 2006)
7	Pulcherriminic acid	5.10_257.1497	Iron chelator, Antimicrobial, Antifungal	1238, 1281,1291	(Randazzo *et al.*, 2016) (Gore-Lloyd *et al.*, 2019)
8	Inotilone	5.42_219.0654	Anti-inflammatory, Anti-cancer	1205,1206, 1238, 1281, 1291	(Huang *et al.*, 2012) (Chao *et al.*, 2019) (Kurma *et al.*, 2020)
9	Isovaleryl 3-nitrotyramide	5.66_253.1185	Anti-cancer	1291	(Teasdale *et al.*, 2011)
10	10-Hydroxy-trans-paspalic acid	5.39_284.1392	Ergot alkaloid with potential pharmacological activity	1205, 1291	(Schiff, 2006) (Flieger *et al.*, 1993)

Table 14: *Leptographium* strains with potential novel ("no-hit") compound

Fungal strains WIN (M)	Ions (row retention time _row m/z)
1238 1281 1290 1293 1291	5.39_252.0868
	6.41_242.2843

3.3.2. Analyzing crude extracts using a high-performance liquid chromatography (HPLC) system

Based on the results from the agar plug based bioactivity assay against *Pseudomonas aeruginosa* PAO750, extracts from two strains, WIN (M) 1238 and 1291, with the largest zones of inhibition were selected for HPLC. Two potential novel compounds as indicated by 'not detected' (ND) on separation, were shared among the two samples (Table 14). The number of peaks in the chromatogram indicate the number of the potential compounds present in the crude mixture. The peaks in the chromatograms corresponding roughly to the number of the potential compounds present in the crude extract of the two selected *Leptographium* strains were compared to the peaks present in the corresponding media controls (ME) (Figure 4). For the strain WIN(M) 1238, nine peaks appeared in the chromatogram (7.092, 7.546, 21.843, 22.860, 23.384, 26.415, 26.937, 48.200, 50.227) (Figure 5). Compounds with similar properties have similar retention times. For sample WIN (M) 1291 HPLC analysis will be carried out in the future.

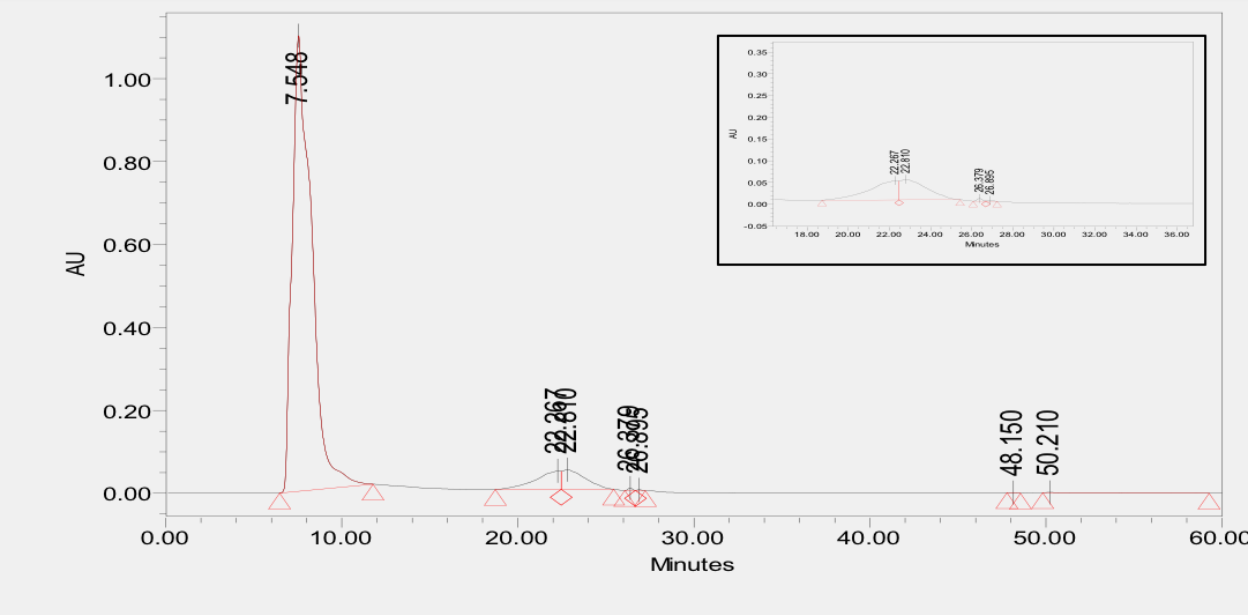


Figure 4: HPLC chromatogram for ME media control; Retention times: 7.548, 22.267, 22.810, 26.379, 26.895, 48.150, 50.210

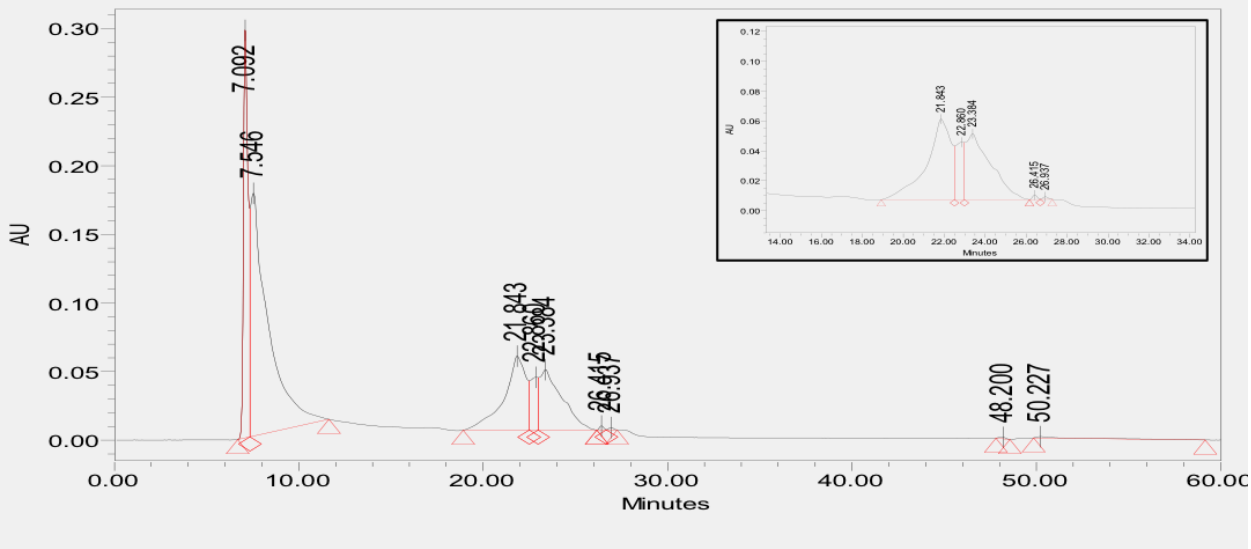


Figure 5: HPLC chromatogram for the crude extract derived from WIN (M) 1238; Retention times: 7.092, 7.546, 21.843, 22.860, 23.384, 26.415, 26.937, 48.200, 50.227

3.3.3. Fractionation of crude extracts using thin layer chromatography (TLC) analyses and the Biotage separation system.

Thin layer chromatography (TLC) was carried out in the lab of Dr. John Sorensen, Department of Chemistry, University of Manitoba. *Leptographium* sp. WIN (M) 1238 and 1291 were grown in 4 L ME media. WIN (M) 1238 has been processed via TLC analysis, but analysis of the WIN (M) 1291 extract will be proceeding in the future. Crude extract weight of WIN (M) 1238 was noted as 1.31g. TLC was initially done on the crude extract to ensure the selection of the proper solvent system according to the migration of the crude extract. The two different TLC systems that were employed were hexane:ethyl acetate (70:30) and dichloromethane:methanol (DCM:methanol) (90:10). It was concluded that a system of dichloromethane:methanol in a ratio of 90:10 was the most ideal system for this extract (see Figure 3 in material and method section).

The Biotage system employs Flash Chromatography for separation of compounds. It adjusts the solvents in a ratio that leads to the optimum Rf (Retention factor = compound migration distance/solvent migration distance) for the target compound for efficient separation. This system showed that proper separation could not be obtained when using the hexane:ethylacetate solvent system as indicated by the closely crowded spots (compounds poorly resolved) , while the dichloromethane:methanol solvent system resulted in clearly resolved spots. The Rfs were calculated for each of the five circled spots of interest (most polar) on the manual TLC column and used as input into the Biotage system.

For the circled spots of interest (see Figure 3), the calculated Rfs are as follows:

Rf1= 0.6 cm/7cm= 0.09

Rf2=1.5 cm/7 cm= 0.21

Rf3=2 cm/7 cm= 0.29

Rf4=3 cm/7 cm= 0.43

Rf5=3.5 cm/7cm= 0.50

Hence, it was concluded that, for obtaining the same pattern of separation as indicated by the circled spots in (Figure 3) dichloromethane:methanol was the optimum solvent system. A total of 117 fractions were collected and TLC plates were performed to identify similar fractions that can be combined and dried (see Figure 6). The patterns for fractions 1-53 and 98-117 on the TLC column resembled the crude extract, indicating that they were not properly resolved and have to be analyzed again in the future, probably with a different suitable solvent system. The remaining fractions were divided further into five categories, based on the similarities in the pattern of spots on the TLC column. They are as follows: F 54-58, F 59-65, F 66- 69, F 70-86 and F 86-97.

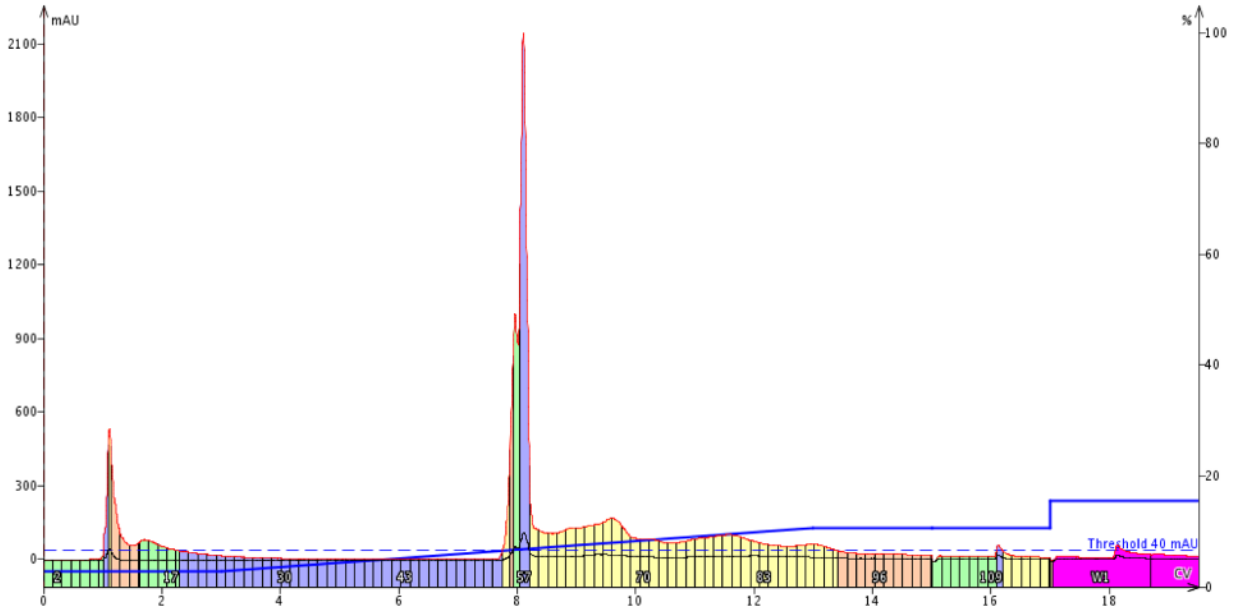


Figure 6: FLASH chromatogram of the fraction of the crude extract of *Leptographium* strain WIN(M) 1238. The Biotage screen showing the peaks corresponding to the retention times. The gradient was performed with dichloromethane:methanol 90:10 resulting in 117 fractions (F1 to F117). X axis is CV (column volume; solvent volume necessary to elute a non-retained solute CV=1/Rf) and Y axis is absorbance in milli-Absorbance Units (mAu). The blue line represents the baseline of the solvent gradient percentage.

3.3.4. Bioactivity assay of the crude extract in the fractions obtained from TLC

There were five fractions obtained from the TLC analysis of the *Leptographium sp.* WIN(M)1238 extracts according to similar separation patterns. Their bioactivity was tested against *Pseudomonas aeruginosa* strain PA0750. The filter paper discs with the fractions of each crude extract dissolved in DMSO at the final concentration of 150 µg / mL, were placed on the bacterial lawn grown on agar plates. The zones of inhibition were measured as described previously. Antimicrobial activity was exhibited by four out of the five fractions (F 54-58, F 59-65, F 66-69 and F 70-86) as noted from their zones of inhibition (Table 15). Further investigation using LC-MS will be required in order to facilitate the identification of the compounds with potential antimicrobial activity in these fractions.

Table 15: showing presence of the bioactivity of the crude extract fractions of the fungal strains WIN(M) 1238 against PA0750.

TLC fractions for WIN (M) 1238	**Zone of inhibition (cm) (PA0750)**
F 54-58	0.5
F 59-65	0.1
F 66-69	0.2
F 70-86	0.1

3.4 Internal transcribed spacer sequence and phylogenetic analysis

Sanger sequencing of the ITS region of thirteen strains of *Leptographium* sp. that showed antimicrobial activity was carried out, along with five additional strains that did not show bioactivity (Table 6). PCR products representing the ITS regions for the various strains were sent to the Manitoba Institute of Cell Biology at the University of Manitoba. The ITS data were applied to confirm the identity of the fungal strains (WIN(M) strains) utilized in this study. The sequences obtained from this work were compared to ITS sequences extracted from NCBI GenBank. All sequences were curated in AliView and aligned. The data set contained ITS sequences obtained in this study (Appendix Table S3) and sequences from NCBI representing five different *Leptographium* species (*Leptographium lundbergii, Leptographium procerum, Leptographium truncatum, Leptographium terebrantis, Leptographium wingfieldii*), in addition sequences were added for strains of *Sporothrix schenckii* (Appendix Table S1) to serve as the outgroup in the phylogenetic analysis.

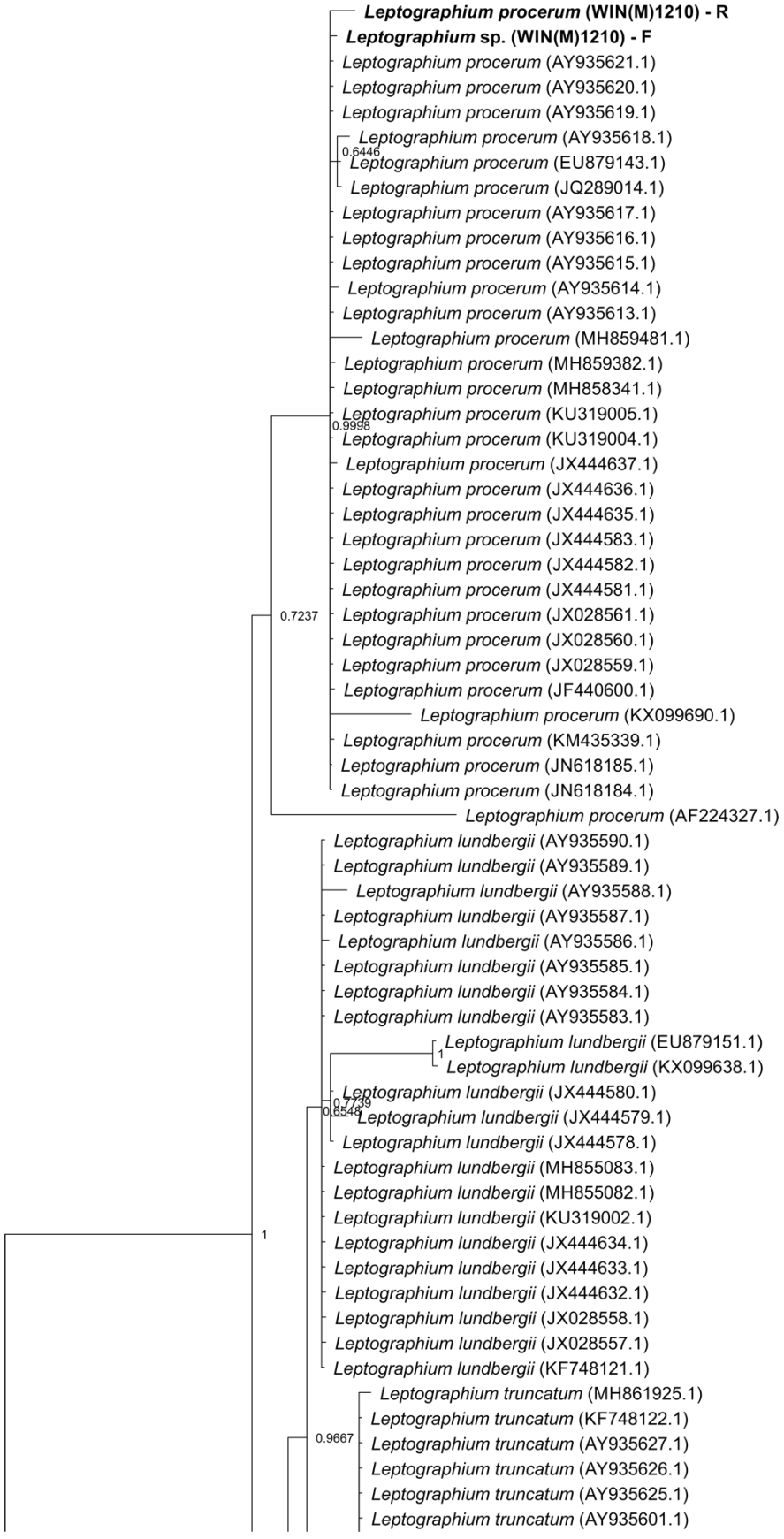
A phylogenetic tree for the ITS region sequences was based on Bayesian estimation utilizing the MrBayes program (Figure 7). The tree consists of five different clades with *Sporothrix schenckii* selected to represent the outgroup. The first clade was formed by the *Leptographium procerum* sequences sharing a common node supported with a high node support value (0.9998). Members of this clade formed one cluster, with one strain denoted as *Leptographium procerum* (accession number: KX099690_1) positioned on a long branch, which could be the result of mislabeling/misidentification or poor sequence quality (by the original authors). Another strain identified as *Leptographium procerum* (accession number: AF224327_1) branched out as an outlier with low node support value (0.7237). Strain WIN (M) 1210 (forward and reverse

fragments) were noted to position within the *Leptographium procerum* clade which confirmed its identity as *Leptographium procerum*.

Strains recorded as *Leptographium truncatum* formed the second clade but the node support values among the members of this clade were poor (< / = approximately 0.95) which indicated poor resolution beyond the species level. However, *Leptographium truncatum* (accession number: A Y935582_1) branched separately from the main clade with a high node support value (0.9996) but it still shared a node with the *Leptographium truncatum* clade. Strains named *Leptographium lundbergii* formed another clade with two strains (accession numbers for ITS sequences: EU879151_1 and KX099638_1) forming long branches but they still appear to be in the same clade but the node support value including the latter two, is low (0.7739).

The next clade observed in the phylogenetic tree was formed by strains assigned to two different species, strains of *Leptographium terebrantis* and *Leptographium wingfieldii*, along with the majority of the strains in this study (Strains: WIN (M) 1212, 1234, 1206, 1268, 1238, 1278, 1290, 1205, 1291, 1208, 1236, 1330, 1282, 1327, 1293, 1240, 1280). In this clade, the ITS sequence for *Leptographium terebrantis* (accession number: KF293983_1) was positioned on a long branch with low node support value (0.8287); this might be indicative of misidentification or a poor sequence (high error rate).

In conclusion, the tree topology shows that the different *Leptographium* strains that produce antimicrobial activities can be placed among several clades within the genus *Leptographium*, however many can be assigned to the species *Leptographium wingfieldii*. It should be noted that one marker (ITS) may not be sufficient for precise species designation, therefore different genetic loci should be sequenced in order to assign the various strains to a species.


