BIOACTIVE NATURAL PRODUCTS FROM MARINE MACROALGAL ENDOPHYTES FROM THE BAY OF FUNDY, CANADA

by

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ABSTRACT

We are approaching a time where antimicrobial drugs may no longer be effective due to the growing global antimicrobial resistance crisis, coupled with the lack of antimicrobial drug discovery and development. New antimicrobial therapies are needed, and endophytes from marine macroalgae have been highlighted as an important biological reservoir for the identification of novel antimicrobial molecules. A preliminary investigation of marine macroalgae from the Bay of Fundy, New Brunswick, Canada for their endophytes indicated this location to be an excellent source of endophytic fungi possessing antimicrobial activity. One hundred and forty fungal endophytes were isolated from 20 species of marine macroalgae collected from the Bay of Fundy. Fifty-four endophytes were identified to the genus or species level, and include eleven fungi not previously isolated as endophytes of marine macroalgae. The identity of 86 isolates could not be confirmed through DNA sequencing due to an inability to amplify or sequence DNA or due to low sequence homology with entries in GenBank. These isolates were designated codes according to their morphology. Each endophyte was fermented to obtain an extract in order to facilitate the discovery of new natural products. In order to prioritise the extracts obtained from these endophytic fungi, an antimicrobial bioactivity profiling technique was developed using nine microorganisms and a panel of 17 antimicrobial standards to not only attempt to identify new antimicrobial natural products, but also those that possess unique antimicrobial targets or modes of action. Principal component analysis of the extract bioactivity profiles revealed that the profiles of 37 extracts were unique within the library. Hierarchical cluster analysis using the profiles of the 37 unique extracts and the 17 antimicrobial standards showed that 26 extracts possessed bioactivity

profiles that were distinct from the antimicrobial standards and thus warranted further investigation. Subsequent bioassay guided fractionation of four fungal extracts led to the isolation of six antimicrobial natural products: penicillic acid, methylenolactocin, fumagillin, fumigatin oxide, poly(3*R*,5*R*-dihydroxyhexanoic acid) and (*P/M*)-maximiscin. These natural products, while being known chemical entities, are all reported to possess antimicrobial activity and may play an important role in future antimicrobial drug discovery and development.

DEDICATION

To my family and friends, for all of their support.

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon nuclear magnetic resonance spectroscopy
1D	One dimensional
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
2D	Two dimensional
$[\alpha]_D$	Specific rotation
AcCl	Acetyl chloride
Ac_2O	Acetic anhydride
ACS	American Chemical Society
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BD	Becton Dickinson
BioMAP	Antibiotic mode of action profile
BLAST	Basic Local Alignment Search Tool
bp	Base pair
brd	Broad doublet
brs	Broad singlet
n-BuOH	1-butanol
c	Concentration (quoted in g/100 ml)
calcd	Calculated
$CDCl_3$	Deuterated chloroform
CD_3OD	Deuterated methanol
$(CD_3)_2SO$	Deuterated dimethyl sulfoxide
CFU	Colony forming units
CH_2Cl_2	Dichloromethane
CH_3CN	Acetonitrile
CI	Confidence interval
CO_2	Carbon dioxide
COSY	Correlation spectroscopy
δ	Chemical shift in ppm
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DCC	1,3-Dicyclohexylcarbodiimide
DHF	Dihydrofolic acid
DHPS	Dihydropteroate synthase
dt	Doublet of triplets
DMAP	4-Dimethylaminopyridine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EtOAc	Ethyl acetate
EtOH	Ethanol
FT-IR	Fourier-transform infrared spectroscopy
GIS N	Geographic information system - North
GIS W	Geographic information system - West