Leptographium procerum (WIN(M)1210) - R
Leptographium sp. (WIN(M)1210) - F
Leptographium procerum (AY935621.1)
Leptographium procerum (AY935620.1)
Leptographium procerum (AY935619.1)
Leptographium procerum (AY935618.1)
0.6446
Leptographium procerum (EU879143.1)
Leptographium procerum (JQ289014.1)
Leptographium procerum (AY935617.1)
Leptographium procerum (AY935616.1)
Leptographium procerum (AY935615.1)
Leptographium procerum (AY935614.1)
Leptographium procerum (AY935613.1)
Leptographium procerum (MH859481.1)
Leptographium procerum (MH859382.1)
Leptographium procerum (MH858341.1)
Leptographium procerum (KU319005.1)
0.9998
Leptographium procerum (KU319004.1)
Leptographium procerum (JX444637.1)
Leptographium procerum (JX444636.1)
Leptographium procerum (JX444635.1)
Leptographium procerum (JX444583.1)
Leptographium procerum (JX444582.1)
Leptographium procerum (JX444581.1)
0.7237
Leptographium procerum (JX028561.1)
Leptographium procerum (JX028560.1)
Leptographium procerum (JX028559.1)
Leptographium procerum (JF440600.1)
Leptographium procerum (KX099690.1)
Leptographium procerum (KM435339.1)
Leptographium procerum (JN618185.1)
Leptographium procerum (JN618184.1)
Leptographium procerum (AF224327.1)
Leptographium lundbergii (AY935590.1)
Leptographium lundbergii (AY935589.1)
Leptographium lundbergii (AY935588.1)
Leptographium lundbergii (AY935587.1)
Leptographium lundbergii (AY935586.1)
Leptographium lundbergii (AY935585.1)
Leptographium lundbergii (AY935584.1)
Leptographium lundbergii (AY935583.1)
1
Leptographium lundbergii (EU879151.1)
Leptographium lundbergii (KX099638.1)
Leptographium lundbergii (JX444580.1)
0.7739
0.6548
Leptographium lundbergii (JX444579.1)
Leptographium lundbergii (JX444578.1)
Leptographium lundbergii (MH855083.1)
Leptographium lundbergii (MH855082.1)
Leptographium lundbergii (KU319002.1)
Leptographium lundbergii (JX444634.1)
Leptographium lundbergii (JX444633.1)
Leptographium lundbergii (JX444632.1)
Leptographium lundbergii (JX028558.1)
Leptographium lundbergii (JX028557.1)
Leptographium lundbergii (KF748121.1)
1
Leptographium truncatum (MH861925.1)
Leptographium truncatum (KF748122.1)
0.9667
Leptographium truncatum (AY935627.1)
Leptographium truncatum (AY935626.1)
Leptographium truncatum (AY935625.1)
Leptographium truncatum (AY935601.1)

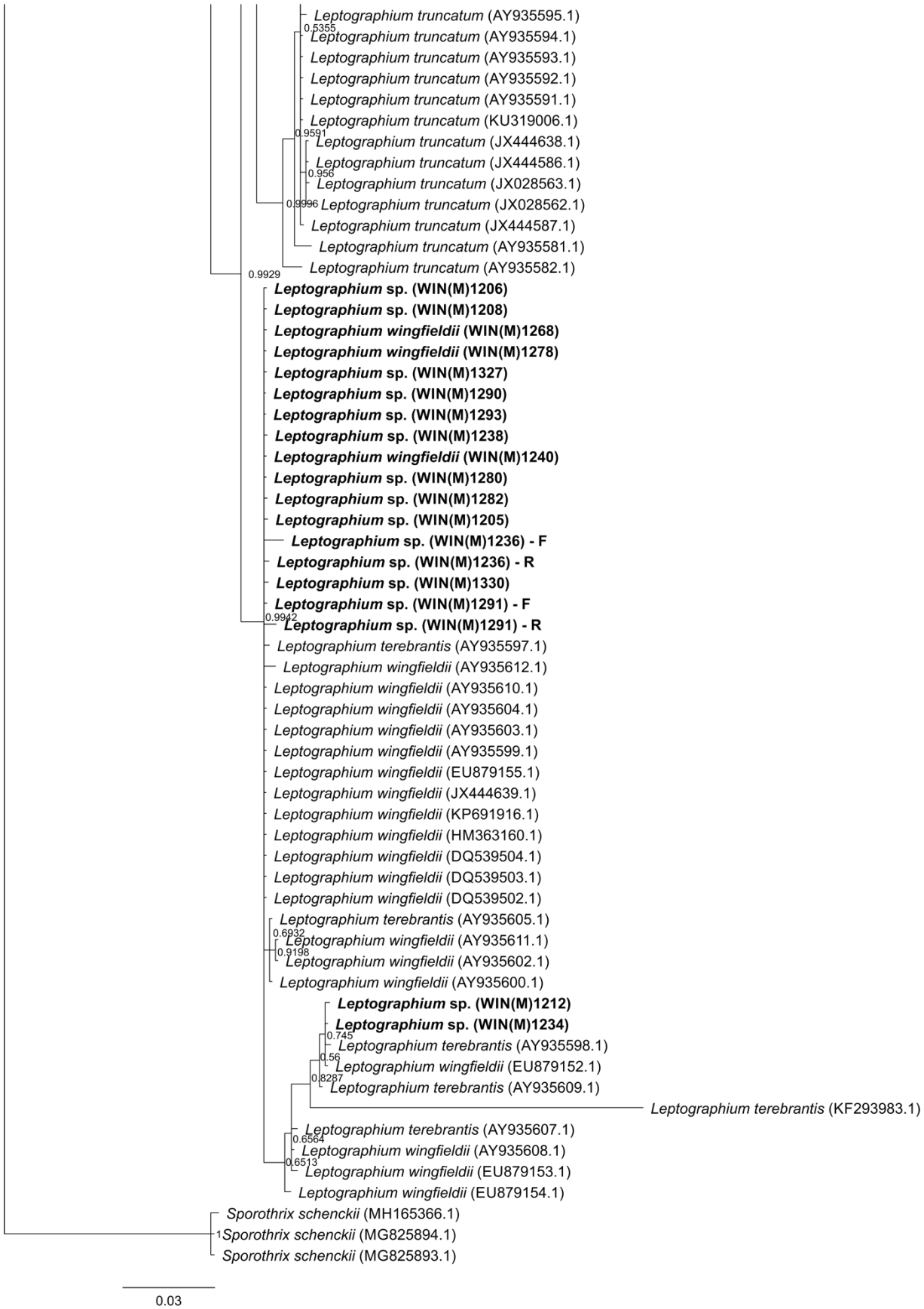

Leptographium truncatum (AY935595.1)
0.5355
Leptographium truncatum (AY935594.1)
Leptographium truncatum (AY935593.1)
Leptographium truncatum (AY935592.1)
Leptographium truncatum (AY935591.1)
Leptographium truncatum (KU319006.1)
0.9591
Leptographium truncatum (JX444638.1)
Leptographium truncatum (JX444586.1)
0.956
Leptographium truncatum (JX028563.1)
0.9996
Leptographium truncatum (JX028562.1)
Leptographium truncatum (JX444587.1)
Leptographium truncatum (AY935581.1)
Leptographium truncatum (AY935582.1)
0.9929
Leptographium sp. (WIN(M)1206)
Leptographium sp. (WIN(M)1208)
Leptographium wingfieldii (WIN(M)1268)
Leptographium wingfieldii (WIN(M)1278)
Leptographium sp. (WIN(M)1327)
Leptographium sp. (WIN(M)1290)
Leptographium sp. (WIN(M)1293)
Leptographium sp. (WIN(M)1238)
Leptographium wingfieldii (WIN(M)1240)
Leptographium sp. (WIN(M)1280)
Leptographium sp. (WIN(M)1282)
Leptographium sp. (WIN(M)1205)
Leptographium sp. (WIN(M)1236) - F
Leptographium sp. (WIN(M)1236) - R
Leptographium sp. (WIN(M)1330)
Leptographium sp. (WIN(M)1291) - F
0.9942
Leptographium sp. (WIN(M)1291) - R
Leptographium terebrantis (AY935597.1)
Leptographium wingfieldii (AY935612.1)
Leptographium wingfieldii (AY935610.1)
Leptographium wingfieldii (AY935604.1)
Leptographium wingfieldii (AY935603.1)
Leptographium wingfieldii (AY935599.1)
Leptographium wingfieldii (EU879155.1)
Leptographium wingfieldii (JX444639.1)
Leptographium wingfieldii (KP691916.1)
Leptographium wingfieldii (HM363160.1)
Leptographium wingfieldii (DQ539504.1)
Leptographium wingfieldii (DQ539503.1)
Leptographium wingfieldii (DQ539502.1)
Leptographium terebrantis (AY935605.1)
0.6932
Leptographium wingfieldii (AY935611.1)
0.9198
Leptographium wingfieldii (AY935602.1)
Leptographium wingfieldii (AY935600.1)
Leptographium sp. (WIN(M)1212)
Leptographium sp. (WIN(M)1234)
0.745
Leptographium terebrantis (AY935598.1)
0.56
Leptographium wingfieldii (EU879152.1)
0.8287
Leptographium terebrantis (AY935609.1)
Leptographium terebrantis (KF293983.1)
Leptographium terebrantis (AY935607.1)
0.6564
Leptographium wingfieldii (AY935608.1)
0.6513
Leptographium wingfieldii (EU879153.1)
Leptographium wingfieldii (EU879154.1)
Sporothrix schenckii (MH165366.1)
1
Sporothrix schenckii (MG825894.1)
Sporothrix schenckii (MG825893.1)
0.03

Figure 7: Phylogenetic tree showing the position of *Leptographium* strains that exhibit antimicrobial activity among different *Leptographium* spp. using ITS sequences.

Chapter 4: Discussion and concluding remarks

4.1. Discussion

Leptographium spp. are blue stain fungi that invade certain pine species they are vectored by bark beetles. *Leptographium wingfieldii* has been introduced into North America from Europe and it is vectored by *Tomicus* species and in some instances this fungus contributes to beetle-associated mortality of trees (Hausner *et al.,* 2005).

Fungi produce different classes of secondary metabolites which are low molecular weight compounds with a wide spectrum of biological activities, which may have either beneficial or harmful consequences (Watve *et al.,* 2001). Some fungi have been noted for their production of antibiotics and recently fungi have been explored in order to find novel antibiotics (Goyal *et al.,* 2016).

Traditionally bacteria were considered to be the major sources of antibiotic specially Actinobacteria (Ventola, 2015; Michael *et al.,* 2014). However, an increasing number of studies have been conducted on fungi that had been previously ignored which has led to the discovery of novel antimicrobial compounds such as polyketides engyodontochones A-F, oxaphenalenone dimers, and talaromycesone A and B, (Imhoff *et al.,* 2018) prenylxanthones (Tortorella *et al.,* 2018) and Ophiosetin (Mahajan and Balachandran, 2017). Ascomycete fungal genomes code for on average of 16 polyketide synthases (PKS), 10 non-ribosomal protein synthases (NRPS), two tryptophan synthetases (TS), and two dimethylallyl tryptophan synthetases (DMATS) with crucial importance in SM synthesis. These SM genes code for the enzymes that can be enriched in BGCs responsible for main synthesis steps of metabolites (Pusztahelyi *et al.,* 2015). Caballero *et al.* (2019) explored the BGCs in the ophiostomoid fungal species *Raffaelea lauricola* and found genes

encoding ABC transporters, which are key components of resistance to antifungal agents, cytochrome P450 genes, and other genes encoding biotic stress-responsive enzymes such as laccases, peroxidases and tannases and proteolytic enzymes, as well as polyketides and non-ribosomal proteins that act as virulence factors in fungal interactions with other plants, animals or insects. This data suggests that ophiostomoid fungi are potentially a rich source of biochemical compounds and SMs that function in defense pathways against other organisms.

In this study, secondary metabolites from a hundred *Leptographium* spp. were explored in terms of the production of potential therapeutic/antimicrobial compounds. Agar plug and disc diffusion assays were conducted to test for antimicrobial effects. The principle of the disc diffusion is based on the agar plug assay, in which the filter papers that contained the test compounds were placed on a lawn of bacteria of interest allowing the molecules to diffuse and create the zone of inhibition around the disc filter paper (Horváth *et al*., 2016). It was observed from the agar plug assay that out of the hundred *Leptographium* spp. eighteen fungal strains showed zones of inhibition that ranged between 0.1 cm to 1.6 cm against the *Pseudomonas aeruginosa* strain PAO750. It is an efflux deficient strain with deletions in five operons encoding the RND (reticulation nodulation division) pumps as well as the *ompH* (outer membrane protein H) coding for the *P. aeruginosa* TolC homolog that functions in tandem with the efflux pump. Out of the different bacterial strains used in this study, all the fungal strains managed to target PAO750, probably because of its already highly drug susceptibility. The inability of the fungal strains to inhibit the majority of other bacteria is probably due to the fact that SMs were isolated from cultures grown on a small scale (100 mL) and the yield and/or concentrations were not sufficient to have a strong antimicrobial effect.

After extraction of the SMs from the thirteen strains, disc diffusion assay was conducted on a lawn of the bacterial strain *P. aeruginosa* PAO750 which was selected as the previous agar plug assay showed potential antimicrobial inhibition by the fungal strains against this weak efflux deficient strain. All of the thirteen strains showed zones of inhibition that vary between 0.1cm to 0.9 cm against the PAO750 strain. From this it was inferred that zones of inhibition indicate the presence of antimicrobial activity in *Leptographium* spp. against the selected bacteria used in this study.

The combination of LC and MS permits the analysis of multiple compounds in a complex mixture in highly selective and specific way. The analytes are separated by their mass-to-charge ratio (*m/z*). One of the advantages of LC-MS is that the analysis requires reduced sample preparation. Moreover, the high sensitivity of the technique leads to reduced noise, allowing detection to very low levels (fg/mL) (Coskun, 2016).

In this study, selected secondary metabolites from *Leptographium* spp. were analysed using LC-MS at the laboratory of Dr. Russell Kerr (Department of Chemistry, University of Prince Edward Island (PEI)) and matched against Antibase 2012 database, which is a comprehensive database of more than 40,000 natural compounds from variety of microorganisms. The corresponding mass-to-charge ratios (*m/z*) were used to predict the potential compounds produced by *Leptographium* spp. Some of the compounds predicted from this screen have potential antimicrobial activity for example, Limazepine B, Limazepine B2, 1-(2-Hydroxy-6-methoxyphenyl)butan-1-one, Pulcherriminic acid (see Table 13 Result section). These compounds might have resulted in the formation of the zones of inhibition in the bioactivity assays. Moreover, 'no-hit' compounds (see Table 14 Result section) were identified which indicates, that these

compounds could not be detected when matched against the database and could possibly be unknown compounds with potential novel biochemical activities. However, further analysis has to be carried out to determine the exact chemical structure and elucidate the function of the compounds in therapeutics antimicrobial context. Though the antimicrobial activity of the fungal strains was evident only for the gram negative PAO750 strain, however, due to the low yield and concentration of the isolated SMs, it cannot be conclusively said if the antimicrobial compounds exclusively target gram negative bacteria.

HPLC facilitates the resolution of the constituents of the crude extracts. The number of peaks in the chromatogram corresponded to the number of the potential compounds present in the crude mixture. HPLC analysis was carried out for WIN(M) 1238 strain to identify the potential compounds in its crude extract. Seven peaks were identified in the chromatogram including the media control peaks. However, further separation is required to resolve the peaks.

Thin layer chromatography (TLC) is used to separate compounds having different solubilities and adsorption to the two phases between which they are partitioned. TLC was used to identify the suitable solvent that manage to separate the contents of WIN(M)1238 crude extract mixture and to select the desired compounds for further analysis through the Biotage. Multiple spots on the TLC column indicate different molecules based on the polarities. In this study, it was observed that proper separation could not be obtained when using the hexane:ethylacetate solvent system as indicated by the closely crowded spots (compounds poorly resolved), while the dichloromethane:methanol solvent system resulted in clearly resolved spots. A total of 117 fractions were collected and TLC plates were performed to identify similar fractions that can be combined and evaporated. The fractions were grouped into Five categories based on the

similarities in polarities. The bioactivity of these fractions was tested using disc diffusion assay and four out of five fractions showed antimicrobial activity against the tested bacteria PAO750. In conclusion, this result suggests that the crude extract of WIN(M) 1238 may contain potential compounds with antimicrobial activity. This assay was carried out to identify the active compounds in the fractions that resulted in the inhibition. However, further investigation and studies based on mass spectrometry and structural elucidation (X-ray crystallography and/or NMR) is required to facilitate the identification and characterization of these compounds.

ITS sequences from the selected thirteen strains that showed antimicrobial activity were sequenced through Sanger sequencing and phylogenetic tree was constructed using the Mr. Bayes program, along with five strains that did not show activity. The ITS data was used to confirm the identities of the fungi that were studied and to examine if there is a link between phylogenetic position and the production of antimicrobials. For comparison, the data set contained sequences from NCBI representing five different *Leptographium* species (*Leptographium lundbergii*, *Leptographium procerum*, *Leptographium truncatum*, *Leptographium terebrantis*, *Leptographium wingfieldii*), and *Sporothrix schenckii* was selected to represent the outgroup. The node support values were used to determine the position of the strains under study in the phylogenetic tree. Strain WIN (M) 1210 (forward and reverse fragments) were noted to position within the *Leptographium procerum* clade which confirmed its identity as *Leptographium procerum*. The remaining seventeen strains under study were positioned within the clade formed by two different species, strains of *Leptographium terebrantis* and *Leptographium wingfieldii*. The latter two species are difficult to separate based on ITS data alone and additional markers are needed to resolve between these two species. However, *L. terebrantis* is usually found in the South Eastern

USA and probably rare in Canada (Hausner *et al*., 2005). Most of the fungi tested in this work were isolated from North Western Ontario or Western Canada.

Since the species not showing antimicrobial activity were clustered into the same clade as the ones that showed activity, this might indicate that the BGCs and other regulatory factors that contribute to SM production might remain cryptic/silent under certain lab conditions and further investigation is required using variable stress/culture conditions to draw conclusive results.

4.2. Concluding remarks

This study presented the opportunities for preliminary screening of novel biochemical compounds from crude extracts obtained from *Leptographium* spp. several of these strains that did not gave positive results in the bioactivity assays may not have been amenable to the selected culturing condition. Hence, for the future experiments, optimization or modification of culture condition maybe required to induce the production of the SMs. Moreover, the BGCs are often selectively activated under a specific culture condition or in the presence of an inducer or stress signal. Recently, it was observed that aflatoxin production in *Aspergillus flavus* could be induced by using variable carbon sources that altered the expression profiles of the isolates in response to oxidative stress (Fountain *et al.,*2016). Water deficit and thermal stress also effectively induced the production of SMs in xerophilic fungi, such as the production of sugar alcohols as a part of the stress response pathway in *A. flavus* (Medina *et al.*, 2015). This also indicative of the differential gene expression in response to an array of stress conditions that can be utilized to stimulate SMs in lab conditions. This information can be used to strategize experiments with variable culture conditions, using nutrient or abiotic stress cues that can lead to the production of SMs in the cultured fungi. Sometimes, the interaction between different organisms in a culture can be a key

factor in the generation of SMs as the production of a secondary metabolite in one organism takes place after sensing of a secondary metabolite produced by another organism (Sonnenbichler *et al.,* 1994). Such a strategy can also be tested for the *Leptographium* spp. strains in this study and co-culturing it with other related fungi can be an effective method for the production of new bioactive compounds.

Overall, this study indicates that *Leptographium* spp. can be utilized as a source of novel biochemical compounds having broad therapeutic potential, and some of them may also exhibit antimicrobial activities that can also be exploited in a therapeutic context. The phenotypic AST approach used in this study complemented with high throughput HPLC and LC-MS experiments can be used as a foundation for additional advanced approaches like de-replication, co-culturing, stress-induced activation of BGCs and computational mining. Most recently, a broad-spectrum antibiotic, halicin, with inhibitory effect against *Mycobaterium tuberculosis* was discovered through deep machine learning from the Drug Repurposing Hub (Stokes *et al.,* 2020). Thus, combining genotypic and phenotypic approaches, there could be a higher probability of discovering novel antimicrobial compounds from fungi, that can contribute to development of new antibiotics in this age of MDR and failure of traditional antibiotics.

References

Abdel-Hameed, M., Bertrand, R. L., Piercey-Normore, M. D., and Sorensen, J. L. (2016). Putative identification of the usnic acid biosynthetic gene cluster by de novo whole-genome sequencing of a lichen-forming fungus. *Fungal biology*, 120(3), 306-316.

Abraham, E. P., and Chain, E. (1940). An enzyme from bacteria able to destroy penicillin. *Review of Infectious Dieseas*,10, 677-678.

Adeyemo, A. A., Oluwatosin, O., and Omotade, O. O. (2016) Study of streptomycin-induced ototoxicity: protocol for a longitudinal study. *SpringerPlus*. 5(1), 758.

Allen, H. K., Moe, L. A., Rodbummer, J., and Gaarder, A. (2008). Functional metagenomics reveals diverse B-lactamases in a remote Alaskan soil. *The ISME Journal*, 3(2), 243-5.

Aminov, R. I. (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in microbiology*, *1*, 134.

Antimicrobial resistance: global report on surveillance 2014. World Health Organization; 2014. Downloaded from http://www.who.int/drugresistance/documents/surveillancereport/en/

Azerang, P., Khalaj, V., Kobarfard, F., Owlia, P., Sardari, S., and Shahidi, S. (2019). Molecular Characterization of a Fungus Producing Membrane Active Metabolite and Analysis of the Produced Secondary Metabolite. *Iran Biomed J.* 23(2), 121-128.

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6 (2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005.

Bbosa, G. S., Mwebaza, N., Odda, J., Kyegombe, D. B., and Ntale, M. (2014). Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance. *Health, 2014*.

Begley, M., Cotter, P. D., Hill, C., and Ross, R. P. (2009). Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. *Applied Environmental Microbiology*, 75, 5451-5460.

Bills, G. F., and Gloer, J. B. (2016). Biologically active secondary metabolites from the fungi. *The Fungal Kingdom*, 1087-1119.

Bisacchi, G. S. (2015) Origins of the Quinolone Class of Antibacterials: An Expanded "Discovery Story". *Medicinal Chemistry*. 58(12), 4874-82.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., and Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology* 13(1), 42–51.

Blanchette, R. A., Farrell, R. L., Burnes, T. A., Wendler, P. A., Zimmerman, W., Brush, T. S., and Snyder, R. A. (1992). Biological control in pulp and paper production by *Ophiostoma piliferum*. *Tappi Journal*. 75 (12), 102-106.

Boruta, T., and Bizukojc, M. (2017). Production of lovastatin and itaconic acid by *Aspergillus terreus*: a comparative perspective. *World Journal of Microbiology and Biotechnology*, *33*(2), 34. https://doi.org/10.1007/s11274-017-2206-9.

Burgess, J. G., Jordan, E. M., Bregu, M., Mearns-Spragg, A. and Boyd, K. G. Microbial antagonism: a neglected avenue of natural products research. (1999). *Journal of Biotechnol*ogy 70, 27–32.

Bushley, K. E., Raja, R., Jaiswal, P., Cumbie, J. S., Nonogaki, M., Boyd, A. E., Owensby, C. A., Knaus, B. J., Elser, J., Miller, D., Di, Y., McPhail, K. L., and Spatafora, J. W. (2013). The genome of *Tolypocladium inflatum*: evolution, organization, and expression of the cyclosporin biosynthetic gene cluster. *PLoS genetics*, 9(6). e1003496.

Bynum, B. (2018). Rediscovering penicillin. *Lancet*. 392,1108–1109.

Carneiro, D. G., Clarke, T., Davies, C. C., and Bailey, D. (2016) Identifying novel protein interactions: Proteomic methods, optimisation approaches and data analysis pipelines. *Methods*. 95, 46-54.

Caron, F. (2012). Antimicrobial susceptibility testing: a four facets tool for the clinician. *Journal Des Anti-Infectieux*, 14, 186-174.

CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019. DOI: http://dx.doi.org/10.15620/cdc:82532. Available at: http://www.cdc.gov/drugresistance/threat-report-2013.

Chambers, H. F., and Deleo, F. R. (2009). Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Review of Microbiol*.0 7(9), 629-41.

Chao, W., Deng, J. S., Li, P. Y., Kuo, Y. H., and Huang, G. J. (2019). Inotilone from *Inonotus linteus* suppresses lung cancer metastasis in vitro and in vivo through ROS-mediated PI3K/AKT/MAPK signaling pathways. *Scientific reports*, *9*(1), 1-13.

Coskun, O. (2016). Separation techniques: Chromatography. *North Clinical Istanbol* 3 (2), 156-160.

Courvalin, P. (2006). Vancomycin resistance in gram-positive cocci. *Clinical Infectious Disease* 42, 25-34.

Cox, G., Sieron, A., King, A. M., De Pascale, G., Pawlowski, A. C., Koteva, K. and Wright, G. D. (2017). A Common Platform for Antibiotic Dereplication and Adjuvant Discovery. *Cell Chemical Biology*, 24, 98-101.

Croucher, N. J., and Thomson, N. R. (2010). Studying bacterial transcriptomes using RNA-seq. *Current Opinion in Microbiology* 13(5), 619-24.

Cyphert, E. L., Wallat, J. D., Pokorski, J. K., von Recum, H. A. (2017). Erythromycin Modification That Improves Its Acidic Stability while Optimizing It for Local Drug Delivery. *Antibiotics (Basel),6(2), 11*.

Dai, J., Krohn, K., Flörke, U., Draeger, S., Schulz, B., Kiss-Szikszai, A., Antus, S., Kurtán, T., and Van Ree, T. (2006). Metabolites from the endophytic fungus *Nodulisporium* sp. from *Juniperus cedre*. *European journal of organic chemistry*, *2006*(15),3498-3506. https://doi.org/10.1002/ejoc.200600261.

de Beer ZW, Seifert KA, Wingfield, MJ. (2013b). A nomenclator for ophiostomatoid genera and species in the Ophiostomatales and Microascales. In: Ophiostomatoid fungi: Expanding frontiers. (Eds.) Seifert, K. A., DeBeer, Z. W., Wingfield, M. J., CBS-KNAW Fungal Biodiversity Centre, Utrecht (The Netherlands). Biodiversity Series 12: 245–322.

de Beer, Z. W., Duong, T. A., Wingfield, M. J. (2016). The divorce of Sporothrix and Ophiostoma: solution to a problematic relationship. *Studies in Mycology* 83:165-91. doi: 10.1016/j.simyco.2016.07.001.

de Beer, Z. W., Seifert, K. A., Wingfield, M. J. (2013a). The ophiostomatoid fungi: their dual position in the Sordariomycetes. In: Ophiostomatoid fungi: Expanding frontiers. Seifert, K. A., De Beer, Z. W.,Wingfield, M. J. (Eds.), CBS-KNAW Fungal Biodiversity Centre, Utrecht (The Netherlands). Biodiversity Series 12: 1–19.

De la Fuente-Nunez, C. (2019). Towards Autonomous Antibiotic Discovery. *mSystems*, 4(3), e00151-19.

DeModena, J. A., Gutierrez, S., Velasco, J., Fernandez, F. J., Fachini, R. A., Galazzo, J. L., Hughes, D.E. and Martin, J. F. (1993). The production of cephalosporin C by *Acremonium chrysogenum* is improved by the intracellular expression of a bacterial hemoglobin. *Nature Biotechnol*, 11(8), 926-929. https://doi.org/10.1038/nbt0893-926.

Dosen, I. (2016). LC-MS based analysis of secondary metabolites from *Chaetomium* and *Stachybotrys* growth in indoor environments. Department of Systems Biology, Technical University of Denmark.

Durand, G. A., Raoult, D., and Dubourg, G. (2019). Antibiotic discovery: history, methods and perspectives. *International Journal of Antimicrobial Agents*, 53(4), 371-382. https://doi.org/10.1016/j.ijantimicag.2018.11.010.

El-Elimat, T., Figueroa, M., Ehrmann, B. M., Cech, N. B., Pearce, C. J., and Oberlies, N. H. (2013). High-resolution MS, MS/MS, and UV database of fungal secondary metabolites as a dereplication protocol for bioactive natural products. *Journal of Natural Products,* 76:1709–1716.

Engelberg, R., Danielson, A., Wang, S., Singh, M., Wai, A., Sorensen, J., Duan, K., Hausner, G., and Kumar, A. (2018). Creation of a drug-sensitive reporter strain of *Pseudomonas aeruginosa* as a tool for the rapid screening of antimicrobial products. *Journal of microbiological methods*, 152, 1-6.

Fair, R. J., and Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, *6*, 2564. https://doi.org/10.4137/PMC.S14459.

Fani, F., Brotherton, M-C., Leprohon, P., and Ouellette, M. (2013). Genomic analysis and reconstruction of cefotaxime resistance in *Streptococcus pneumoniae*. *Journal of Antimicrobial Chemotherapy*. 68(8), 1718–1727.

Fernando, D., Zhanel, G., and Kumar, A. (2013). Antibiotic resistance and expression of resistance-nodulation-division pump-and outer membrane porin-encoding genes in Acinetobacter species isolated from Canadian hospitals. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *24*(1), 17-21.

Ferrie, J. E. (2014). Arsenic, antibiotics and interventions. *Int J Epidemiol.* 43(4), 977-82.

Fetzner, S. and Drees, S. L. (2013). Old molecules, new biochemistry. *Chemistry and Biology.* 20(12), 1438-40.

Fleming, A. (1945). Nobel Lecture: Penicillin.

Flieger, M., Sedmera, P., Havlíček, V., Cvak, L., and Stuchlík, J. (1993). 10-hydroxy-cis-and 10-hydroxy-trans-paspalic acid amide: New alkaloids from *Claviceps paspali*. *Journal of Natural Products*, *56*(6), 810-814.

Floss, H. G., and Yu, T. W. (2005). Rifamycin-mode of action, resistance, and biosynthesis. *Chemical Reviews* 105(2), 621-32.

Fotso, S., Zabriskie, T. M., Proteau, P. J., Flatt, P. M., Santosa, D. A., Sulastri, and Mahmud, T. (2009). Limazepines A− F, Pyrrolo [1, 4] benzodiazepine Antibiotics from an Indonesian *Micrococcus* sp. *Journal of natural products*, *72*(4), 690-695.

Foulkes, J. (2002). Elitra pharmaceuticals: new paradigms for antimicrobial drug discovery. *Drug Discovery Today, 7*(5), S12-S15. doi: 10.1016/S1359-6446(02)02175-X.

Gao, P., and Xu, G. (2015). Mass-spectrometry-based microbial metabolomics: recent developments and applications. *Analytical and Bioanalytical Chemistry.* 407(3), 669-80

Gill, K. A., Berrué, F., Arens, J. C., and Kerr, R. G. (2014). Isolation and structure elucidation of cystargamide, a lipopeptide from *Kitasatospora cystarginea*. *Journal of natural products*, 77(6), 1372-1376.

Gore-Lloyd, D., Sumann, I., Brachmann, A. O., Schneeberger, K., Ortiz-Merino, R. A., Moreno-Beltrán, M., Schläfli, M., Kirner, P., Kron, A., Rueda-Mejia, M., Somerville, V., Wolfe, K. H., Piel, J., Ahrens, C. H., Henk, D., and Freimoser, F. (2019). Snf2 controls pulcherriminic acid biosynthesis and antifungal activity of the biocontrol yeast *Metschnikowia pulcherrima*. *Molecular microbiology*, *112*(1), 317-332.

Goyal, S., Ramawat, K. G., and Mérillon, J. M. (2016). Different shades of fungal metabolites: an overview. *Fungal metabolites. Reference series in phytochemistry. Springer, Cham*, 1-29.

Gröger, H., Pieper, M., König, B., Bayer, T., and Schleich, H. (2017). Industrial landmarks in the development of sustainable production processes for the β-lactam antibiotic key intermediate 7-aminocephalosporanic acid (7-ACA) Sustainable *Chemistry and Pharm*acy 5, 72–79.

Han, Y., Li, Y., Shen, Y., Li, J., Li, W., and Shen, Y. (2013). Oxoprothracarcin, a novel pyrrolo [1, 4] benzodiazepine antibiotic from marine *Streptomyces* sp. M10946. *Drug discoveries* and *therapeutics*, *7*(6), 243-247.

Hausner, G., Eyjólfsdóttir, G. G., and Reid, J. (2003). Three new species of *Ophiostoma* and notes on *Cornuvesica falcata*. *Candian Journal of Botany,* 81, 40-48.

Hausner, G., Iranpour, M., Kim, J. J., Breuil, C., Gibb, E. A., Loewen, P. C., Hopkin, A. A. (2005). Fungi vectored by the introduced bark beetle *Tomicus piniperda* in Ontario, Canada, and comments on the taxonomy of *Leptographium lundbergii*, *Leptographium*

terebrantis, *Leptographium truncatum*, and *Leptographium wingfieldii*. *Candian Journal of Botany*, 83 (10), 1222-1237.

Hausner, G., Reid, J., and Klassen, G. R. (1992). Do galeate-ascospore members of the Cephaloascaceae, Endomycetaceae and Ophiostomataceae share a common phylogeny?. *Mycologia*, 84(6), 870-881.

Hollenbeck, B. L., and Rice, L. B. (2012). Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence.* 3(5):421-33.

Holloway, B. W. (1955). Genetic recombination in *Pseudomonas aeruginosa*. *Microbiology, 13*(3), 572-581.

Hooper, D. C. (2002). Fluoroquinolone resistance among Gram-positive cocci. *Lancet Infectious Disease.* 2(9), 530-8.

Horváth, G., Bencsik, T., Ács, K., & Kocsis, B. (2016). Sensitivity of ESBL-producing gram-negative bacteria to essential oils, plant extracts, and their isolated compounds. *Academic Press, Amsterdam*, 239-269.

Huang, G. J., Huang, S. S., and Deng, J. S. (2012). Anti-inflammatory activities of inotilone from *Phellinus linteus* through the inhibition of MMP-9, NF-κB, and MAPK activation in vitro and in vivo. PloS one, 7(5). PLoS ONE 7(5): e35922.

Imhoff, J. F., Labes, A., and Wiese, J. (2011). Bio-mining the microbial treasures of the ocean: new natural products. *Biotechnology advances*, *29*(5), 468-482.

Jacobs, K., and Wingfield, M.J. (2001). *Leptographium* species: tree pathogens, insect associates and agents of blue-stain. APS Press Saint Paul, MN.

Jacobs, K., Bergdahl, D. R., Wingfield, M. J., Halik, S., Seifert, K. A., Bright, D. E., and Wingfield, B. D. (2004). *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. *Mycological Research*, 108: 411-418.

James, R. C., Pierce, J. G., Okano, A., Xie, J., and Boger, D. L. (2012) Redesign of glycopeptide antibiotics: back to the future. *ACS Chemical Biology* 7(5), 797-804.

Jankowiak, R., Ostafińska, A., Aas, T., Solheim, H., Bilanksi, P., Linnakosi, R., and Hausner, G. (2018). Three new *Leptographium* spp. (Ophiostomatales) infecting hardwood trees in Norway and Poland. *Antonie Van Leeuwenhoek*. 111(12), 2323-2347. doi:10.1007/s10482-018-1123-8.

Jiménez-Esquilín, A. E., and Roane, T. M. (2005). Antifungal activities of *actinomycete* strains associated with high-altitude Sagebrush Rhizosphere. *Journal of Industrial Microbiology Biotechnol.*, 32, 378-381.

Jorgensen, J. H., and Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical Infectious Diseases*, 49(11):1749-1755.

Katoh, K., Rozewicki, J., and Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics*, *20*(4), 1160-1166.

Khan, Z. A., Siddiqui, M. F., & Park, S. (2019). Current and Emerging Methods of Antibiotic Susceptibility Testing. *Diagnostics (Basel, Switzerland)*, 9(2), 49. https://doi.org/10.3390/diagnostics9020049.

Kim, S., Harrington, T. C., Lee, J. C., and Seybold, S. J. (2011) *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle *Hylurgus ligniperda* in California. *Mycologia*, 103:152–163. doi:10.3852/10-096.

King, A., Reid-Yu, S., Wang, W., King, D. T., De Pascale, G., Strynadka, N. C., Walsh, T. R., Coombes, B., and Wright, G. D. (2014). Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature*, 510, 503–506. https://doi.org/10.1038/nature13445.

Kochanowska-Karamyan, A. J., and Hamann, M. T. (2010). Marine indole alkaloids: potential new drug leads for the control of depression and anxiety. *Chemical reviews*, 110(8), 4489-4497.

Kumar, A., Chua, K. L., and Schweizer, H. P. (2006). Method for regulated expression of single-copy efflux pump genes in a surrogate *Pseudomonas aeruginosa* strain: identification of the BpeEF-OprC chloramphenicol and trimethoprim efflux pump of *Burkholderia pseudomallei* 1026b. *Antimicrobial Agents and Chemotherapy*, 50(10), 3460-3463.

Kurma, S. H., Karri, S., Kuncha, M., Sistla, R., and Bhimapaka, C. R. (2020). Synthesis and anti-inflammatory activity of 2-oxo-2H-chromenyl and 2H-chromenyl-5-oxo-2, 5-dihydrofuran-3-carboxylates. *Bioorganic & Medicinal Chemistry Letters*, 127341.

Land, M., Hauser, L., Jun, S. R., Nookaew, I., Leuze, M. R., Ahn, T. H., Karpinets, T., Lund, O., Kora, G., Wassenaar, T., Poudel, S., and Ussery, D. W. (2015) Insights from 20 years of bacterial genome sequencing.. *Functional and Integrative Genomics,* 15(2), 141-61.

Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics,* 30(22), 3276-3278.

Lewis, K. (2013). Platforms for antibiotic discovery. *Nat Rev Drug Discov*, 12, 371-387.

Li Y., Fan P., Zhou S., Zhang L. (2017). Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microbial Pathogenesis*, 107, 54–61.

Ligon, B. L. (2004). Penicillin: its discovery and early development. *Seminars in Pediatric Infectious Diseases,* 15(1), 52-7.

Lindon, J. C., Nicholson, J. K. (2008). Analytical technologies for metabonomics and metabolomics, and multi-omic information recovery. *Trends in Analytical Chemistry*, 27, 194–204.

Liu, F., and Myers, A. G. (2016). Development of a platform for the discovery and practical synthesis of new tetracycline antibiotics. *Current Opinions in Chemical Biology,* 32, 48-57.

Liu, X-W., Wang, H-M., Lu, Q., and Decock, C. (2017). Taxonomy and pathogenicity of *Leptographium* species associated with *Ips subelongatus* infestations of *Larix* spp. in northern China, including two new species. *Mycological Progress*. 16 (1), 1-13.

Liu, X. Q., Liu, H. X., Li, Y. Q., and Hao, C. J. (2016). High prevalence of beta-lactamase and plasmid-mediated quinolone resistance genes in extended-spectrum cephalosporin-resistant *Escherichia coli* from dogs in Shaanxi, China. *Frontiers in Microbiol*ogy, 7, 1843.

Lodhi, A. F., Zhang, Y., Adil, M., and Deng, Y. (2018). Antibiotic discovery: combining isolation chip (iChip) technology and co-culture technique. *Applied Microbiology and Biotechnology*, 102(17), 7333-7341.

Long, K. S., Poehlsgaard, J., Kehrenberg, C., Schwarz, S., and Vester, B. (2006) The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrobial Agents and Chemotherapy,* 50(7), 2500-2505.

Magaldi, S., Mata-Essayag, S., de Capriles, C. H., Perez, C., Colella, M. T., and Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8, 39-45. https://doi.org/10.1016/j.ijid.2003.03.002.

Mahajan, G., and Balachandran, L. (2015). Biodiversity in production of antibiotics and other bioactive compounds. *Advances in Biochemical Engineering/Biotechnol*, 147:37-58. doi:10.1007/10_2014_268.

Marin-Felix, Y., Groenewald, J. Z., Cai, L., Chen, Q., Marincowitz, S., Barnes, I., ... and De Beer, Z. W. (2017). Genera of phytopathogenic fungi: GOPHY 1. *Studies in mycology*, *86*, 99-216.

Mast, Y., and Wohlleben, W. (2014) Streptogramins - two are better than one!. *International Journal of Medical Microbiology*, 304(1), 44-50.

McGettigan, P. A. (2013). Transcriptomics in the RNA-seq era. *Current Opinions in Chemical Biology*, 17(1), 4-11.

Michael, C. A., Dominey-Howes, D., and Labbate, M. (2014). The antimicrobial resistance crisis: causes, consequences, and management. *Front Public Health*. 2, 145.

Mills, S. D. (2006). When will the genomics investment pay off for antibacterial discovery?. *Biochemal Pharmacology*, 71(7), 1096-102.

Milshteyn, A., Schneider, J. S., and Brady, S. F. (2014). Mining the metabiome: identifying novel natural products from microbial communities. *Chemistry and Biology*, 21(9), 1211-1223.

Moellering, R. C. Jr. (2012). MRSA: the first half century. *Journal of Antimicrobial Chemotherapy*, 67(1), 4-11.

Mooi, C. M. Y., Koh, S. P., and Long, K. (2015). Simultaneous detection and quantification of zeatin and kinetin in coconut water using ultra performance liquid chromatography coupled with a simple step solid phase extraction. *Journal of analytical chemistry*, 70(7), 819-824.

Munita, J. M., and Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology spectrum*. 4(2): 10.1128/microbiolspec.VMBF-0016-2015.

Munteanu, F. D., Titoiu, A. M., Marty, J. L., and Vasilescu, A. (2018). Detection of antibiotics and evaluation of antibacterial activity with screen-printed electrodes. *Sensors (Basel, Switzerland*, *18*(3), 901. https://doi.org/10.3390/s18030901.

Murray, E. M., Allen, C. F., Handy, T. E., Huffine, C. A., Craig, W. R., Seaton, S. C., and Wolfe, A. L. (2019). Development of a Robust and Quantitative High-Throughput Screening Method for Antibiotic Production in Bacterial Libraries. *ACS omega*, 4(13), 15414-15420. https://doi.org/10.1021/acsomega.9b01461.

Nagana, Gowda., and GA, Raftery, D. (2015). Can NMR solve some significant challenges in metabolomics? *Journal of Magnetic Resonance*, 260, 144-60.

Newsom, S. W. (2003). Pioneers in infection control-Joseph Lister. *Journal of Hospitao Infection*, 55(4), 246-53.

Nielsen, J. C., Grijseels, S., Prigent, S., Ji, B., Dainat, J., Nielsen, K. F., Frisvad, J. C., Workman, M., and Nielsen, J. (2017). Global analysis of biosynthetic gene clusters reveals vast potential of secondary metabolite production in *Penicillium* species. *Nature microbiology*, 2(6), 1-9.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews*, 67(4), 593-656.

Onaka, H., Mori, Y., Igarashi, Y. and Furumai, T. (2011). Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces species*. *Applied and Environmental Microbiology*, 77, 400–406.

Osugi, A., Kojima, M., Takebayashi, Y., Ueda, N., Kiba, T., and Sakakibara, H. (2017). Systemic transport of trans-zeatin and its precursor have differing roles in *Arabidopsis* shoots. *Nature plants*, *3*(8), 1-6.

Pan, Y., Zhao, T., Krokene, P., Yu, Z. F., Qiao, M., Lu, J., Chen, P., and Ye, H. (2018). Bark beetle-associated blue-Stain fungi increase antioxidant enzyme activities and monoterpene concentrations in *Pinus yunnanensis*. *Frontiers in plant science*. 9, 1731. https://doi.org/10.3389/fpls.2018.01731.

Patel, G., Patil, M. D., Soni, S., Khobragade, T. P., Chisti, Y., and Banerjee, U. C. (2016). Production of mycophenolic acid by *Penicillium brevicompactum*-A comparison of two methods of optimization. *Biotechnology reports (Amsterdam, Netherlands)*, *11*, 77–85. https://doi.org/10.1016/j.btre.2016.07.003.

Pećanac, M., Janjić, Z., Komarcević, A., Pajić, M., Dobanovacki, D., and Misković, S. S. (2013). Burns treatment in ancient times. *Medicinski Pregled*, 66, 263-267.

Piddock, L. J. (2015). Teixobactin, the first of a new class of antibiotics discovered by iChip technology? *Journalof Antimicrobial Chemotherapy*, 70(10), 2679-80.

Piechaud, M., and Second, L. (1951, January). Studies of 26 strains of *Moraxella Iwoffi*. In *Annales de l'Institut Pasteur*, 80(1), 97.

Pohl, C., Polli, F., Schütze, T., Viggiano, A., Mózsik, L., Jung, S., de Vries, M., Bovenberg, R. A. L., Meyer, V. and Driessen, A. J. (2020). A *Penicillium rubens* platform strain for secondary metabolite production. *Scientific reports*, 10(1), 1-16

Pusztahelyi, T., Holb, I. J., & Pócsi, I. (2015). Secondary metabolites in fungus-plant interactions. *Frontiers in plant science*, *6*, 573.

Queenan, A. M., and Bush, K. (2007). Carbapenemases: the versatile beta-lactamases. *Clinical Microbiology Reviews*, 20(3), 440-58.

Ramirez, M. S., and Tolmasky, M. E. (2010). Aminoglycoside modifying enzymes. *Drug Resistance Updat*es, 13(6), 151-71.

Randazzo, P., Aubert-Frambourg, A., Guillot, A., and Auger, S. (2016). The MarR-like protein PchR (YvmB) regulates expression of genes involved in pulcherriminic acid biosynthesis and in the initiation of sporulation in *Bacillus subtilis*. *BMC microbiology*, 16(1), 190.

El-Gamal MI, Brahim I, Hisham N, Aladdin R, Mohammed H, Bahaaeldin A. (2017). Recent updates of carbapenem antibiotics. *European Journal of Medical Chemistry*, 131, 185-195.

Ribeiro da Cunha, B., Fonseca, L. P., and Calado, C. R. (2019). Antibiotic discovery: where have we come from, where do we go? *Antibiotics*, *8*(2), 45.

Rice, A. V., Thormann, M. N., and Langor, A. V. (2008). Mountain pine beetle-associated blue-stain fungi are differentially adapted to boreal temperatures. *Forest Pathology*, 38, 113-123.

Roberts, M. C. (2008). Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. *FEMS Microbiology Letters*, 282(2), 147-59.

Ronquist, F., and Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12), 1572-1574.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Bret Larget, Liang Liu, Marc A. Suchard, John P., and Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology*, 61(3), 539-542.

Rossolini, G. M., Arena, F., Pecile, P., and Pollini, S. (2014). Update on the antibiotic resistance crisis. *Current Opinions in Pharmacol*ogy, 18, 56-60.

Sandra, K., and Sandra, P. (2013). Lipidomics from an analytical perspective. *Current Opinions in Chemical Biology*, 17(5), 847-53.

Saravolatz, L.D., Markowitz, N., Arking, L., Pohlod, D., and Fisher, E. (1982). Methicillin-resistant *Staphylococcus aureus*: epidemiologic observations during a community-acquired outbreak. *Annals of Internal Medicine*, *96*(1), 11-16.

Schiff Jr, P. L. (2006). Ergot and its alkaloids. *American journal of pharmaceutical education*, 70(5): 98.

Sedmak, J. J., Weerasinghe, D. K., and Jolly, S. O. (1990). Extraction and quantitation of astaxanthin from *Phaffia rhodozyma*. *Biotechnology techniques*, 4(2), 107-112. https://doi.org/10.1007/BF00163282

Senyuva, H. Z., Gilbert, J., and Hutton, S. (2007). Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC/TOF-MS–A New Approach to Fungal Characterization. Applications (Food), Agilent Technologies.

Senyuva, H. Z., Gilbert, J., and Hutton, S. (2007). Rapid Analysis of Crude Fungal Extracts for Secondary Metabolites by LC/TOF-MS–A New Approach to Fungal Characterization. Applications (Food), Agilent Technologies.

Sievert, D. M., Rudrik, J. T., Patel, J. B., McDonald, L. C., Wilkins, M. J., and Hageman, J. C. (2008). Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002-2006. *Clin Infect Dis*. 46(5), 668-74.

Silva, E. M. S., Silva, I. R. D., Ogusku, M. M., Carvalho, C. M., Maki, C. S., and Procópio, R. E. D. L. (2018). Metabolites from endophytic *Aspergillus fumigatus* and their in vitro effect against the causal agent of tuberculosis. *Acta Amazonica*, 48(1), 63-69.

Singh, M., Yau, Y. C., Wang, S., Waters, V., and Kumar, A. (2017). MexXY efflux pump overexpression and aminoglycoside resistance in cystic fibrosis isolates of *Pseudomonas aeruginosa* from chronic infections. *Canadian journal of microbiology*, 63(12), 929-938.