H	Hydrogen
HCl	Hydrochloric acid
HEK	Human embryonic kidney cells
H_2O	Water
HPLC	High performance liquid chromatography
HRESIMS	High resolution electron spray ionization mass spectroscopy
Hz	Hertz
IC_{50}	Median inhibitory concentration
IF	Isolation frequency
int	Integration
IR	Infrared spectroscopy
ITS	Internal transcribed spacer
J	Coupling constant
KOH	Potassium hydroxide
LC-MS	Liquid chromatography, mass spectrometry
Ltd.	Limited
m	Multiplet
MDR-TB	Multidrug-resistant Tuberculosis
MEA	Malt extract agar
MEA-SW	Malt extract agar with seawater
MEB	Malt extract broth
MeOH	Methanol
MGIT	Mycobacteria growth indicator tube
MHz	Megahertz
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mult	Multiplicity
m/z	Mass to charge ratio
Na	Sodium
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
NCBI	National Center for Biotechnology Information
NMR	Nuclear magnetic resonance spectroscopy
NP	Normal phase
NPRG	Natural Products Research Group
NRPS	Non-ribosomal peptide synthetases
OD	Optical density
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
ppm	Parts per million
q	Quartet
®	Registered trade mark
RNA	Ribonucleic acid
RP	Reversed phase

rpm	Revolutions per minute
RT	Room temperature
s	Singlet
SE	Standard error of the mean
sex	Sextet
SM	Service mark
sp.	Species
spp.	Multiple species of the same genus
subsp.	Subspecies
t	Triplet
tt	Triplet of triplets
TB	Tuberculosis
TBE	Tris borate ethylenediaminetetraacetic acid
td	Triplet of doublets
TFA	Trifluoroacetic acid
THF	Tetrahydrofolic acid
TLC	Thin layer chromatography
TM	Trade mark
tRNA	Transfer RNA
UNB	University of New Brunswick
UNB-SJ	University of New Brunswick, Saint John campus
UV	Ultraviolet
VRE	Vancomycin-resistant *Enterococcus*
WHO	World Health Organization
ΔOD	Change in optical density
Δppm	Mass error in parts per million

Chapter 1: General introduction

1.1 The need for new antimicrobial therapies

For the last 80 years, antimicrobial drugs have revolutionized medical treatment (Andersson and Hughes 2010; Davies and Davies 2010; World Health Organization 2014a). Since their introduction, these drugs have seen use as a panacea for the treatment of infectious diseases (World Health Organization 2014a). The introduction of antimicrobial drugs into human healthcare has facilitated major advances in medicine by not only providing treatment for community-acquired infections, but also by having a pivotal role in reducing post-surgical infections (*e.g.* organ transplantation surgeries and the implantation of medical devices), improving neonatal care, and advancing cancer chemotherapy (Gould and Bal 2013; Rossolini et al. 2014; Ventola 2015; Wright 2014; Zaffiri et al. 2012). The use of antimicrobial drugs in medicine has improved the quality of life for millions and has had a direct impact on extending life expectancy worldwide (Ventola 2015; Wright 2014; Zaffiri et al. 2013).

Our current antimicrobial drug regime can be divided into drug classes based upon the target and mode of action of each drug, ultimately targeting one of five broad biological pathways: metabolism, cell-wall synthesis, protein synthesis, RNA synthesis and DNA synthesis (Table 1.1) (Levy and Marshall 2004; Lewis 2013). The first antimicrobial class introduced for clinical use were the sulfonamides, which indirectly target the metabolism of the pathogen by competitively inhibiting the production of folate (Lewis 2013; McCullough and Maren 1973; White 2012; Zaffiri et al. 2012). The sulfa-drugs were initially found to possess activity against streptococcal infections, and from 1937 to 1943, these compounds were successful at reducing mortality associated with

child birth (24%-36%), pneumonia (17%-32%) and scarlet fever (52%-65%) (Jayachandran et al. 2010).