Sonnenbichler, J., Dietrich, J. and Peipp, H. (1994). Secondary fungal metabolites and their biological activities, V. Investigations concerning the induction of the biosynthesis of toxic secondary metabolites in basidiomycetes. *Biological Chemistry Hoppe-Seyler*, 375, 71–79.

Stierle, A., Strobel, G., and Stierle, D. (1993). Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science*, *260*(5105), 214-216. www.jstor.org/stable/2881310.

Stokes, J. M., Yang, K., Swanson, K., Jin, W., Cubillos-Ruiz, A., Donghia, N. M., MacNair, C. R., French, S., Carfrae, L. A., Bloom-Ackermann, Z., Tran, V. M., Chiappino-Pepe, A., Badran, A. H., Andrews, I. W., Chory, E. J., Church, G. M., Brown, E. D., Jaakkola, T. S., Barzilay, R., and Collins, J. J. (2020). A deep learning approach to antibiotic discovery. *Cell*, *180*(4), 688-702. https://doi.org/10.1016/j.cell.2020.04.001.

Strebhardt, K., and Ullrich, A. Paul Ehrlich's magic bullet concept: 100 years of progress. *Nature Reviews in Cancer*. 8(6), 473-80.

Sugiyama, Y., Ito, Y., Suzuki, M., and Hirota, A. (2009). Indole derivatives from a marine sponge-derived yeast as DPPH radical scavengers. *Journal of natural products*, *72*(11), 2069-2071.

Tamae, C., Liu, A., Kim, K., Sitz, D., Hong, J., Becket, E., Bui, A., Solaimani, P., Tran, K. P., Yang, H., and Miller, J. H. (2008). Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *Journal of Bacteriology*, 190(17), 5981-5988.

Taylor, S. D., and Palmer, M. (2016). The action mechanism of daptomycin. *Bioorganic Medical Chemistry*, 24(24), 6253-6268.

Teasdale, M. E., Donovan, K. A., Forschner-Dancause, S. R., and Rowley, D. C. (2011). Gram-positive marine bacteria as a potential resource for the discovery of quorum sensing inhibitors. *Marine biotechnology*, *13*(4), 722-732.

Tortorella, E., Tedesco, P., Palma Esposito, F., January, G. G., Fani, R., Jaspars, M., and De Pascale, D. (2018). Antibiotics from deep-sea microorganisms: current discoveries and perspectives. *Marine drugs*, *16*(10), 355.

Ueda, K., and Beppu, T. (2017). Antibiotics in microbial coculture. *Journal of Antibiotics (Tokyo)*. 70(4), 361-365.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therpuetics*, 40(4), 277-83.

Vihervaara, T., Suoniemi, M., and Laaksonen, R. (2014) Lipidomics in drug discovery. *Drug Discov Today*. 19(2), 164-70.

Wainwright M., and Kristiansen J. E. (2011). On the 75th anniversary of Prontosil. *Dyes and Pigments*, 8, 231–234,

Wainwright, M. (1991). Streptomycin: discovery and resultant controversy. *History of Philosophical Life Sciences*, 13(1), 97-124.

Waksman, S. (1947). Microbial antagonisms and antibiotic substances. Oxford University Press, Oxford, UK.

Walsh, C. (2003). Antibiotics: Actions, Origins, Resistance. American Society for Microbiology (ASM) Press; Washington, DC

Wang, Z., Chen, T., Gao, Y., Breuil, C. and Hiratsuka, Y. (1995). Biological degradation of resin acids in wood chips by wood inhabiting fungi. *Applied Environmental Microbiology*, 61, 222–225.

Watve, M. G., Tickoo, R., Jog, M. M., and Bhole, B. D. (2001). How many antibiotics are produced by the genus *Streptomyces*? *Archives of microbiology*, 176(5), 386-390.

Weisblum, B. (1995). Erythromycin resistance by ribosome modification. *Antimicrobial Agents in Chemotherapy*, 39(3), 577-85.

Wiest, D. B., Cochran, J. B., and Tecklenburg, F. W. J. (2012). Chloramphenicol toxicity revisited: a 12-year-old patient with a brain abscess., *Pediatric Pharmacology and Therapeutics*, 17(2), 182-8.

Williams, K. J. (2009). The introduction of 'chemotherapy' using arsphenamine - the first magic bullet. *Journal of the Royal Society of Medicine*, 102(8), 343-8.

Williston, E. H., Zia-Walrath, P., and Youmans, G. P. (1947). Plate methods for testing antibiotic activity of *Actinomycetes* against virulent human type tubercle bacilli. *Journal of Bacteriology*, 54, 563-568.

Wingfield, M. J. (2000). *Leptographium* Species: Tree Pathogens, Insect Associates, And Agents of Blue-stain. In *APS Press.*

Woodruff, H. B. (2014). Selman, A. Waksman, winner of the 1952 Nobel Prize for physiology or medicine. *Appl Environ Microbiol.* 80(1), 2-8.

Zhang, D., Zhao, L., Wang, L., Fang, X., Zhao, J., Wang, X., Li, l., Liu, H., Wei, Y., You, X., Cen, S. and Yu, L. (2017). Griseofulvin derivative and indole alkaloids from *Penicillium griseofulvum* CPCC 400528. *Journal of natural products*, 80(2), 371-376.

Zhang, H., Deng, Z., Luo, D., Guo, Z., Peng, Y., and Zou, K. (2016). Secondary Metabolites from the Endophytic Fungus *Penicillium citrinum*. *Chemistry of Natural Compounds*, 52(2), 304-305.

Zhou, X., de Beer, Z. W., & Wingfield, M. J. (2006). DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae sp. nov*., associated with pine bark beetles in South Africa. *Studies in Mycology*, *55*, 269-277.

Zipfel, R. D., de Beer, Z. W., Jacobs, K., Wingfield, B. D., and Wingfield, M. J. (2006). Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Studies in mycology*, 55, 75–97. https://doi.org/10.3114/sim.55.1.75.

Appendix (Supplementary (S) information, tables and figures)

Item 1: Tables contain crude extracts yields amounts, sequences obtained from the NCBI database and analysis of ITS sequences for members of the genus *Leptographium*.

Table S1: Indicating the net weights of the crude extracts for 100 strains of *Leptographium*.

Table S2: Sequences obtained from the NCBI database

Table S3: ITS sequences for selected members of the genus *Leptographium* spp.

Item 2: Optimization of fungal growth culture/conditions and extraction methods

2.1. Culture media

2.2. Extraction methods

Item 3: Optimization of the antibiotic resistance platform (ARP) to be used to screen for novel antibiotics isolation from fungi

3.1. ARP: Agar Overlay Method

3.2 ARP: Spent-Media Infusion Method3.3 ARP: High throughput (96-well plate)

3.4 ARP: Agar plug assay

Item 4: Combinations of extracts from different *Leptographium wingfieldii* strains and test their antimicrobial activity in different combinations.

Item 5: Identification of potential compounds within the crude fungal extracts using Liquid chromatography–mass spectrometry (LC-MS)

Item 1: Tables contain crude extracts yields amounts, sequences obtained from the NCBI database and analysis of ITS sequences for members of the genus *Leptographium*.

Table S1: Indicating the net weights of the crude extracts

Fungal strains WIN (M)	Net weight/mg	Fungal strains WIN (M)	Net weight/mg
1202	23	1265	26.8
1204	39.3	1266	30.3
1205	32.5	1267	63.5
1206	41.9	1268	58.4
1207	48.8	1269	22.3
1208	97.4	1270	33.6
1209	53.7	1271	28.2
1210	35.3	1272	67
1211	38	1273	30.5
1212	33	1275	33.5
1214	11.2	1276	30.6
1215	31.7	1277	27.5
1216	29.2	1278	39.9
1217	21.2	1279	30.3
1218	26.5	1280	76.1
1227	62.3	1281	15.8
1228	17.7	1282	80.2
1230	26.2	1283	34.9
1231	12.6	1284	39
1232	30.8	1285	48
1234	26.1	1286	21.5
1235	31.9	1287	34
1236	27.9	1288	31.4
1237	26.4	1290	23.9

1238	17.6	1291	17.5
1239	27.1	1293	18.8
1240	20.5	1294	30.6
1241	34.3	1295	38.1
1242	12.4	1296	34.8
1243	43.6	1297	71.3
1244	34.7	1298	52.8
1245	24.5	1301	34.7
1246	62	1302	42.2
1247	27.3	1303	45.7
1248	18.8	1304	48.4
1249	35.9	1306	52.3
1250	78.2	1307	26.4
1253	22.6	1311	80.8
1254	27.6	1312	5.7
1255	56.3	1321	31.7
1256	26.3	1322	39
1257	33.5	1323	31.3
1258	105	1324	34.6
1259	35.4	1327	29.8
1260	39.3	1330	41.9
1261	43	1331	54.1
1262	21.3	1335	39.3
1263	39.2	1342	29.5
1264	31.2	1375	31.3

Table S2: Sequences obtained from the NCBI database for ITS phylogenetic analysis (see Figure 7).

Organism name	**NCBI accession number**	**Organism name**	**NCBI accession number**
Sporothrix schenckii	MH165366.1	*Leptographium procerum*	JX444637.1
Sporothrix schenckii	MG825894.1	*Leptographium procerum*	JX444636.1
Sporothrix schenckii	MG825893.1	*Leptographium procerum*	JX444635.1
Grosmannia aurea	AY935606.1	*Leptographium procerum*	JX444583.1
Grosmannia aurea	KF748108.1	*Leptographium procerum*	JX444582.1
Leptographium procerum	JN618184.1	*Leptographium procerum*	JX444581.1
Leptographium procerum	AY935621.1	*Leptographium procerum*	JX028561.1
Leptographium procerum	AY935620.1	*Leptographium procerum*	JX028560.1
Leptographium procerum	AY935619.1	*Leptographium procerum*	JX028559.1
Leptographium procerum	AY935618.1	*Leptographium procerum*	JF440600.1
Leptographium procerum	AY935617.1	*Leptographium procerum*	KX099690.1
Leptographium procerum	AY935616.1	*Leptographium procerum*	KM435339.1
Leptographium procerum	AY935615.1	*Leptographium procerum*	JN618185.1
Leptographium procerum	AY935614.1	*Leptographium wingfieldii*	AY935599.1
Leptographium procerum	AY935613.1	*Leptographium wingfieldii*	AY935612.1
Leptographium procerum	EU879143.1	*Leptographium wingfieldii*	AY935611.1
Leptographium procerum	AF224327.1	*Leptographium wingfieldii*	AY935610.1
Leptographium procerum	MH859481.1	*Leptographium wingfieldii*	AY935608.1
Leptographium procerum	MH859382.1	*Leptographium wingfieldii*	AY935604.1
Leptographium procerum	MH858341.1	*Leptographium wingfieldii*	AY935603.1

Leptographium procerum	KU319005.1	*Leptographium wingfieldii*	AY935602.1
Leptographium procerum	KU319004.1	*Leptographium wingfieldii*	AY935600.1
Leptographium procerum	JQ289014.1	*Leptographium lundbergii*	JX444632.1
Leptographium wingfieldii	EU879154.1	*Leptographium lundbergii*	JX444580.1
Leptographium wingfieldii	EU879153.1	*Leptographium lundbergii*	JX444579.1
Leptographium wingfieldii	EU879152.1	*Leptographium lundbergii*	JX444578.1
Leptographium wingfieldii	JX444639.1	*Leptographium lundbergii*	JX028558.1
Leptographium wingfieldii	KP691916.1	*Leptographium lundbergii*	JX028557.1
Leptographium wingfieldii	HM363160.1	*Leptographium lundbergii*	KX099638.1
Leptographium wingfieldii	DQ539504.1	*Leptographium lundbergii*	KF748121.1
Leptographium wingfieldii	DQ539503.1	*Leptographium terebrantis*	AY935609.1
Leptographium wingfieldii	DQ539502.1	*Leptographium terebrantis*	AY935607.1
Leptographium lundbergii	AY935590.1	*Leptographium terebrantis*	AY935605.1
Leptographium lundbergii	AY935589.1	*Leptographium terebrantis*	AY935598.1
Leptographium lundbergii	AY935588.1	*Leptographium terebrantis*	AY935597.1
Leptographium lundbergii	AY935587.1	*Leptographium terebrantis*	KF293983.1
Leptographium lundbergii	AY935586.1	*Leptographium truncatum*	MH861925.1
Leptographium lundbergii	AY935585.1	*Leptographium truncatum*	KF748122.1
Leptographium lundbergii	AY935584.1	*Leptographium truncatum*	AY935627.1
Leptographium lundbergii	AY935583.1	*Leptographium truncatum*	AY935626.1
Leptographium lundbergii	EU879151.1	*Leptographium truncatum*	AY935625.1
Leptographium lundbergii	MH855083.1	*Leptographium truncatum*	AY935601.1
Leptographium lundbergii	MH855082.1	*Leptographium truncatum*	AY935595.1

Leptographium lundbergii	KU319002.1	*Leptographium truncatum*	AY935594.1
Leptographium lundbergii	JX444634.1	*Leptographium truncatum*	AY935593.1
Leptographium lundbergii	JX444633.1	*Leptographium truncatum*	AY935592.1
Leptographium truncatum	AY935591.1	*Leptographium truncatum*	JX028562.1
Leptographium truncatum	AY935582.1	*Leptographium truncatum*	JX444587.1
Leptographium truncatum	AY935581.1	*Leptographium truncatum*	JX444586.1
Leptographium truncatum	KU319006.1	*Leptographium truncatum*	JX028563.1
Leptographium truncatum	JX444638.1		

Table S3: ITS sequences obtained during this study for selected members of the genus *Leptographium*.

Fungi	WIN(M) /TOM number	Sequences	Bioactivity
Leptographium sp.	WIN(M)1206/TOM 56.27	>Leptographium WIN(M)1206 TAGCGAGTTCACAGTGACTCCCAACCCGTGCAAACCTTA CCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGC CGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGG TTGGACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCC GCTCCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTA TCGTCTGAGCAAAACCACAGAATCGTTAAAACTTTCAAC AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC GAACTGCGATAAGTAATGCGAATTGCAGAATTCAGCGA GCCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCAT TCTGGCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCA CGCAGCGCGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCA GGCCTGCGCCCAGCGCAGGCCGCCGCAGCCCCCGAAAG CCAGTGGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAG CATCACGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTG CCCCACAGACCGGCAGACGCGAGTCTGCCTCCTCTCAA	Detected
Leptographium sp.	WIN(M)1208/ TOM 62.53	>Leptographium WIN(M)1208 AGCGAGTTCACAGTGACTCCCAACCCGTGCAAACCTTAC CGCATCCTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGCC GGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGGG TTGGACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCC GCTCCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTA TCGTCTGAGCAAAACCACAGAATCGTTAAAACTTTCAAC AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC GAACTGCGATAAGTAATGCGAATTGCAGAATTCAGCGA GCCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCAT TCTGGCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCA CGCAGCGCGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCA GGCCTGCGCCCAGCGCAGGCCGCCGCAGCCCCCGAAAG CCAGTGGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAG CATCACGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTG CCCCACAGACCGGCAGACGCGAGTCTGCCTCN	Not detected
Leptographium winfieldii	WIN(M)1268/ TOM 78.22 04	>Leptographium WIN(M)1268 GCGAGTTCACAGTGACTCCCAACCCGTGCAAACCTTACC GCATCCTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGCCG GGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGGTT GGACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCCGC TCCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTATC GTCTGAGCAAAACCACAGAATCGTTAAAACTTTCAACAA CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA ACTGCGATAAGTAATGCGAATTGCAGAATTCAGCGAGCC ATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT	Detected

		GGCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCACG CAGCGCGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCAGG CCTGCGCCCAGCGCAGGCCGCCGCAGCCCCCGAAAGCC AGTGGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAGCA TCACGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTGCC CCACAGACCGGCAGACGCGAGTCTGCCTCCTN	
Leptographium wingfieldii	WIN(M)1278/ TOM 80.21	>Leptographium WIN(M)1278 AATGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGG GATCATTAGCGAGTTCACAGTGACTCCCAACCCGTGCAA ACCTTACCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCT TCCTGCCGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGG GGGGGGTTGGACGGGCGCCCGCCCGCCGGGGGTGCGGC GCGGCCGCTCCCTCTCGCCGCGAACCTTCTTTGCAGTAT AATTGTATCGTCTGAGCAAAACCACAGAATCGTTAAAAC TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA ACGCAGCGAACTGCGATAAGTAATGCGAATTGCAGAAT TCAGCGAGCCATCGAATCTTTGAACGCACATTGCGCCCG CCAGCATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTC CTCCCTCACGCAGCGCGCCTGCGTGGTGTTGGGGTGTTC TGCGGCCAGGCCTGCGCCCAGCGCAGGCCGCCGCAGCC CCCGAAAGCCAGTGGCGGGCCGGCAGCGGGCTCCGAGC GCAGTAAGCATCACGCCCTCGCTCTGGACGCTCCCGCCT GCGCCCTGCCCCACAGACCGGCAGACGCGAGTCTGCCTC CTCT	Detected
Leptographium sp.	WIN(M)1327/ TOM 90.20	>Leptographium WIN(M)1327 GAGTTCACAGTGACTCCCAACCCGTGCAAACCTTACCGC ATCCTTTCTGAGAGACGAGCGCCCGTTGCTTCCTGCCGG GCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGGTTG GACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCCGCT CCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTATCG TCTGAGCAAAACCACAGAATCGTTAAAACTTTCAACAAC GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAA CTGCGATAAGTAATGCGAATTGCAGAATTCAGCGAGCCA TCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTG GCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCACGC AGCGCGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCAGGC CTGCGCCCAGCGCAGGCCGCCGCAGCCCCCGAAAGCCA GTGGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAGCAT CACGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTGCCC CACAGACCGGCAGACGCGAGTCTGCCTCC	Detected
Leptographium sp.	WIN(M)1290/ TOM 81.16	>Leptographium WIN(M)1290 CCGGAAAGTTATCCAAACTCGGTCATTTAGAGGAAGTAA AAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGG GATCATTAGCGAGTTCACAGTGACTCCCAACCCGTGCAA ACCTTACCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCT TCCTGCCGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGG	Not Detected

		GGGGGGTTGGACGGGCGCCCGCCCGCCGGGGGTGCGGC GCGGCCGCTCCCTCTCGCCGCGAACCTTCTTTGCAGTAT AATTGTATCGTCTGAGCAAAACCACAGAATCGTTAAAAC TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA ACGCAGCGAACTGCGATAAGTAATGCGAATTGCAGAAT TCAGCGAGCCATCGAATCTTTGAACGCACATTGCGCCCG CCAGCATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTC CTCCCTCACGCAGCGCGCCTGCGTGGTGTTGGGGTGTTC TGCGGCCAGGCCTGCGCCCAGCGCAGGCCGCCGCAGCC CCCGAAAGCCAGTGGCGGGCCGGCAGCGGGCTCCGAGC GCAGTAAGCATCACGCCCTCGCTCTGGACGCTCCCGCCT GCGCCCTGCCCCACAGACCGGCAGACGCGAGTCTGCCTC CTTCTCAAGGTTGACCTCGGATCAGGTAGGACTACCCGC TGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACC AACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGCAA CAGCTCAGATTTGGAATCTGGCCCCCAGGGCCCGAGTTG TAATCTGGAGAGGATGCTTCTGGCGCGGCGCCTTCCGAG TTCCCTGGAACGGGACGCCGGAGAGGGTGAGAGCCCCG TACGGTTGGACGCCTAGCCTTTGTGAAGCTCCTTCGACG AGTCGAGTAGTTTGGGAATGCTGCTCAAAAT	
Leptographium sp.	WIN(M)1293/ TOM 82.17	>Leptographium WIN(M)1293 AAGTTATCCAAACTCGGTCATTTAGAGGAAGTAAAAGTC GTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCAT TAGCGAGTTCACAGTGACTCCCAACCCGTGCAAACCTTA CCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGC CGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGG TTGGACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCC GCTCCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTA TCGTCTGAGCAAAACCACAGAATCGTTAAAACTTTCAAC AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC GAACTGCGATAAGTAATGCGAATTGCAGAATTCAGCGA GCCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCAT TCTGGCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCA CGCAGCGCGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCA GGCCTGCGCCCAGCGCAGGCCGCCGCAGCCCCGAAAGC CAGTGGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAGC ATCACGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTGC CCCACAGACCGGCAGACGCGAGTCTGCCTCCTTCTCAAG GTTGACCTCGGATCAGGTAGGACTACCCGCTGAACTTAA GCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGAT TGCCCCAGTAACGGCGAGTGAAGCGGCAACAGCTCAGA TTTGGAATCTGGCCCCCAGGGCCCGAGTTGTAATCTGGA GAGGATGCTTCTGGCGCGGCGCCTTCCGAGTTCCCTGGA ACGGGACGCCGGAGAGGGTGAGAGCCCCGTACGGTTGG ACGCCTAGCCTTTGTGAAGCTCCTTCGACGAGTCGAGTA GT	Not Detected

Leptographium sp.	WIN(M)1212/ TOM 64.19	>Leptographium WIN(M)1212 CAGTGACTCCCAACCCGTGCAAACCTTACCGCATCCTTT CTGAGAGAGAGCGCCCCCGTTGCTTTCTGCCGGGCGGCG TGCCCTCCTCCCTCCCCCTCTGCAGGGGGGGGGTTGGAC GGGCGCCCGCCCGCCGGGGGCGCGGCGCGGCCGCTCCC TCTCGCCGCGAACCTTCTTTGCAGTATAATTGTATCGTCT GAGCAAAACCACAGAATCGTTAAAACTTTCAACAACGG ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAACT GCGATAAGTAATGCGAATTGCAGAATTCAGCGAGCCATC GAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGC GGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCACGCAG CGCGCCTGCGTGGTGTTGGGGCGTTCTGCGGCCAGGCCT GCGCCCAGCGCAGGCCGCCGCAGCCCCCGAAAGCCAGT GGCGGGCCGGCAGCGGGCTCCGAGCGCAGTAAGCATCA CGCCCTCGCTCTGGACGCTCCCGCCTGCGCCCTGTCCCA CAGACCGGCAGACGCGAGTCTGCCTCCTTCTCAAGGTTG ACCTCGGATCAGGTAGGACTACCCGCTGAACTAAAGCAT ATCAA	Not Detected
Leptographium sp.	WIN(M)1234/ TOM 68.11	>Leptographium WIN(M)1234 CCCGTTGCTTTCTGCCGGGCGGCGTGCCCTCCTCCCTCCC CCTCTGCAGGGGGGGGGTTGGACGGGCGCCCGCCCGCC GGGGGCGCGGCGCGGCCGCTCCCTCTCGCCGCGAACCTT CTTTGCAGTATAATTGTATCGTCTGAGCAAAACCACAGA ATCGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGC ATCGATGAAGAACGCAGCGAACTGCGATAAGTAATGCG AATTGCAGAATTCAGCGAGCCATCGAATCTTTGAACGCA CATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTCCG AGCGTCATTTCCTCCCTCACGCAGCGCGCCTGCGTGGTG TTGGGGCGTTCTGCGGCCAGGCCTGCGCCCAGCGCAGGC CGCCGCAGCCCCCGAAAGCCAGTGGCGGGCCGGCAGCG GGCTCCGAGCGCAGTAAGCATCACGCCCTCGCTCTGGAC GCTCCCGCCTGCGCCCTGTCCCACAGACCGGCAGACGCG AGTCTGCCTCCTTCTCAAGGTTGACCTCGGATCAGGTAG GACTACCCGCTGAACTTAAGCATATCAA	Detected
Leptographium sp.	WIN(M)1238/ TOM 69.28	>Leptographium WIN(M)1238 GGTCTCCGTTGGTGAACCAGCGGAGGGATCATTAGCGAG TTCACAGTGACTCCCAACCCGTGCAAACCTTACCGCATC CTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGCCGGGCGG CGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGGGTTGGACG GGCGCCCGCCCGCCGGGGGTGCGGCGCGGCCGCTCCCTC TCGCCGCGAACCTTCTTTGCAGTATAATTGTATCGTCTGA GCAAAACCACAGAATCGTTAAAACTTTCAACAACGGATC TCTTGGTTCTGGCATCGATGAAGAACGCAGCGAACTGCG ATAAGTAATGCGAATTGCAGAATTCAGCGAGCCATCGA ATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGG GCATGCCTGTCCGAGCGTCATTTCCTCCCTCACGCAGCG	Detected

		CGCCTGCGTGGTGTTGGGGTGTTCTGCGGCCAGGCCTGC GCCCAGCGCAGGCCGCCGCAGCCCCCGAAAGCCAGTGG CGGGCCGGCAGCGGGCTCCGAGCGCAGTAAGCATCACG CCCTCGCTCTGGACGCTCCCGCCTGCGCCCTGCCCCACA GACCGGCAGACGCGAGTCTGCCTCCTCTCAAGGTG	
Leptographium wingfieldii	WIN(M)1240/ T0M70.25	>Leptographium WIN(M)1240 TTGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGG ATCATTAGCGAGTTCACAGTGACTCCCAACCCGTGCAAA CCTTACCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCTT CCTGCCGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGG GGGGGTTGGACGGGCGCCCGCCCGCCGGGGGTGCGGCG CGGCCGCTCCCTCTCGCCGCGAACCTTCTTTGCAGTATA ATTGTATCGTCTGAGCAAAACCACAGAATCGTTAAAACT TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA CGCAGCGAACTGCGATAAGTAATGCGAATTGCAGAATTC AGCGAGCCATCGAATCTTTGAACGCACATTGCGCCCGCC AGCATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCCT CCCTCACGCAGCGCGCCTGCGTGGTGTTGGGGTGTTCTG CGGCCAGGCCTGCGCCCAGCGCAGGCCGCCGCAGCCCC CGAAAGCCAGTGGCGGGCCGGCAGCGGGCTCCGAGCGC AGTAAGCATCACGCCCTCGCTCTGGA	Detected
Leptographium sp.	WIN(M)1280/ TOM80.30	>Leptographium WIN(M)1280 TTGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGG ATCATTAGCGAGTTCACAGTGACTCCCAACCCGTGCAAA CCTTACCGCATCCTTTCTGAGAGAGAGCGCCCGTTGCTT CCTGCCGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGG GGGGGTTGGACGGGCGCCCGCCCGCCGGGGGTGCGGCG CGGCCGCTCCCTCTCGCCGCGAACCTTCTTTGCAGTATA ATTGTATCGTCTGAGCAAAACCACAGAATCGTTAAAACT TTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA CGCAGCGAACTGCGATAAGTAATGCGAATTGCAGAATTC AGCGAGCCATCGAATCTTTGAACGCACATTGCGCCCGCC AGCATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCCT CCCTCACGCAGCGCGCCTGCGTGGTGTTGGGGTGTTCTG CGGCCAGGCCTGCGCCCAGCGCAGGCCGCCGCAGCCCC CGAAAGCCAGTGGCGGGCCGGCAGCGGGCTCCGAGCGC AGTAAGCATCACGCCCTCGCTCTGGACGCTCCCGCCTGC GCCCTGCCCCACAGACCGGCAGACGCGAGTCT	Not Detected
Leptographium sp.	WIN(M)1282/ TOM80.4	>Leptographium WIN(M)1282 TTCCTGCCGGGCGGCGTGCCCTCCTCCCCTCCCTCTGCGG GGGGGGTTGGACGGGCGCCCGCCCGCCGGGGGTGCGGC GCGGCCGCTCCCTCTCGCCGCGAACCTTCTTTGCAGTAT AATTGTATCGTCTGAGCAAAACCACAGAATCGTTAAAAC TTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA ACGCAGCGAACTGCGATAAGTAATGCGAATTGCAGAAT TCAGCGAGCCATCGAATCTTTGAACGCACATTGCGCCCG	Detected

		CCAGCATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTC CTCCCTCACGCAGCGCGCCTGCGTGGTGTTGGGGTGTTC TGCGGCCAGG	
Leptogr aphium sp.	WIN(M) 1205/ TOM 58.10	>Leptographium sp WIN(M) 1205 TCTCCGTTGGTGAACCAGTGGAGGGATCATTAGCGAGTT CACAGTGACTCCCAACCCGTGCAAACCTTACCGCATCCT TTCTGAGAGAGAGCGCCCGTTGCTTCCTGCCGGGCGGCG TGCCCTCCTCCCCTCCCTCTGCGGGGGGGGTTGGACGGG CGCCCGCCCGCCGGGGGTGCGGCGCGGCCGCTCCCTCTC GCCGCGAACCTTCTTTGCAGTATAATTGTATCGTCTGAG CAAAACCACAGAATCGTTAAAACTTTCAACAACGGATCT CTTGGTTCTGGCATCGATGAAGAACGCAGCGAACTGCGA TAAGTAATGCGAATTGCAGAATTCAGCGAGCCATCGAAT CTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGC ATGCCTGTCCGAGCGTCATTTCCTCCCTCACGCAGCGCG CCTGCGTGGTGTTGGGGTGTTCTGCGGCCAGGCCTGCGC CCAGCGCAGGCCGCCGCAGCCCCCGAAAGCCAGTGGCG GGCCGGCAGCGGGCTCCGAGCGCAGTAAGCATCACGCC CTCGCTCTGGACGCTCCCGCCTGCGCCCTGCCCCACAGA CCGGCAGACGCGAGTCTGCCTCCTTCTCAAGGTTGACCT CGGATCAGGTAGGACTACCCGCTGAACTTAAGCATATCA	Detected
Leptogr aphium sp.	WIN(M)12 36/ TOM 68.29	>Leptographium sp. WIN(M)1236 GCGAGTTCACAGTGACTCCCAACCCGTGCAAACCTTACC GCATCCTTTCTGAGAGAGAGCGCCCGTTGCTTCCTGCCG GGCGGCGTGCCCTCCTCCCCTCCCTCTGCGGGGGGGGTT GGACGGGCGCCCGCCCGCCGGGGGTGCGGCGCGGCCGC TCCCTCTCGCCGCGAACCTTCTTTGCAGTATAATTGTATC GTCTGAGCAAAACCACAGAATCGTTAAAACTTTCAACAA CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGA ACTGCGATAAGTAATGCGAATTGCAGAATTCAGCGAGCC ATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCT GGCGGGCATGCCTGTCCGAGCGTCATTTCCTCCCTCACG CAGCGCGCCTGCGTGGTGTTGGGGTGTTTCTG	Detected Forward Fragmen t only
Leptogr aphium sp.	WIN(M)12 36/ TOM 68.29	>Leptographium sp.WIN(M)1236 CCGCCGCAGCCCCCGAAAGCCAGTGGCGGGCCGGCAGC GGGCTCCGAGCGCAGTAAGCATCACGCCCTCGCTCTGGA CGCTCCCGCCTGCGCCCTGCCCCACAGACCGGCAGACGC GAGTCTGCCTCCT	Detected /Reverse fragment only
Leptogr aphium proceru m	WIN(M)12 10/ TOM 62.30	>Leptographium sp.WIN(M) 1210 CGCCGGGGGCGCGGCGCGGCCGCTCTCCCACCGAACCTT CTATTAGTATACCTTTACCGTCTGAGCAAAACCACTGAA TCGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCAT CGATGAAGAACGCAGCGAACTGCGATAAGTAATGCGAA TTGCAGAATTCAGCGAGCCATCGAATCTTTGAACGCACA TTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTCCGAG CGTCATTTCCTCCCTCACGCAGCGCGCCTGCGTGGTGTTG	Not Detected / Reverse fragment only

		GGGCGTTTCTGCGGCCAGGCCTGCGCCTAGCAGGCCTCC GCAGCCCCCGAAAGCCAGTGGCGGGCCGGCAGCGGGCT CCGAGCGCAGTAAGCAGCAGTGCCCTCGCTCTGGACGCC TCCGCCTGCGCCCTGCCCCAAATGACCGGCAGACGCAAG TCTGCCCCTCCTCTCAA	
Leptographium proceru m	WIN(M)1210/ TOM 62.30	>Leptographium sp. WIN(M)1210 CGAGTTCACAGCGACTCCCAACCCGTGCATACCTTACCG CATCTTCTGAGAGCGCCCGTTGCCTCCTGGCGGG	Not Detected / Forward fragment only
Leptographium sp.	WIN(M) 1330/TOM 90.27	>Leptographium sp. WIN(M) 1330 CAGTGACTCCCAACCCGTGCAAACCTTACCGCATCCTTT CTGAGAGAGAGCGCCCGTTGCTTCCTGCCGGGCGGCGTG CCCTCCTCCCCTCCCTCTGCGGGGGGGGTTGGACGGGCG CCCGCCCGCCGGGGGTGCGGCGCGGCCGCTCCCTCTCGC CGCGAACCTTCTTTGCAGTATAATTGTATCGTCTGAGCA AAACCACAGAATCGTTAAAACTTTCAACAACGGATCTCT TGGTTCTGGCATCGATGAAGAACGCAGCGAACTGCGATA AGTAATGCGAATTGCAGAATTCAGCGAGCCATCGAATCT TTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCAT GCCTGTCCGAGCGTCATTTCCTCCCTCACGCAGCGCGCC TGCGTGGTGTTGGGGTGTTCTGCGGCCAGGCCTGCGCCC AGCGCAGGCCGCCGCAGCCCCCGAAAGCCAGTGGCGGG CCGGCAGCGGGCTCCGAGCGCAGTAAGCATCACGCCCTC GCTCTGGACGCTCCCGCCTGCGCCCTGCCCCACAGACCG GCAGACGCGAGTCTGCCTCCTTCTCAAGGTTGACCTCGG ATCAAGTAGGACTACCCACTGAACTTAAGCATAT	Detected
Leptographium sp.	WIN(M) 1291/ TOM 81.19	> Leptographium sp. WIN(M) 1291 ACAGTGACTCCCAACCCGTGCAAACCTTACCGCATCCTT TCTGAGAGAGAGCGCCCGTTGCTTCCTGCCGGGCGGCGT GCCCTCCTCCCCTCCCTCTGCGGGGGGGGTTGGACGGGC GCCCGCCCGCCGGGGGTGCGGCGCGGCCGCTCCCTCTCG CCGCGAACCTTCTTTGCAGTATAATTGTATCGTCTGAGC AAAACCACAGAATCGTTAAAACTTTCAACAACGGATCTC TTGGTTCTGGCATCGATGAAGAACGCAGCGAACTGCGAT AAGTAATGCGAATTGCAGAATTCAGCGAGCCATCGAATC TTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCA TGCCTGTCCGAGCGTCATTTCCTCCCTCACGCAGCGCGC CTGCGTGGTGTTGGGGTGTTCTGCGGCCAGGCC	Detected / Forward fragment only
Leptographium sp.	WIN(M) 1291/ TOM 81.19	> Leptographium sp. WIN(M) 1291 GCAGGCCGCCGCAGCCCCCGAAAGCCAGTGGCGGGCCG GCAGCGGGCTCCGAGCGCAGTAAGCATCACGCCCTCGCT CTGGACGCTCCCGCCTGCGCCCTGCCCCACAGACCGGCA GACGCGAGTCTCCCTCCT	Detected / Reverse fragment only

Note: WIN(M) = Fungal collection maintained at the University of Manitoba, Department of Microbiology, Hausner Lab; TOM = fungi collected from Northwestern Ontario isolated from *Tomicus piniperda* (common pine shoot beetle);

Item 2: Optimization of fungal growth culture/conditions and extraction methods

Various media were used to culture fungi, and multiple extraction methods were examined.

2.1. Culture media

Different batches of ME media from various suppliers were used to cultured fungi and extract their secondary metabolites. The first media was composed of 30 g malt extract (VWR life science, Ohio, USA), 1 g yeast extract (Bacto™, Dickinson & Co., Maryland, USA) and 20 g bacteriological agar (Bacto™ BD). The second media was composed of 30 g malt extract (OXOID, Fisher Scientific, Ottawa, ON, Canada), 1 g yeast extract (Bacto™ BD) and 20 g bacteriological agar (Bacto™ BD). Fungal culturing was carried out, first in ME agar plate, and then in ME broth to facilitate extraction of secondary metabolites. After that, the bioactivities of the crude extracts obtained from the two ME media (provided by the respective suppliers) were assayed, and no significant differences in the bioactivities were noted.

2.2. Extraction methods

Secondary metabolites were extracted from *Leptographium wingfieldii* strain 1290 and *Penicillium rubens* UAMH CENTRE FOR GLOBAL MICROFUNGAL BIODIVERSITY (UHMH) strain 9184 using two extraction methods (from liquid and solid ME media). Samples were inoculated in 300 mL ME broth and/or 12 ME agar plates (12 x 25 ml the capacity of the plate = 300 ml). After reaching the optimal growth, 1.5 ml HCL was used to acidify the media and secondary metabolites were extracted using 300 mL ethyl acetate. Crude extracts were obtained,

and their bioactivities were tested (see Table S4). It was noted that the best method for extracting fungal secondary metabolites from liquid ME medium was to acidify the media first using 10 ml HCL followed by extraction with ethyl acetate (with equal volume of spent ME medium). It was noticed that the antimicrobial activities appear to increase using this extraction method.

Table S4: Crude extracts from *L. wingfieldii* 1290 and *Penicillium rubens* (UAMH 9184) showing antimicrobial activity against the selected bacterial strains (Table 5 material and method section) in material and methods section 2.2) at 20% crude extracts concentration. Zones of inhibition (diameter) were measured in cm.

Fungal strains	MRSA	ACCT17978	AB030	PAO1	PA750
1290 liquid	No	0.5	0.2	0.1	0.3
1290 solid	No	0.1	No	0.1	0.2
9184 Liquid fresh	0.2	0.1	0.2	0.1	0.45
9184 Solid	No	No	No	No	No

3.1 Item 3: Optimization of the antibiotic resistance platform (ARP) to be used to screen for novel antibiotics isolation from fungi

The antibiotic resistance platform (ARP) was constructed in Dr. Wright's lab at McMaster University (Cox *et al.*, 2017). This platform consists of different strains of *Escherichia coli* that lack outer membrane protein coding genes *BamB* (of the β-barrel assembly machine family) and *TolC*; these mutants are highly susceptible to antibiotics due to their changed permeabilities and efflux properties. Each strain contains a different resistance gene that corresponds to a different antibiotic class, see (Figure 1). If each strain is challenged with an unknown antimicrobial compound, only the strain containing the corresponding resistance gene would survive the exposure. So, by identifying the resistant strains, one can theoretically identify the class of the antibiotics. They generated the ARP to eliminate the rediscovery of previously known antibiotics and to promote adjuvant discovery. This ARP was originally designed to work with Actinobacteria. However, we utilized this platform for different approaches to be applied to fungi.

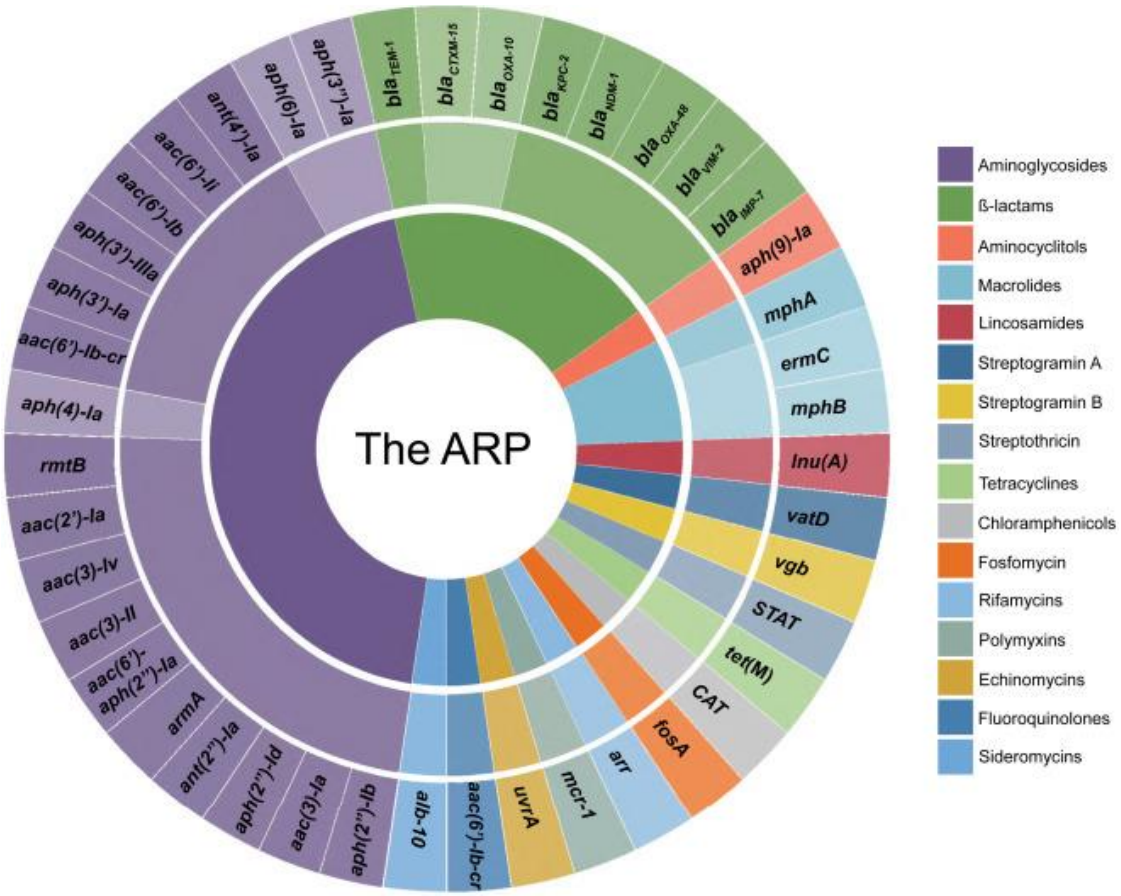


Figure S1: Antibiotic Resistance Spectrum of the ARP Figure adopted from (Cox *et al.*, 2017) under the License number: 4858360249380.

3.1. ARP: Agar Overlay Method

The first method that we used was agar overlay method which was modified from the protocol in the original paper. Fungal plugs were suspended in sterile water and vortexed vigorously to suspend the fungal spores. Then, the fungal spore suspension was swabbed on top of sterile filter paper that was placed on top of ME agar plates and incubated for 7 days at 24 ^{0}C. After obtaining optimal growth, the fungal growth formed on the filter paper was peeled off. An agar overlay of lysogeny broth (LB) agar (Bacto™ BD) is then applied onto the fermented media, and the plates were incubated overnight to allow diffusion of any compounds secreted into the

overlay. One colony of each ARP strain was inoculated in 2 mL LB broth and incubated at 37 ^{0}C overnight. From each of the overnight culture, 5 µl was spotted onto the overlay and incubated at 37 ^{0}C overnight. However, one of the concerns of using this method is the uneven distribution of the fungal growth on the filter paper which results in variable concentrations of metabolites across the plate. Moreover, during the peeling off of the filter paper containing the fungal growth, there is high probability of contamination of the plates with fungal spores, see Figure 2. The spotting of bacteria is further complicated by the variable growth times of the fungal species, especially with fast-growing fungi, like *Penicillium* spp. for which significant growth is achieved by overnight incubation.

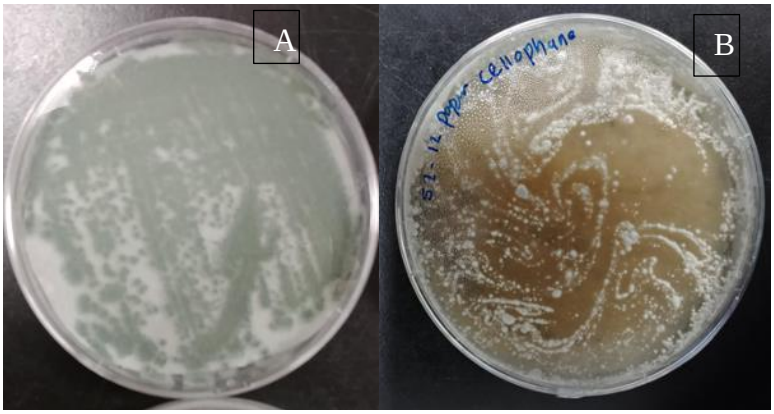


Figure S2: Plates A shows an even distribution of fungal growth on filter paper; plate B shows fungal contamination, probably due to residual fungal spores, in the agar overlay method

3.2 ARP: Spent-Media Infusion Method

In an alternative approach, the fungal strains were grown in small volumes of ME broth (100 mL) for 7 days. The spent media was filter sterilized using Nalgene Syringe Filter (0.45 µm nylon membrane) (Thermo Scientific) and combined with molten (100 mL) LB agar with double the amount of agar. There was no significant result; all the ARP strains manage to grow in the presence of *Leptographium* spp. spent media (Table S 5). This method offered the advantage of incorporation of sterile spent media directly into the plates facilitating the even distribution of metabolites throughout the plate, and also eliminated the contamination with residual fungal spores. However, the limitation of this method is that it is time consuming. The combination of the molten agar and spent media might also dilute the concentration of the secondary metabolites secreted into the media. There might be some cross contamination among the different fungal strains during the process of combination of the spent media with the molten agar and transfer into the plates.