Following the serendipitous discovery of penicillin from a fungus belonging to the genus *Penicillium* by Fleming (1929), its antibacterial assessment (Chain et al. 1940; Florey and Jennings 1942) and its approval for widespread use in the 1940s, the β-lactam class of antimicrobials has been used extensively to treat a broad range of infections that were previously deemed "untreatable" (Aminov 2017; Page 2012). Considerable work has been conducted since the discovery of penicillin to improve upon its structure in order to broaden its spectrum of activity towards Gram positive and Gram negative bacteria (Aminov 2017; Page 2012). The β-lactam class of antimicrobial drugs inhibit cell wall synthesis of the pathogen and since the discovery of penicillin has grown to include the cephalosporins, monobactams and carbapenams (Aminov 2017; Page 2012; Zaffiri et al. 2012; Zaffiri et al. 2013).

Actinomycetes have been shown to be a rich source of numerous antimicrobial drugs (Brown and Wright 2016; Golinska et al. 2015; Lewis 2013, 2017; Nalini and Prakash 2017; Wright 2017). Streptomycin, a protein synthesis inhibitor in the aminoglycoside class, significantly reduces the mortality rate of diseases such as Tuberculosis (TB), caused by the pathogen *Mycobacterium tuberculosis* (Aminov 2017; Begg and Barclay 1995; Zaffiri et al. 2012). The discovery of other classes of protein synthesis inhibitors, namely the tetracyclines, chloramphenicols, and macrolides, in the 1940s and 1950s broadened the spectrum of activity towards a wide range of pathogenic microorganisms and provided additional options to clinicians for treating infection (Aminov 2017; Nelson and Levy 2011; White 2012; Zaffiri et al. 2012). The RNA

polymerase inhibitor rifampin, discovered in the late 1950s, provided a unique target for treating *M. tuberculosis* infection (Aminov 2017; White 2012). The approval of the antifungal drugs amphotericin B and nystatin in the 1950s provided treatment for numerous mycoses and function by competitively binding with ergosterol and creating irreparable pores in the fungal cell membrane (Kathiravan et al. 2012; Perfect 2017). Indeed, the study of actinobacteria in the 1940s and 1950s has been invaluable for the discovery of both antibacterial and antifungal drug classes (Lewis 2013, 2017; Zaffiri et al. 2012).

While many antimicrobial drugs were derived from natural sources, several synthetically derived antimicrobial drugs have been discovered (*e.g.* the TB drugs isoniazid, pyrazinamide and ethambutol, the oxazolidinones, as well as the quinolones) (Andriole 2005; White 2012; Zaffiri et al. 2013). The discovery of the quinolones, a synthetic class of antimicrobial drugs that disrupt DNA synthesis, in the 1960s has allowed for the treatment of a broad spectrum of infectious pathogens (Aminov 2017; Andriole 2005). Due to their broad-spectrum activity, the fluorinated derivatives of the quinolones (*i.e.* the fluoroquinolones) have been reported to be among the most widely prescribed antimicrobial drugs for the treatment of respiratory and urinary tract infections (Aminov 2017; Andriole 2005).

While several other antibiotic classes have been approved for clinical use (Table 1.1), there has been a lack of new discoveries since the approval of the fluoroquinolones, with daptomycin, the last clinically useful antibiotic belonging in a new class, having been discovered in 1986 and approved for clinical use in 2003 (Figure 1.1) (Kealey et al. 2017; Lewis 2013; World Health Organization 2014a). While antimicrobial drugs have been

revolutionary in advancing human healthcare, the limited number of antimicrobial agents available, combined with a general complacency for current antimicrobial pharmaceutical treatment regimes, has resulted in increased prevalence and incidence of multidrug resistant infections (Brown and Wright 2016; Lewis 2013; Lewis 2015; Lewis 2017; Neu 1992; Norrby et al. 2005; Public Health Agency of Canada 2016; World Health Organization 2014a; Wright 2017).

Table 1.1. Classes of antimicrobial agents and their primary mode of action.