Table S 5: Results of spent media infusion approach for different *Leptographium* strains

Fungal strain WIN(M)	Antibiotic resistance genes in the ARP *E. coli*																	
	E. coli BW25113Δ*amB*Δ*tolC*	*E. coli* BW25113	*aph(3')-IA*	*vatD*	*STAT*	*arr*	*rmtB*	*NDM-1*	*fhuB mut.*	*ermC*	*CAT*	*uvrA*	*MCR-1*	*aph(9)-IA*	*apmA*	*tet(A)*	*vph*	*fosA*
1205	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1206	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1234	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

P = indicated positive growth

3.3 ARP: High throughput (96-well plate)

High throughput methods allow for testing more than one sample, test triplicates, and require less material usage. The ARP cell suspensions were made (ARP cells were normalized to 0.5 McFarland scale and diluted to 1:100 in LB broth); fungal culture broths were filter sterilized to obtained spent media. In a 96-well plate, 75 µl of the ARP cell suspension and 75 µl of fungal spent media were mixed and incubated for 24h at 37 °C. OD_{600} was measured. There were no significant results; all ARP strains showed growth in the presence of *L. wingfieldii* strains spent culture broth. In contrast, only the ARP strain that contains the *mcr-1* gene (Mobilized Colistin Resistance) managed to grow along with the wild type *E. coli* in the presence of the environmental fungal strain 51-9 (*Penicillium* sp. based on ITS data) in the spent media, and this suggests that the 51-9 strain might produce either colistin or a colistin-like compound. The fungal strain 51-9 was an isolated from an environmental sample (Falcon beach, Falcon Lake). For validation of this method, the ARP strains were challenged with LB and ME only media. Figure 3. The limitation of this method is that it is tedious and time-consuming, further limited by the small volume and the dilution of the spent media.

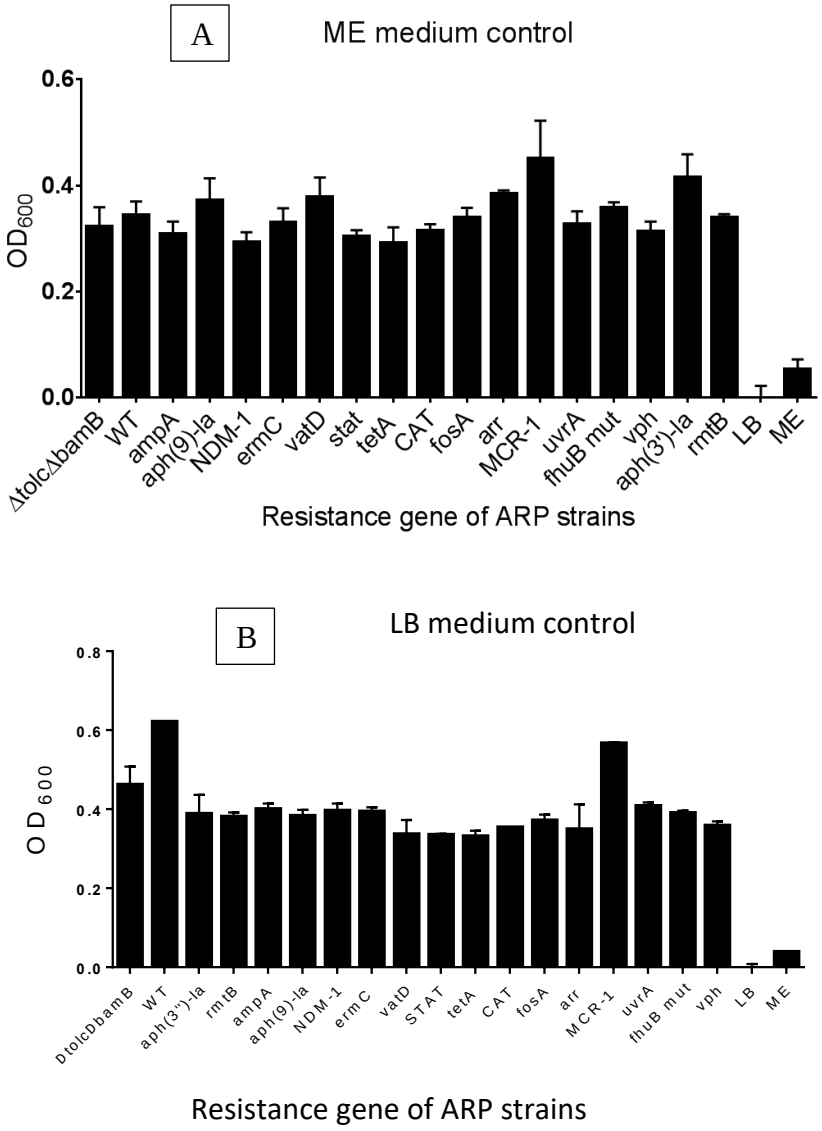


Figure S3: ARP strains challenged with (A) ME media, and (B) LB media, as control

3.4. ARP: Agar plug assay

ARP strains were inoculated in (LB) agar plates containing 50 mg/ml gentamycin or 100mg/ml ampicillin and incubated at 37C^{o} for overnight. Once the optimum growth was attained, a single colony from each ARP strains were used to inoculated a 2 ml LB broth that contain antibiotic markers to maintain the plasmid and then incubated overnight at 37 C^{o}. Overnight cultures were normalized to 50 MacFarland units, (as described in material and methods section 2.2). The normalized bacterial cells were spread on LB plates and agar plug from selected fungal strains (see Table S 6) were placed on top of the bacterial lawn. All the ARP strains managed to grow in the presence of twelve *Leptographium* spp. that showed antibacterial activity (Table 12; result section 3.1). However, all the ARP strains did not manage to grow in the presence of the environmental fungal strains 51-9 (51-9A and 51-9B; *Penicillium* sp. based on ITS data) except the wild type *E. coli* BW25113 and the strain that contains the *mcr-1* gene (Mobilized Colistin Resistance).

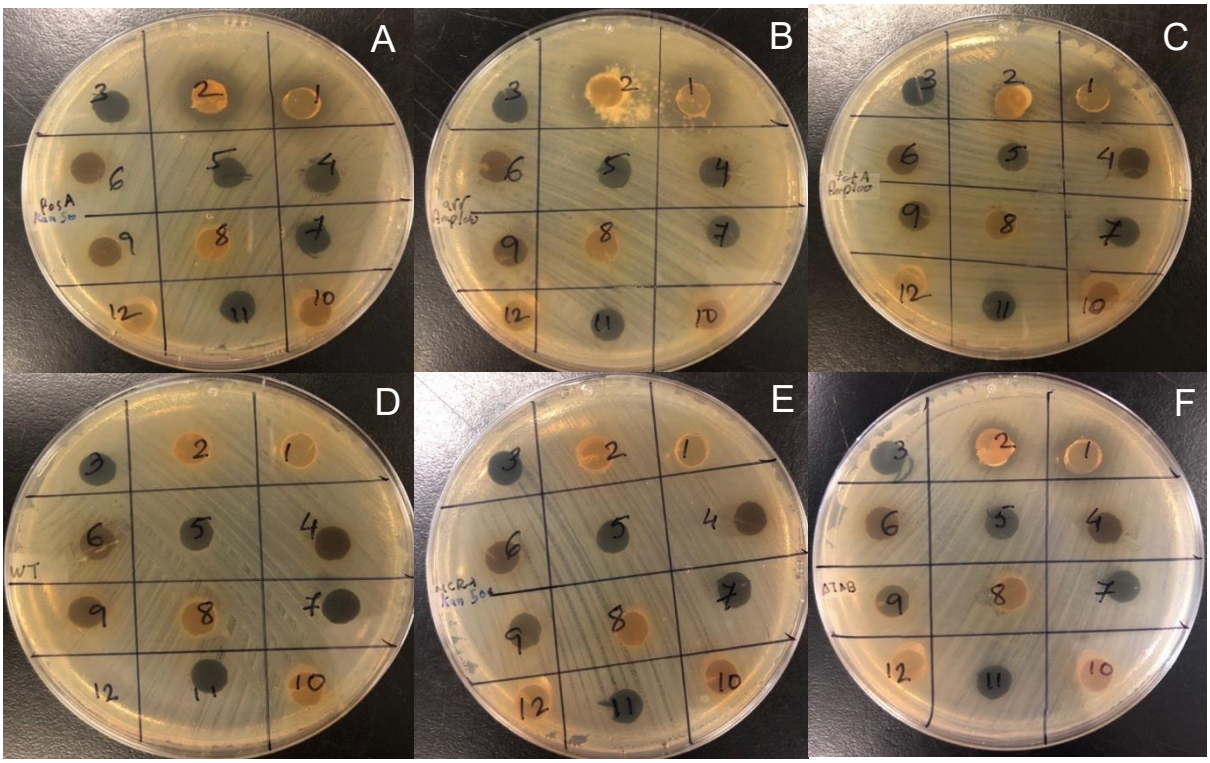


Figure S3: Representative results of ARP: agar plug assay approach for different fungal strains. (A) *fosA,* (B) *arr,* (C) *tetA,* (D) WT; *E. coli* BW25113, (E) *MRC-1*, (F) ΔTΔB; *E. coli* BW25113 Δ*bamB*Δ*tolC*

Table S6: Fungal strains numbered corresponding to the numbering on the plates (Figure S3) used at ARP agar plug assay

#	Fungal strains
1	51-9A
2	51-9B
3	WIN(M) 1330
4	WIN(M) 1282
5	WIN(M) 1280
6	WIN(M) 1281
7	WIN(M) 1278
8	WIN(M) 1271
9	WIN(M) 1238
10	WIN(M) 1236
11	WIN(M) 1240
12	ME (Malt extract medium) control

Item 4: Combinations of extracts from different *Leptographium wingfieldii* strains and test their synergistic antimicrobial activity in different combinations.

Combining different antibiotics or antibiotic with compounds with no antibiotic activity (such as an adjuvant) is an approach that can lead to an improvement in the activity of existing antibiotics (Tyers and Wright, 2019). This approach was tried for extracts from *L. wingfieldii*. Various combinations resulted in a modulation of bioactivity; i.e. synergies where observed among various extract combinations. Examples of these results are shown in Table S7; here it can be noted that some extracts on their own show no antimicrobial activity but in combination with extracts from another strain zones of clearing were observed. The combined potential of the different *Leptographum* spp. strains may be due to the similar antimicrobial activities of the compounds produced by the individual strains, as indicated by the LC-MS results (see Result section 3.3)

Table S7: Representative result of combining extracts from different *Leptographium* strains that were tested against different bacteria. Zones of inhibition (diameter) were measured in cm

Combination	Bacterial strains				
	P.aeruginosa PAO750	*P.aeruginosa* PAO1	MRSA	*A.baummanii* ABO30	*A.baummanii* 17978
1286+1288	--	--	--	--	0.4
1286+1290	0.2	0.3			0.2
1288+1293	--	--	0.6	0.2	0.2

Item 5: Identifying potential compounds within the crude fungal extracts using Liquid chromatography–mass spectrometry (LC-MS)

Thirteen fungal strains (Table 12; Results section 3.1) that showed antimicrobial activity in the agar plug assay and/or with crude extracts were prepared (see material and methods section 2.3) and sent for analysis by LC-MS (Dr. Russell Kerr, Department of Chemistry, University of Prince Edward Island (PEI)). However, due to time limitation in the current situation, only four strains showing positive antimicrobial activity were sent. In addition, twelve samples that did not show antimicrobial activity were sent for analysis (Table S8). Among these additional twelve samples, one strain, WIN (M) 1281 had its WGS (whole genome sequence) data generate by the Kumar/Hausner research group. The potential compounds detected from the LC-MS analysis were summarized in (Table S9) and the mass:ion charge ratios are represented in the form of a heatmap (Figure 4) indicating the relative abundances of the different compounds. In the heatmap, some potential compounds were shared among all the fungal strains. For example: trans-Zeatin which is aPlant Growth Regulator, also has shown antiaging effect on human fibroblast cells and 2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate which is an indole derivative; anti-oxidative and has potentially antimicrobial activity (specific tests not conducted, but other indole derivatives have shown antibiotic activity).

The LC-MS analysis predicted a variety of potential compounds that might be produced by *Leptographium* spp. in addition to being produced by previously known organisms. The potential compounds were arranged based on the closest similarity of mass:ion charge ratios to the predicted compound in ten part per million range (10 ppm). LC-MS analysis result showed “not determined (ND) compounds” which are the compounds produced by *Leptographium* spp. but did not match

a previously known compound in their databases. We hypothesized that they might be novel compounds.

Table S8: Fungal strains sent for LC-MS analysis at Dr. Russell Kerr, Department of Chemistry, University of Prince Edward Island (PEI)

Fungal strains WIN(M)
1290
1291
1238
1293
1281*
1288
1217
1210
1212
1207
1211
1214
1209
1208
1206
1205
ME-Media control

*whole genome available

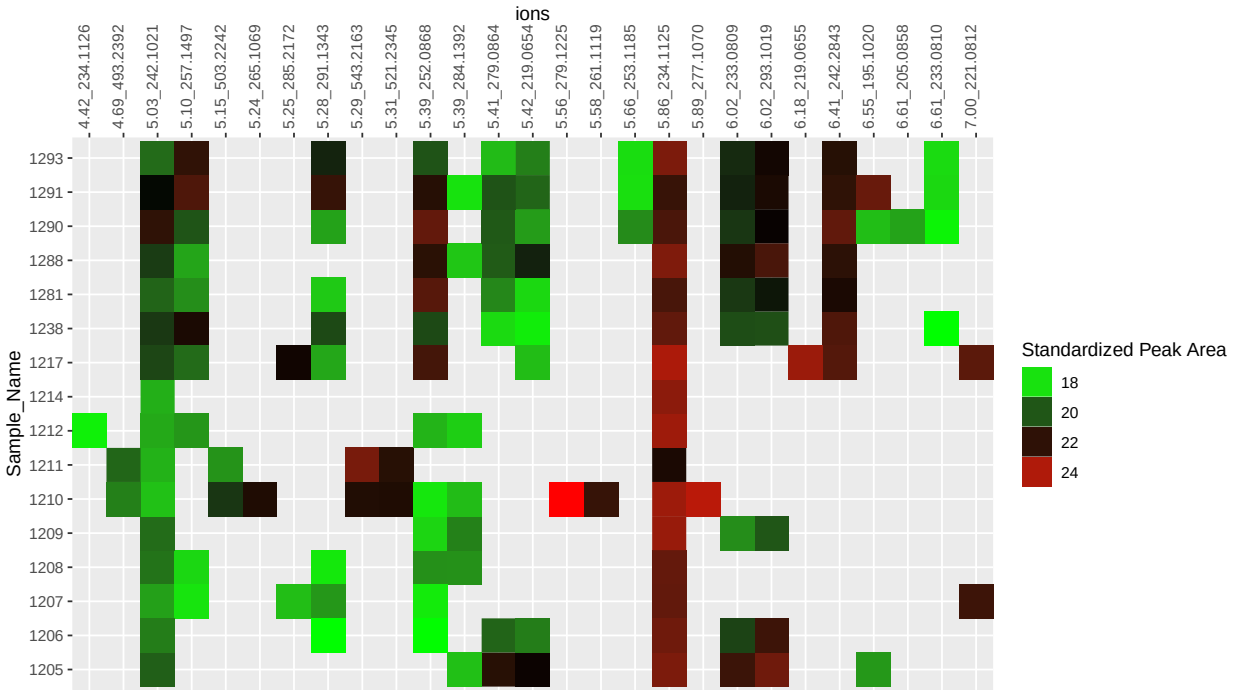
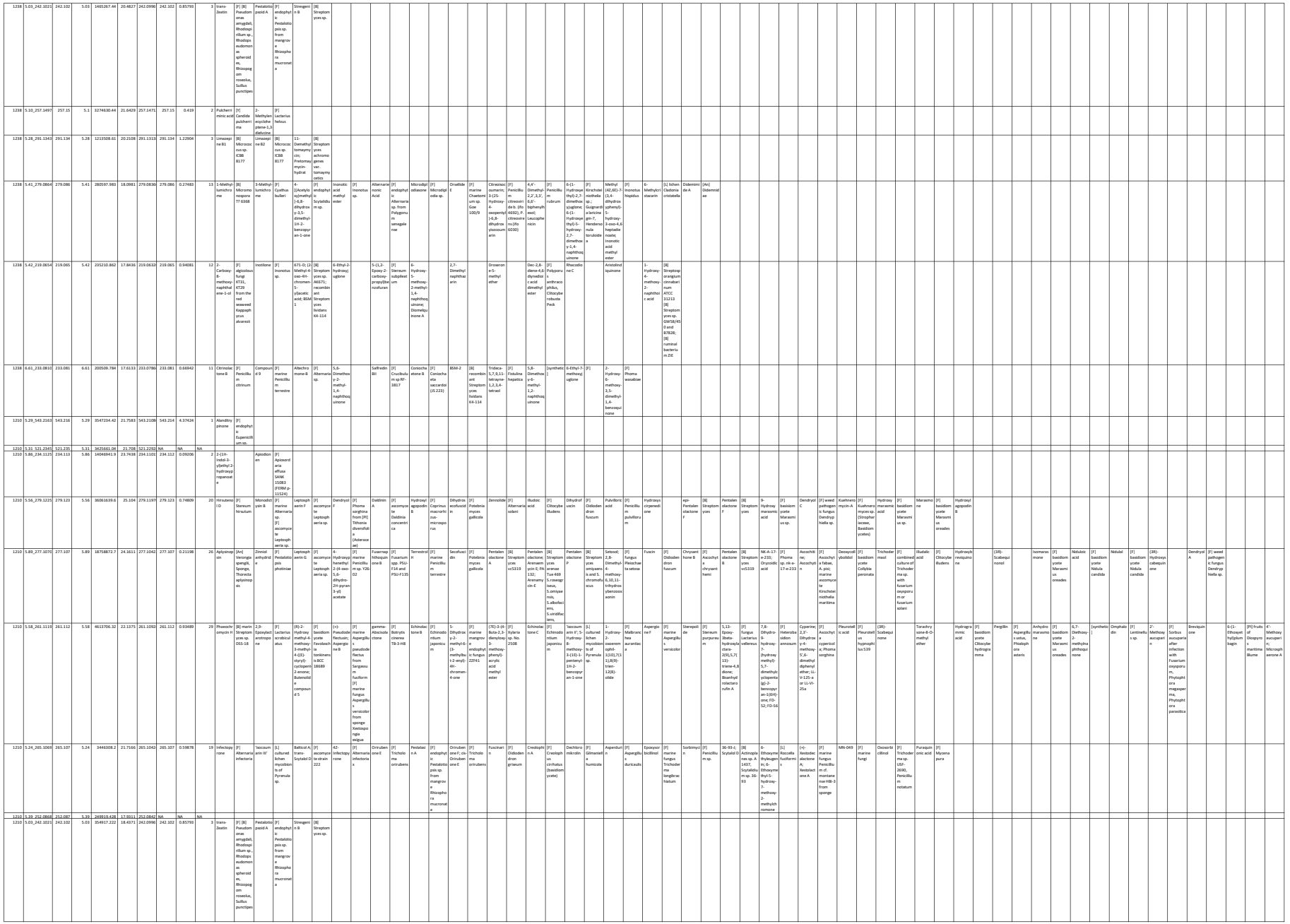


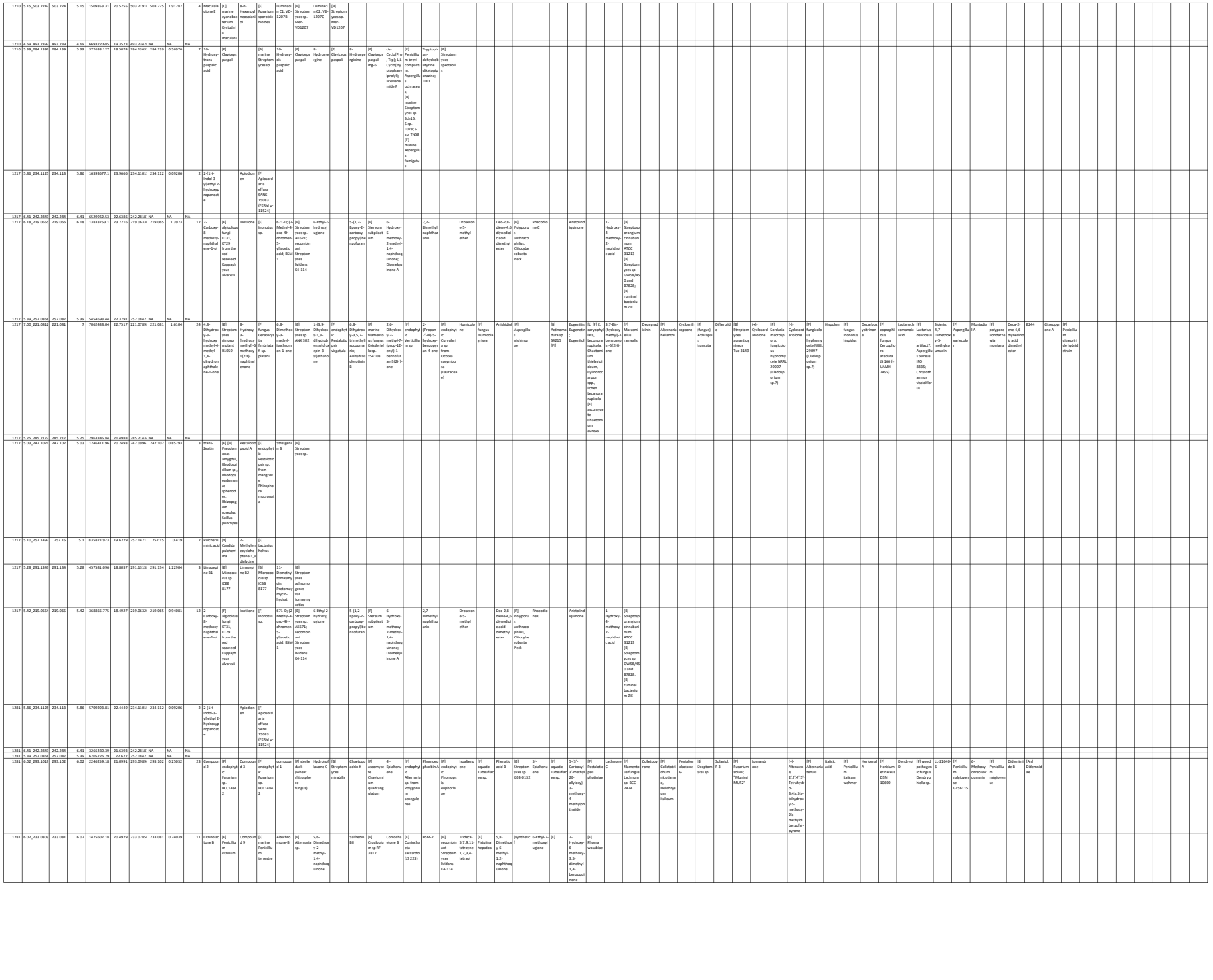
Figure S4: Heatmap showed the abundance of potential compounds produced by the *Leptographium* strains. *Green* color represents the lowest abundance level, while *Red* color represents the highest abundance level of secondary metabolites.

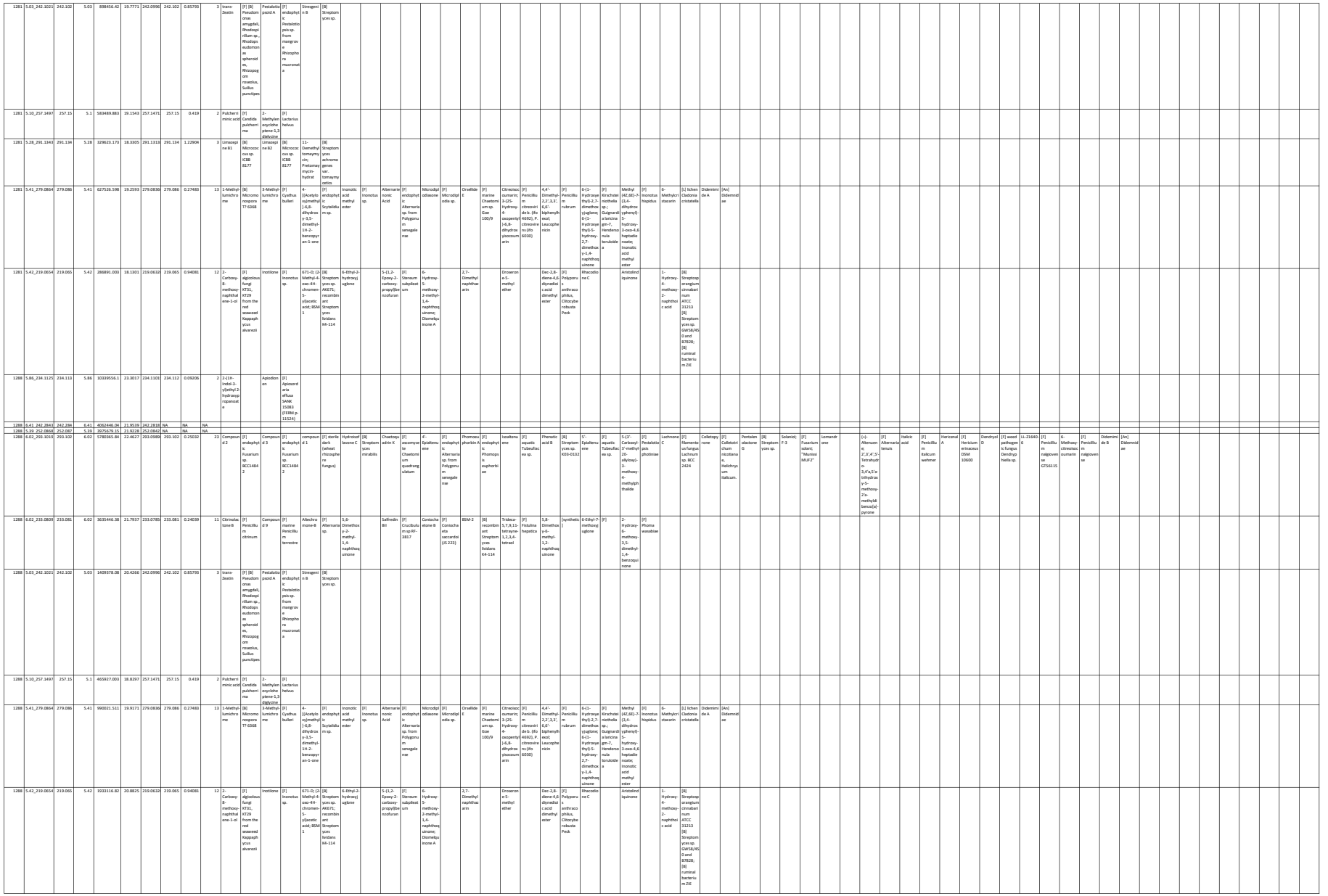
Table S 9: LC-MS result corresponding to fungal strains sent to PEI

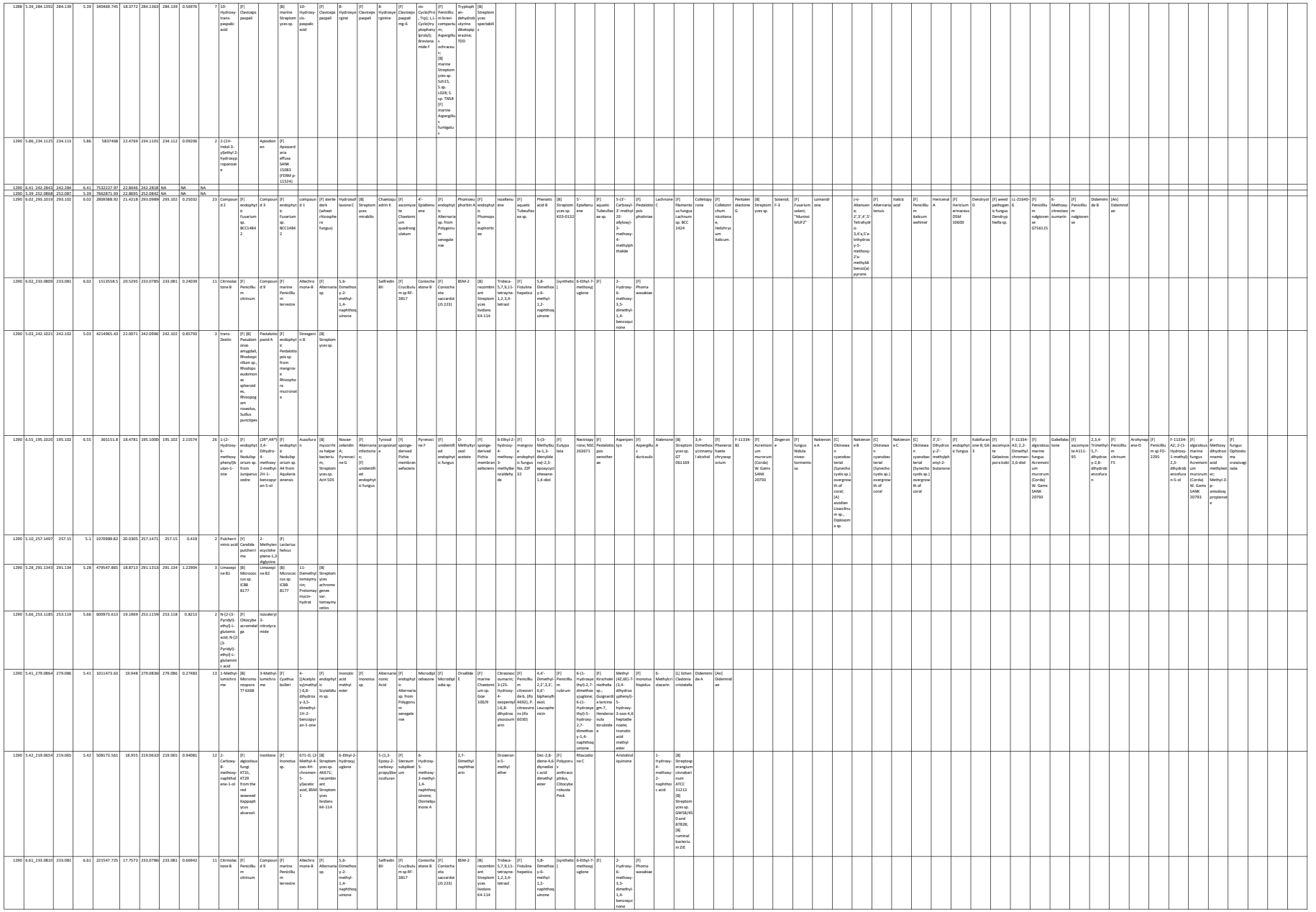
Sample_N	ions	row.m.z	row.reter	Peak_Area	Log_2_Pe	range_10	Closest.N	ppm_diff	Number_	X1	X1.1	X2	X2.1	X3	X3.1	X4	X4.1	X5	X5.1	X6	X6.1	X7	X7.1	X8	X8.1	X9	X9.1	X10	X10.1	X11	X11.1	X12	X12.1	X13	X13.1	X14	X14.1	X15	X15.1	X16	X16.1	X17	X17.1	X18	X18.1	X19	X19.1	X20	X20.1	X21	X21.1	X22	X22.1	X23	X23.1	X24	X24.1	X25	X25.1	X26	X26.1	X27	X27.1	X28
1211	5.29_543.2163	543.216	5.29	9639674.11	23.2006	543.2108	543.214	4.37424	1	Alanditrypinone	[F] endophytic Eupenicillium sp.																																																					
1211	5.31_521.2345	521.235	5.31	3845920.48	21.8749	521.2292	NA	NA	NA																																																							
1211	5.86_234.1125	234.113	5.86	3255740.29	21.6346	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																			
1211	5.03_242.1021	242.102	5.03	411873.996	18.6518	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1211	5.15_503.2242	503.224	5.15	553498.921	19.0782	503.2191	503.225	1.91287	4	Maculalactone E	[C] marine cyanobacterium Kyrtuthrix maculans	8-n-Hexanoylneosolaniol	[F] Fusarium sporotrichioides	Luminacin C1; VD-1207B	[B] Streptomyces sp. Mer-VD1207	Luminacin C2; VD-1207C	[B] Streptomyces sp. Mer-VD1207																																															
1211	4.69_493.2392	493.239	4.69	882482.622	19.7512	493.2342	NA	NA	NA																																																						
1208	5.86_234.1125	234.113	5.86	7742569.35	22.8844	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																				
1208	5.39_252.0868	252.087	5.39	574704.821	19.1325	252.0842	NA	NA	NA																																																						
1208	5.03_242.1021	242.102	5.03	771101.821	19.5566	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1208	5.10_257.1497	257.15	5.1	287696.401	18.1342	257.1471	257.15	0.419	2	Pulcherriminic acid	[Y] Candida pulcherrima	2-Methylenecycloheptene-1,3-diglycine	[F] Lactarius helvus																																																			
1208	5.28_291.1343	291.134	5.28	246606.913	17.9119	291.1313	291.134	1.22904	3	Limazepine B1	[B] Micrococcus sp. ICBB 8177	Limazepine B2	[B] Micrococcus sp. ICBB 8177	11-Demethyltomaymycin; Pretomaymycin-hydrat	[B] Streptomyces achromogenes var. tomaymycetics																																																	
1208	5.39_284.1392	284.139	5.39	565005.879	19.1079	284.1363	284.139	0.56976	7	10-Hydroxy-trans-paspalic acid	[F] Claviceps paspali		[B] marine Streptomyces sp.	10-Hydroxy-cis-paspalic acid	[F] Claviceps paspali	8-Hydroxyergine	[F] Claviceps paspali	8-Hydroxyerginine	[F] Claviceps paspali mg-6	cis-Cyclo(Pro, Trp); L,L-Cyclo(tryptophanylprolyl); Brevianamide F	[F] Penicillium brevi-compactum; Aspergillus ochraceus; [B] marine Streptomyces sp. Sch15, S.sp. L028; S. sp. TN58 [F] marine Aspergillus fumigatus	Tryptophan-dehydrobutyrine diketopiperazine; TDD	[B] Streptomyces spectabilis																																								
1212	5.86_234.1125	234.113	5.86	14226676.6	23.7621	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																			
1212	5.39_252.0868	252.087	5.39	402079.895	18.6171	252.0842	NA	NA	NA																																																						
1212	5.03_242.1021	242.102	5.03	447062.96	18.7701	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1212	5.10_257.1497	257.15	5.1	536449.486	19.0331	257.1471	257.15	0.419	2	Pulcherriminic acid	[Y] Candida pulcherrima	2-Methylenecycloheptene-1,3-diglycine	[F] Lactarius helvus																																																			
1212	4.42_234.1126	234.113	4.42	227547.414	17.7958	234.1102	234.112	0.51921	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																			

1212	5.39_284.1392	284.139	5.39	315681.597	18.2681	284.1363	284.139	0.56976	7	10-Hydroxy-trans-paspalic acid	[F] Claviceps paspali		[B] marine Streptomyces sp.	10-Hydroxy-cis-paspalic acid	[F] Claviceps paspali	8-Hydroxyergine	[F] Claviceps paspali	8-Hydroxyerginine	[F] Claviceps paspali mg-6	cis-Cyclo(Pro, Trp); L,L-Cyclo(tryptophanylprolyl); Brevianamide F	[F] Penicillium brevi-compactum; Aspergillus ochraceus; [B] marine Streptomyces sp. Sch15, S.sp. L028; S. sp. TN58 [F] marine Aspergillus fumigatus	Tryptophan-dehydrobutyrine diketopiperazine; TDD	[B] Streptomyces spectabilis																																									
1209	5.86_234.1125	234.113	5.86	13315655.1	23.6666	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																			
1209	5.39_252.0868	252.087	5.39	294284.965	18.1669	252.0842	NA	NA	NA																																																							
1209	6.02_293.1019	293.102	6.02	1044945.11	19.995	293.0989	293.102	0.25032	23	Compound 2	[F] endophytic Fusarium sp. BCC14842	Compound 3	[F] endophytic Fusarium sp. BCC14842	compound 1	[F] sterile dark (wheat rhizosphere fungus)	Hydroisoflavone C	[B] Streptomyces mirabilis	Chaetoquadrin K	[F] ascomycete Chaetomium quadrangulatum	4'-Epialtenuene	[F] endophytic Alternaria sp. from Polygonum senegalense	Phomoeuphorbin A	[F] endophytic Phomopsis euphorbiae	Isoaltenuene	[F] aquatic Tubeufiacea sp.	Phenatic acid B	[B] Streptomyces sp. K03-0132	5'-Epialtenuene	[F] aquatic Tubeufiacea sp.	5-(3'-Carboxyl-3'-methyl-2E-allyloxy)-3-methoxy-4-methylphthalide	[F] Pestalotiopsis photiniae	Lachnone C	[F] filamentous fungus Lachnum sp. BCC 2424	Colletopyrone	[F] Colletotrichum nicotianae, Helichrysum italicum.	Pentalenolactone G	[B] Streptomyces sp.	Solaniol; F-3	[F] Fusarium solani; "Munissi MUF2"	Lomandrone		(+)-Altenuene; 2',3',4',5'-Tetrahydro-3,4'a,5'a-trihydroxy-5-methoxy-2'a-methyldibenzo[a]-pyrone	[F] Alternaria tenuis	Italicic acid	[F] Penicillium italicum wehmer	Hericenal A	[F] Hericium erinaceus DSM 10600	Dendryol D	[F] weed pathogenic fungus Dendryphiella sp.	LL-Z1640-6	[F] Penicillium nalgiovense GT56115	6-Methoxy-citreoisocoumarin	[F] Penicillium nalgiovense	Didemimide B	[An] Didemnidae									
1209	6.02_233.0809	233.081	6.02	588209.849	19.166	233.0785	233.081	0.24039	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																																	
1209	5.03_242.1021	242.102	5.03	831817.891	19.6659	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogon roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1209	5.39_284.1392	284.139	5.39	664843.046	19.3427	284.1363	284.139	0.56976	7	10-Hydroxy-trans-paspalic acid	[F] Claviceps paspali		[B] marine Streptomyces sp.	10-Hydroxy-cis-paspalic acid	[F] Claviceps paspali	8-Hydroxyergine	[F] Claviceps paspali	8-Hydroxyerginine	[F] Claviceps paspali mg-6	cis-Cyclo(Pro, Trp); L,L-Cyclo(tryptophanylprolyl); Brevianamide F	[F] Penicillium brevi-compactum; Aspergillus ochraceus; [B] marine Streptomyces sp. Sch15, S.sp. L028; S. sp. TN58 [F] marine Aspergillus fumigatus	Tryptophan-dehydrobutyrine diketopiperazine; TDD	[B] Streptomyces spectabilis																																									
1214	5.86_234.1125	234.113	5.86	11882723	23.5024	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																				
1214	5.03_242.1021	242.102	5.03	422263.164	18.6878	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogon roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1238	5.86_234.1125	234.113	5.86	7553352.23	22.8487	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																				
1238	6.41_242.2843	242.284	6.41	6144942.81	22.551	242.2818	NA	NA	NA																																																							
1238	5.39_252.0868	252.087	5.39	1215319.27	20.2129	252.0842	NA	NA	NA																																																						
1238	6.02_293.1019	293.102	6.02	1135437.74	20.1148	293.0989	293.102	0.25032	23	Compound 2	[F] endophytic Fusarium sp. BCC14842	Compound 3	[F] endophytic Fusarium sp. BCC14842	compound 1	[F] sterile dark (wheat rhizosphere fungus)	Hydroisoflavone C	[B] Streptomyces mirabilis	Chaetoquadrin K	[F] ascomycete Chaetomium quadrangulatum	4'-Epialtenuene	[F] endophytic Alternaria sp. from Polygonum senegalense	Phomoeuphorbin A	[F] endophytic Phomopsis euphorbiae	Isoaltenuene	[F] aquatic Tubeufiacea sp.	Phenatic acid B	[B] Streptomyces sp. K03-0132	5'-Epialtenuene	[F] aquatic Tubeufiacea sp.	5-(3'-Carboxyl-3'-methyl-2E-allyloxy)-3-methoxy-4-methylphthalide	[F] Pestalotiopsis photiniae	Lachnone C	[F] filamentous fungus Lachnum sp. BCC 2424	Colletopyrone	[F] Colletotrichum nicotianae, Helichrysum italicum.	Pentalenolactone G	[B] Streptomyces sp.	Solaniol; F-3	[F] Fusarium solani; "Munissi MUF2"	Lomandrone		(+)-Altenuene; 2',3',4',5'-Tetrahydro-3,4'a,5'a-trihydroxy-5-methoxy-2'a-methyldibenzo[a]-pyrone	[F] Alternaria tenuis	Italicic acid	[F] Penicillium italicum wehmer	Hericenal A	[F] Hericium erinaceus DSM 10600	Dendryol D	[F] weed pathogenic fungus Dendryphiella sp.	LL-Z1640-6	[F] Penicillium nalgiovense GT56115	6-Methoxy-citreoisocoumarin	[F] Penicillium nalgiovense	Didemimide B	[An] Didemnidae									
1238	6.02_233.0809	233.081	6.02	1157059.4	20.142	233.0785	233.081	0.24039	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																																	