Biological pathway targeted	Antibiotic class	Example therapeutic	Mode of action
Cell wall synthesis	β-lactams	Penicillin	Inhibition of cell wall biosynthesis
	Glycopeptides	Vancomycin	Inhibition of cell wall biosynthesis
	Lipopeptides	Daptomycin	Depolarization of cell membrane
Protein synthesis	Aminoglycosides	Gentamicin	Binding of 30S ribosomal subunit
	Tetracyclines	Tetracycline	Binding of 30S ribosomal subunit
	Macrolides	Erythromycin	Binding of 50S ribosomal subunit
	Chloramphenicols	Chloramphenicol	Binding of 50S ribosomal subunit
	Lincosamides	Clindamycin	Binding of 50S ribosomal subunit
	Streptogramins	Synercid	Binding of 50S ribosomal subunit
	Oxazolidinones	Linezolid	Binding of 50S ribosomal subunit
DNA transcription and synthesis	Fluoroquinolones	Ciprofloxacin	Inhibition of DNA synthesis
RNA synthesis	Rifamycins	Rifampin	Binding of RNA polymerase β-subunit
	Fidaxomicin	Fidaxomicin	Inhibition of RNA polymerase
Metabolism	Sulfonamides	Sulfamethazine	Competitive inhibitor for DHPS (folate synthesis)
	Pyrimidines	Trimethoprim	Inhibitor of DHF to THF, involved in folate synthesis
	Diarylquinolines	Bedaquiline	Inhibition of F_1F_0-ATPase