1290	6.61_205.0858	205.086	6.61	475291.728	18.8585	205.0837	205.086	0.63066	17	1-Methoxy-6-methyl-3-oxo-indan-4-carbaldehyde	[C] marine Lyngbya majuscula	2,4-Dihydroxy-6-[(1'E,3'E)-penta-1',3'-dienyl]-benzaldehyde	[F] endophytic Periconia atropurpurea of Xylopia aromatica	Botryosphaeridione	[F] endophytic Botryosphaeria rhodina PSU-M35 and PSU-M114	(S)-2-(3'-Hydroxyisoprop-1'-enyl)-2,3-dihydrobenzofuran-5-carbaldehyde	[F] basidiomycete Bondarzewia berkeleyi (Bondarzewiace)	Anhydro-talomone	acid treatement of talomone	Benzophomopsin A	[F] endophytic Phomopsis sp.	8-Hydroxy-6-methoxy-3-methyl-1(2H)-naphthalenone	[F] fungus Ceratocystis fimbriata f. sp. platani	2,2-Dimethyl-2H-1-benzopyran-6-carboxylic acid; Anofinic acid	[F] Lactarius deliciosus, Curvularia fallax [F] endophytic Penicillium sp. GT6105	Methylnemotinate	[F] Poria subacida	Frustulosinol	[F] Stereum frustulosum	Odyssic acid	[F] Poria sp.	2,3-Dihydro-2-isopropenyl-5-benzofurancarboxylic acid; Fomannoxic acid; FD-78b	[F] Heterobasidion annosum; Fomes annosus	Lactarochromal; 3,4-Dihydro-2,2-dimethyl-2H-1-benzopyran-4-one-6-carbaldehyde	[F] Lactarius deliciosus	Cladoacetal B	[F] fungicolous hyphomycete NRRL 29097 (Cladosporium sp.?)	Cladoacetal A	[F] fungicolous hyphomycete NRRL 29097 (Cladosporium sp.?)	H-31	[F] basidiomycete Heterobasidion annosum, Gloeophyllum abietinum	5-Formyl-3-hydroxy-2-isopropenyl-2,3-dihydrobenzofuran	[F] Fomes annosus												
1293	5.86_234.1125	234.113	5.86	10034035.2	23.2584	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																										
1293	6.41_242.2843	242.284	6.41	3802245.97	21.8584	242.2818	NA	NA	NA																																														
1293	5.39_252.0868	252.087	5.39	1084024.8	20.048	252.0842	NA	NA	NA																																														
1293	6.02_293.1019	293.102	6.02	3012005.24	21.5223	293.0989	293.102	0.25032	23	Compound 2	[F] endophytic Fusarium sp. BCC14842	Compound 3	[F] endophytic Fusarium sp. BCC14842	compound 1	[F] sterile dark (wheat rhizosphere fungus)	Hydroisoflavone C	[B] Streptomyces mirabilis	Chaetoquadrin K	[F] ascomycete Chaetomium quadrangulatum	4'-Epialtenuene	[F] endophytic Alternaria sp. from Polygonum senegalense	Phomoeuphorbin A	[F] endophytic Phomopsis euphorbiae	Isoaltenuene	[F] aquatic Tubeufiacea sp.	Phenatic acid B	[B] Streptomyces sp. K03-0132	5'-Epialtenuene	[F] aquatic Tubeufiacea sp.	5-(3'-Carboxyl-3'-methyl-2E-allyloxy)-3-methoxy-4-methylphthalide	[F] Pestalotiopsis photiniae	Lachnone C	[F] filamentous fungus Lachnum sp. BCC 2424	Colletopyrone	[F] Colletotrichum nicotianae, Helichrysum italicum.	Pentalenolactone G	[B] Streptomyces sp.	Solaniol; F-3	[F] Fusarium solani; "Munissi MUF2"	Lomandrone		(+)-Altenuene; 2',3',4',5'-Tetrahydro-3,4'a,5'a-trihydroxy-5-methoxy-2'a-methyldibenzo[a]-pyrone	[F] Alternaria tenuis	Italicic acid	[F] Penicillium italicum wehmer	Hericenal A	[F] Hericium erinaceus DSM 10600	Dendryol D	[F] weed pathogenic fungus Dendryphiella sp.	LL-Z1640-6	[F] Penicillium nalgiovense GT56115	6-Methoxy-citreoisocoumarin	[F] Penicillium nalgiovense	Didemimide B	[An] Didemnidae
1293	6.02_233.0809	233.081	6.02	1738743.56	20.7296	233.0785	233.081	0.24039	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																							
1293	5.03_242.1021	242.102	5.03	836776.662	19.6745	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																								
1293	5.10_257.1497	257.15	5.1	4264524.83	22.024	257.1471	257.15	0.419	2	Pulcherriminic acid	[Y] Candida pulcherrima	2-Methylenecycloheptene-1,3-diglycine	[F] Lactarius helvus																																										
1293	5.28_291.1343	291.134	5.28	1901971.05	20.8591	291.1313	291.134	1.22904	3	Limazepine B1	[B] Micrococcus sp. ICBB 8177	Limazepine B2	[B] Micrococcus sp. ICBB 8177	11-Demethyltomaymycin; Pretomaymycin-hydrat	[B] Streptomyces achromogenes var. tomaymycetics																																								
1293	5.66_253.1185	253.119	5.66	273466.959	18.061	253.1159	253.118	0.8213	2	N-[2-(3-Pyridyl)-ethyl]-L-glutamic acid; N-[2(3-Pyridyl)-ethyl]-L-glutaminic acid	[F] Clitocybe acromelalga	Isovaleryl 3-nitrotyramide																																									
1293	5.41_279.0864	279.086	5.41	377564.912	18.5264	279.0836	279.086	0.27483	13	1-Methyl-lumichrome	[B] Micromonospora T? 6368	3-Methyl-lumichrome	[F] Cyathus bulleri	4-[(Acetyloxy)methyl]-6,8-dihydroxy-3,5-dimethyl-1H-2-benzopyran-1-one	[F] endophytic Scytalidium sp.	Inonotic acid methyl ester	[F] Inonotus sp.	Alternarienonic Acid	[F] endophytic Alternaria sp. from Polygonum senegalense	Microdiplodiasone	[F] Microdiplodia sp.	Orsellide E	[F] marine Chaetomium sp. Goe 100/9	Citreoisocoumarin; 3-(2S-Hydroxy-4-oxopentyl)-6,8-dihydroxyisocoumarin	[F] Penicillium citreoviride b. (ifo 4692), P. citreovirens (ifo 6030)	4,4'-Dimethyl-2,2',3,3',6,6'-biphenylhexol; Leucophenicin	[F] Penicillium rubrum	6-(1-Hydroxyethyl)-2,7-dimethoxyjuglone; 6-(1-Hydroxyethyl)-5-hydroxy-2,7-dimethoxy-1,4-naphthoquinone	[F] Kirschsteiniothelia sp.; Guignardia laricina gm-7, Hendersonula toruloidea	Methyl (4Z,6E)-7-(3,4-dihydroxyphenyl)-5-hydroxy-3-oxo-4,6 heptadienoate; Inonotic acid methyl ester	[F] Inonotus hispidus	6-Methylcristazarin	[L] lichen Cladonia cristatella	Didemimide A	[An] Didemnidae																				
1293	5.42_219.0654	219.065	5.42	677292.621	19.3694	219.0632	219.065	0.94081	12	2-Carboxy-8-methoxy-naphthalene-1-ol	[F] algicolous fungi KT31, KT29 from the red seaweed Kappaphycus alvarezii	Inotilone	[F] Inonotus sp.	671-D; (2-Methyl-4-oxo-4H-chromen-5-yl)acetic acid; BSM-1	[B] Streptomyces sp. AK671; recombinant Streptomyces lividans K4-114	6-Ethyl-2-hydroxyjuglone		5-(1,2-Epoxy-2-carboxy-propyl)benzofuran	[F] Stereum subpileatum	6-Hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone; Diomelquinone A		2,7-Dimethylnaphthazarin		Droserone-5-methyl ether		Dec-2,8-diene-4,6-diynedioic acid dimethyl ester	[F] Polyporus anthracophilus, Clitocybe robusta Peck	Rhacodione C		Aristolindiquinone		1-Hydroxy-4-methoxy-2-naphthoic acid	[B] Streptosporangium cinnabarinum ATCC 31213 [B] Streptomyces sp. GW58/450 and B7828; [B] ruminal bacterium ZIE																						
1293	6.61_233.0810	233.081	6.61	278760.243	18.0887	233.0786	233.081	0.66942	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																							
1291	5.86_234.1125	234.113	5.86	4677039.62	22.1572	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																										
1291	6.41_242.2843	242.284	6.41	4230545.82	22.0124	242.2818	NA	NA	NA																																													
1291	5.39_252.0868	252.087	5.39	3783149.43	21.8512	252.0842	NA	NA	NA																																														
1291	6.02_293.1019	293.102	6.02	3278763.41	21.6447	293.0989	293.102	0.25032	23	Compound 2	[F] endophytic Fusarium sp. BCC14842	Compound 3	[F] endophytic Fusarium sp. BCC14842	compound 1	[F] sterile dark (wheat rhizosphere fungus)	Hydroisoflavone C	[B] Streptomyces mirabilis	Chaetoquadrin K	[F] ascomycete Chaetomium quadrangulatum	4'-Epialtenuene	[F] endophytic Alternaria sp. from Polygonum senegalense	Phomoeuphorbin A	[F] endophytic Phomopsis euphorbiae	Isoaltenuene	[F] aquatic Tubeufiacea sp.	Phenatic acid B	[B] Streptomyces sp. K03-0132	5'-Epialtenuene	[F] aquatic Tubeufiacea sp.	5-(3'-Carboxyl-3'-methyl-2E-allyloxy)-3-methoxy-4-methylphthalide	[F] Pestalotiopsis photiniae	Lachnone C	[F] filamentous fungus Lachnum sp. BCC 2424	Colletopyrone	[F] Colletotrichum nicotianae, Helichrysum italicum.	Pentalenolactone G	[B] Streptomyces sp.	Solaniol; F-3	[F] Fusarium solani; "Munissi MUF2"	Lomandrone		(+)-Altenuene; 2',3',4',5'-Tetrahydro-3,4'a,5'a-trihydroxy-5-methoxy-2'a-methyldibenzo[a]-pyrone	[F] Alternaria tenuis	Italicic acid	[F] Penicillium italicum wehmer	Hericenal A	[F] Hericium erinaceus DSM 10600	Dendryol D	[F] weed pathogenic fungus Dendryphiella sp.	LL-Z1640-6	[F] Penicillium nalgiovense GT56115	6-Methoxy-citreoisocoumarin	[F] Penicillium nalgiovense	Didemimide B	[An] Didemnidae
1291	6.02_233.0809	233.081	6.02	1918035.9	20.8712	233.0785	233.081	0.24039	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																							

1291	5.03_242.1021	242.102	5.03	2543440.62	21.2783	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																																	
1291	6.55_195.1020	195.102	6.55	8098807.36	22.9493	195.1000	195.102	2.15574	26	1-(2-Hydroxy-6-methoxyphenyl)butan-1-one	[F] endophytic Nodulisporium sp. from Juniperus cedre	(2R*,4R*)-3,4-Dihydro-4-methoxy-2-methyl-2H-1-benzopyran-5-ol	[F] endophytic Nodulisporium sp. A4 from Aquilaria sinensis	Auxofuran	[B] mycorrhiza helper bacterium, Streptomyces sp. AcH 505	Novae-zelandin A; Pyrenocine G	[F] Alternaria infectoria x; [F] unidentified endophytic fungus	Tyrosol propionate	[Y] sponge-derived Pichia membranaefaciens	Pyrenocine F	[F] unidentified endophytic fungus	O-Methyltyrosol acetate	[Y] sponge-derived Pichia membranaefaciens	6-Ethyl-2-hydroxy-4-methoxy-3-methylbenzaldehyde	[F] mangrove endophytic fungus No. ZZF 32	5-(3-Methylbuta-1,3-dienylidene)-2,3-epoxycyclohexane-1,4-diol	[F] Eutypa lata	Nectriapyrone; NSC 263671	[F] Pestalotiopsis oenotherae	Asperpentyn	[F] Aspergillus duricaulis	Xialenone A	[B] Streptomyces sp. GT 061169	3,4-Dimethoxycinnamyl alcohol	[F] Phanerochaete chrysosporium	F-11334-B1	[F] Acremonium murorum (Corda) W. Gams SANK 20793	Zingerone	[F] fungus Nidula niveo-tormentosa	Nakienone A	[C] Okinawan cyanobacterial (Synechocystis sp.) overgrowth of coral; [A] ascidian Lissoclinum sp., Diplosoma sp.	Nakienone B	[C] Okinawan cyanobacterial (Synechocystis sp.) overgrowth of coral	Nakienone C	[C] Okinawan cyanobacterial (Synechocystis sp.) overgrowth of coral	3',5'-Dihydroxy-2'-methylphenyl-2-butanone	[F] endophytic fungus	Kobifuranone B; GK-3	[F] ascomycete Gelasinospora kobi	F-11334-A3; 2,2-Dimethylchroman-3,6-diol	[F] algicolous marine fungus Acremonium murorum (Corda) W. Gams SANK 20793	Galiellalactone	[F] ascomycete A111-95	2,3,4-Trimethyl-5,7-dihydroxy-2,8-dihydrobenzofuran	[F] Penicillium citrinum F5	Arohynapene-D	[F] Penicillium sp FO-2295	F-11334-A2; 2-(1-Hydroxy-1-methyl)-2,3-dihydrobenzofuran-5-ol	[F] algicolous marine fungus Acremonium murorum (Corda) W. Gams SANK 20793	p-Methoxydihydrocinnamic acid methylester; Methyl-3-p-anisoloxypropionate	[F] fungus Ophiostoma crassivaginata			
1291	5.10_257.1497	257.15	5.1	6065139.74	22.5321	257.1471	257.15	0.419	2	Pulcherriminic acid	[Y] Candida pulcherrima	2-Methylenecycloheptene-1,3-diglycine	[F] Lactarius helvus																																																			
1291	5.28_291.1343	291.134	5.28	4628698.59	22.1422	291.1313	291.134	1.22904	3	Limazepine B1	[B] Micrococcus sp. ICBB 8177	Limazepine B2	[B] Micrococcus sp. ICBB 8177	11-Demethyltomaymycin; Pretomaymycin-hydrat	[B] Streptomyces achromogenes var. tomaymycetics																																																			
1291	5.66_253.1185	253.119	5.66	268833.466	18.0364	253.1159	253.118	0.8213	2	N-[2-(3-Pyridyl)-ethyl]-L-glutamic acid; N-[2(3-Pyridyl)-ethyl]-L-glutaminic acid	[F] Clitocybe acromelalga	Isovaleryl 3-nitrotyramide																																																					
1291	5.41_279.0864	279.086	5.41	1084382.29	20.0484	279.0836	279.086	0.27483	13	1-Methyl-lumichrome	[B] Micromonospora T? 6368	3-Methyl-lumichrome	[F] Cyathus bulleri	4-[(Acetyloxy)methyl]-6,8-dihydroxy-3,5-dimethyl-1H-2-benzopyran-1-one	[F] endophytic Scytalidium sp.	Inonotic acid methyl ester	[F] Inonotus sp.	Alternarienonic Acid	[F] endophytic Alternaria sp. from Polygonum senegalense	Microdiplodiasone	[F] Microdiplodia sp.	Orsellide E	[F] marine Chaetomium sp. Goe 100/9	Citreoisocoumarin; 3-(2S-Hydroxy-4-oxopentyl)-6,8-dihydroxyisocoumarin	[F] Penicillium citreoviride b. (ifo 4692), P. citreovirens (ifo 6030)	4,4'-Dimethyl-2,2',3,3',6,6'-biphenylhexol; Leucophenicin	[F] Penicillium rubrum	6-(1-Hydroxyethyl)-2,7-dimethoxyjuglone; 6-(1-Hydroxyethyl)-5-hydroxy-2,7-dimethoxy-1,4-naphthoquinone	[F] Kirschsteiniothelia sp.; Guignardia laricina gm-7, Hendersonula toruloidea	Methyl (4Z,6E)-7-(3,4-dihydroxyphenyl)-5-hydroxy-3-oxo-4,6 heptadienoate; Inonotic acid methyl ester	[F] Inonotus hispidus	6-Methylcristazarin	[L] lichen Cladonia cristatella	Didemimide A	[An] Didemnidae																													
1291	5.42_219.0654	219.065	5.42	886690.816	19.7581	219.0632	219.065	0.94081	12	2-Carboxy-8-methoxy-naphthalene-1-ol	[F] algicolous fungi KT31, KT29 from the red seaweed Kappaphycus alvarezii	Inotilone	[F] Inonotus sp.	671-D; (2-Methyl-4-oxo-4H-chromen-5-yl)acetic acid; BSM 1	[B] Streptomyces sp. AK671; recombinant Streptomyces lividans K4-114	6-Ethyl-2-hydroxyjuglone		5-(1,2-Epoxy-2-carboxy-propyl)benzofuran	[F] Stereum subpileatum	6-Hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone; Diomelquinone A		2,7-Dimethylnaphthazarin		Droserone-5-methyl ether		Dec-2,8-diene-4,6-diynedioic acid dimethyl ester	[F] Polyporus anthracophilus, Clitocybe robusta Peck	Rhacodione C		Aristolindiquinone		1-Hydroxy-4-methoxy-2-naphthoic acid	[B] Streptosporangium cinnabarinum ATCC 31213 [B] Streptomyces sp. GW58/450 and B7828; [B] ruminal bacterium ZIE																															
1291	6.61_233.0810	233.081	6.61	286649.442	18.1289	233.0786	233.081	0.66942	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																																	
1291	5.39_284.1392	284.139	5.39	260446.959	17.9906	284.1363	284.139	0.56976	7	10-Hydroxy-trans-paspalic acid	[F] Claviceps paspali		[B] marine Streptomyces sp.	10-Hydroxy-cis-paspalic acid	[F] Claviceps paspali	8-Hydroxyergine	[F] Claviceps paspali	8-Hydroxyerginine	[F] Claviceps paspali mg-6	cis-Cyclo(Pro, Trp); L,L-Cyclo(tryptophanylprolyl); Brevianamide F	[F] Penicillium brevi-compactum; Aspergillus ochraceus; [B] marine Streptomyces sp. Sch15, S.sp. L028; S. sp. TN58 [F] marine Aspergillus fumigatus	Tryptophan-dehydrobutyrine diketopiperazine; TDD	[B] Streptomyces spectabilis																																									
1207	5.86_234.1125	234.113	5.86	7633264.48	22.8639	234.1101	234.112	0.09206	2	2-(1H-Indol-3-yl)ethyl 2-hydroxypropanoate		Apiodionen	[F] Apiosordaria effusa SANK 15083 (FERM p-11524)																																																			
1207	5.39_252.0868	252.087	5.39	240365.705	17.8749	252.0842	NA	NA	NA																																																									
1207	7.00_221.0812	221.081	7	5009691.55	22.2563	221.0789	221.081	1.6104	24	4,8-Dihydroxy-3-hydroxymethyl-4-methyl-1,4-dihydronaphthalene-1-one	[B] Streptomyces rimosus mutant R1059	8-Hydroxy-3-(hydroxymethyl)-6-methoxy-1(2H)-naphthalenone	[F] fungus Ceratocystis fimbriata f. sp. platani	6,8-Dimethoxy-3-methyl-isochromen-1-one	[B] Streptomyces sp. ANK 302	1-(3,9-Dihydroxy-1,3-dihydrobenzo[c]oxepin-3-yl)ethanone	[F] endophytic Pestalotiopsis virgatula	6,8-Dihydroxy-3,5,7-trimethylisocoumarin; Anhydrosclerotinin B	[F] marine filamentous fungus Keissleriella sp. YS4108	2,6-Dihydroxy-2-methyl-7-(prop-1E-enyl)-1-benzofuran-3(2H)-one	[F] endophytic Verticillium sp.	2-(Propan-2'-ol)-5-hydroxy-benzopyran-4-one	[F] endophytic Curvularia sp. from Ocotea corymbosa (Lauraceae)	Humicolone	[F] fungus Humicola grisea	Anishidiol	[F] Aspergillus nishimurae		[B] Actinomadura sp. S4215 [Pl]	Eugenitin; Eugenetin; Eugenitol	[L] [F] E. caryophyllata, Leconora rupicola, Chaetomium thielavioideum, Cylindrocarpon spp., lichen Lecanora rupicola [F] ascomycete Chaetomium aureus	3,7-Bis-(hydroxymethyl)-1-benzoxepin-5(2H)-one	[F] Marasmiellus ramealis	Deoxyradicinin	[F] Alternaria helianthi	Cycloarthropsone	[F] (fungus) Arthropsis truncata	Differolide	[B] Streptomyces aurantiogriseus Tue 3149	(+)-Cyclosordariolone	[F] Sordaria macrospora, fungicolous hyphomycete NRRL 29097 (Cladosporium sp.?)	(-)-Cyclosordariolone	[F] fungicolous hyphomycete NRRL 29097 (Cladosporium sp.?)	Hispolon	[F] fungus Inonotus hispidus	Decarboxycitrinone	[F] coprophilous fungus Cercophora areolata JS 166 (= UAMH 7495)	Lactarochromanoic acid	[F] Lactarius deliciosus, artifact?; Aspergillus terreus IFO 8835; Chrysothamnus viscidiflorus	Siderin; 4,7-Dimethoxy-5-methylcoumarin	[F] Aspergillus variecolor	Montadial A	[F] polypore Bondarzewia montana	Deca-2-ene-4,6-diynedinoic acid dimethyl ester	B244	Citreopyrone A	[F] Penicillium citreoviride hybrid strain							
1207	5.25_285.2172	285.217	5.25	367292.265	18.4866	285.2143	NA	NA	NA																																																									

1206	6.02_293.1019	293.102	6.02	5013888.25	22.2575	293.0989	293.102	0.25032	23	Compound 2	[F] endophytic Fusarium sp. BCC14842	Compound 3	[F] endophytic Fusarium sp. BCC14842	compound 1	[F] sterile dark (wheat rhizosphere fungus)	Hydroisoflavone C	[B] Streptomyces mirabilis	Chaetoquadrin K	[F] ascomycete Chaetomium quadrangulatum	4'-Epialtenuene	[F] endophytic Alternaria sp. from Polygonum senegalense	Phomoeuphorbin A	[F] endophytic Phomopsis euphorbiae	Isoaltenuene	[F] aquatic Tubeufiacea sp.	Phenatic acid B	[B] Streptomyces sp. K03-0132	5'-Epialtenuene	[F] aquatic Tubeufiacea sp.	5-(3'-Carboxyl-3'-methyl-2E-allyloxy)-3-methoxy-4-methylphthalide	[F] Pestalotiopsis photiniae	Lachnone C	[F] filamentous fungus Lachnum sp. BCC 2424	Colletopyrone	[F] Colletotrichum nicotianae, Helichrysum italicum.	Pentalenolactone G	[B] Streptomyces sp.	Solaniol; F-3	[F] Fusarium solani; "Munissi MUF2"	Lomandrone		(+)-Altenuene; 2',3',4',5'-Tetrahydro-3,4'a,5'a-trihydroxy-5-methoxy-2'a-methyldibenzo[a]-pyrone	[F] Alternaria tenuis	Italicic acid	[F] Penicillium italicum wehmer	Hericenal A	[F] Hericium erinaceus DSM 10600	Dendryol D	[F] weed pathogenic fungus Dendryphiella sp.	LL-Z1640-6	[F] Penicillium nalgiovense GT56115	6-Methoxy-citreoisocoumarin	[F] Penicillium nalgiovense	Didemimide B	[An] Didemnidae									
1206	6.02_233.0809	233.081	6.02	1294949.94	20.3045	233.0785	233.081	0.24039	11	Citrinolactone B	[F] Penicillium citrinum	Compound 9	[F] marine Penicillium terrestre	Altechromone-B	[F] Alternaria sp.	5,6-Dimethoxy-2-methyl-1,4-naphthoquinone		Salfredin BII	[F] Crucibulum sp RF-3817	Coniochaetone B	[F] Coniochaeta saccardoi (JS 223)	BSM-2	[B] recombinant Streptomyces lividans K4-114	Trideca-5,7,9,11-tetrayne-1,2,3,4-tetraol	[F] Fistulina hepatica	5,8-Dimethoxy-6-methyl-1,2-naphthoquinone	[synthetic]	6-Ethyl-7-methoxyjuglone	[F]	2-Hydroxy-6-methoxy-3,5-dimethyl-1,4-benzoquinone	[F] Phoma wasabiae																																	
1206	5.03_242.1021	242.102	5.03	692057.177	19.4005	242.0996	242.102	0.85793	3	trans-Zeatin	[F] [B] Pseudomonas amygdali, Rhodospirillum sp., Rhodopseudomonas spheroides, Rhizopogom roseolus, Suillus punctipes	Pestalotiopsoid A	[F] endophytic Pestalotiopsis sp. from mangrove Rhizophora mucronata	Stresgenin B	[B] Streptomyces sp.																																															
1206	5.28_291.1343	291.134	5.28	202705.434	17.629	291.1313	291.134	1.22904	3	Limazepine B1	[B] Micrococcus sp. ICBB 8177	Limazepine B2	[B] Micrococcus sp. ICBB 8177	11-Demethyltomaymycin; Pretomaymycin-hydrat	[B] Streptomyces achromogenes var. tomaymycetics																																															
1206	5.41_279.0864	279.086	5.41	899449.23	19.7787	279.0836	279.086	0.27483	13	1-Methyl-lumichrome	[B] Micromonospora T? 6368	3-Methyl-lumichrome	[F] Cyathus bulleri	4-[(Acetyloxy)methyl]-6,8-dihydroxy-3,5-dimethyl-1H-2-benzopyran-1-one	[F] endophytic Scytalidium sp.	Inonotic acid methyl ester	[F] Inonotus sp.	Alternarienonic Acid	[F] endophytic Alternaria sp. from Polygonum senegalense	Microdiplodiasone	[F] Microdiplodia sp.	Orsellide E	[F] marine Chaetomium sp. Goe 100/9	Citreoisocoumarin; 3-(2S-Hydroxy-4-oxopentyl)-6,8-dihydroxyisocoumarin	[F] Penicillium citreoviride b. (ifo 4692), P. citreovirens (ifo 6030)	4,4'-Dimethyl-2,2',3,3',6,6'-biphenylhexol; Leucophenicin	[F] Penicillium rubrum	6-(1-Hydroxyethyl)-2,7-dimethoxyjuglone; 6-(1-Hydroxyethyl)-5-hydroxy-2,7-dimethoxy-1,4-naphthoquinone	[F] Kirschsteiniothelia sp.; Guignardia laricina gm-7, Hendersonula toruloidea	Methyl (4Z,6E)-7-(3,4-dihydroxyphenyl)-5-hydroxy-3-oxo-4,6 heptadienoate; Inonotic acid methyl ester	[F] Inonotus hispidus	6-Methylcrystazarin	[L] lichen Cladonia cristatella	Didemimide A	[An] Didemnidae																													
1206	5.42_219.0654	219.065	5.42	692427.288	19.4013	219.0632	219.065	0.94081	12	2-Carboxy-8-methoxy-naphthalene-1-ol	[F] algicolous fungi KT31, KT29 from the red seaweed Kappaphycus alvarezii	Inotilone	[F] Inonotus sp.	671-D; (2-Methyl-4-oxo-4H-chromen-5-yl)acetic acid; BSM-1	[B] Streptomyces sp. AK671; recombinant Streptomyces lividans K4-114	6-Ethyl-2-hydroxyjuglone		5-(1,2-Epoxy-2-carboxy-propyl)benzofuran	[F] Stereum subpileatum	6-Hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone; Diomelquinone A		2,7-Dimethylnaphthazarin		Droserone-5-methyl ether		Dec-2,8-diene-4,6-diynedioic acid dimethyl ester	[F] Polyporus anthracophilus, Clitocybe robusta Peck	Rhacodione C		Aristolindiquinone		1-Hydroxy-4-methoxy-2-naphthoic acid	[B] Streptosporangium cinnabarinum ATCC 31213 [B] Streptomyces sp. GW58/450 and B7828; [B] ruminal bacterium ZIE																															