Adapted from Lewis 2013, Davies & Davies 2010

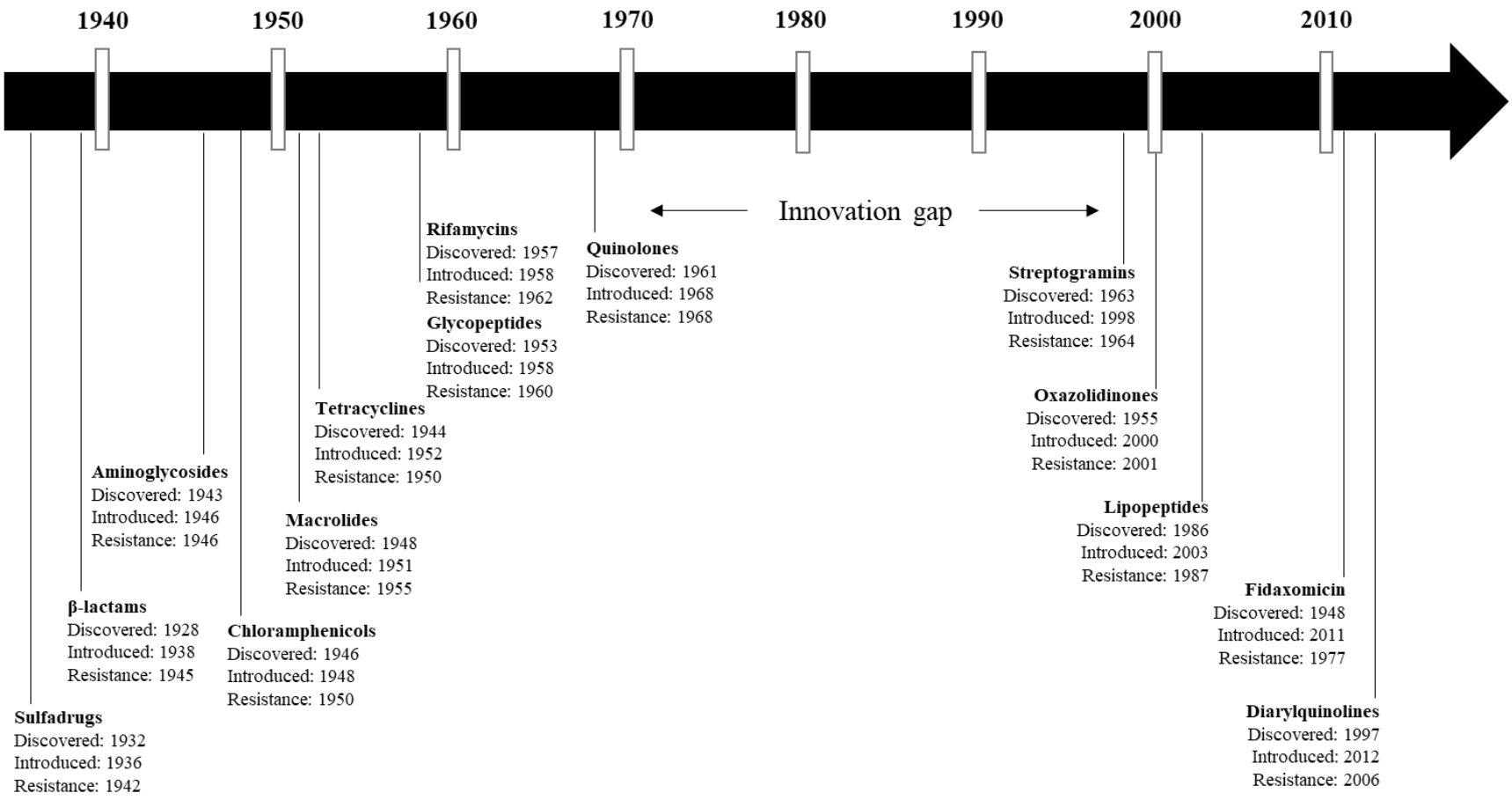


Figure 1.1. Timeline of antibiotic discovery, approval and first antimicrobial resistance (adapted from Lewis 2013). The timeline was created using the year the antibiotics were introduced for human usage.

Despite the identification of a plethora of biological targets, drug resistance has been observed for each of the major classes of antibiotics (Figure 1.1), which severely limits the availability of effective anti-infective agents (Kealey et al. 2017; Lewis 2013). Many antimicrobial resistance mechanisms exist that allow pathogens to circumvent chemotherapy (Davies and Davies 2010; Lewis 2013). These mechanisms include the destruction of the antimicrobial chemical by enzymes, modification of the drug target, limited penetration into the cells of the pathogen, or efflux of the drug before it can take effect (D'Costa et al. 2011; Davies and Davies 2010; Fischbach and Walsh 2009; Kanafani and Perfect 2008; Kontoyiannis and Lewis 2002; Lewis 2013; Lupetti et al. 2002; Neu 1992). Resistance mechanisms have been studied extensively and are known to be acquired by pathogens, either through mutation or by horizontal gene transfer into a recipient either by cell to cell conjugation, transformation through plasmids, or phage-mediated transduction (Andersson and Hughes 2010; Davies and Davies 2010; Levy and Marshall 2004).

In their 2016 report on the Canadian antimicrobial resistance surveillance system, the Public Health Agency of Canada identified several priority pathogens that are of greatest concern to the health of Canadian citizens due to their pervasive reoccurrence in healthcare and community settings (Public Health Agency of Canada 2016). These include *Clostridium difficile*, *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. (Public Health Agency of Canada 2016). In Canada, rates of drug resistant infections vary by pathogen; *N. gonorrhoeae* infections, for example, increased 43% from 2004 to 2013 (Public Health Agency of Canada 2016).

On average, one third of the *N. gonorrhoeae* infections reported in 2014 (32%) showed resistance to penicillin, tetracycline, erythromycin or ciprofloxacin (Public Health Agency of Canada 2016). It was also reported that infection rates have remained stable in recent years for drug resistant strains of *M. tuberculosis*, methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) (Public Health Agency of Canada 2015, 2016). Despite the recent stability of antimicrobial resistance trends in Canada, however, antimicrobial drug resistance remains of paramount concern globally and has prompted warnings from the World Health Organization that a post-antibiotic era in this century is a real possibility (World Health Organization 2014a).

Worldwide, the outlook for antimicrobial drug resistance remains markedly different to that of Canada. For example, it has been estimated that each year more than half a million cases of MDR-TB have been reported globally over the last decade (World Health Organization 2007, 2008, 2009a, b, 2010, 2011, 2012, 2013, 2014b, 2015, 2016). Rates of this drug resistant infection are reported to be highest in Eastern European and central Asian countries, where nearly one third of new TB cases in this region are classified as MDR-TB (World Health Organization 2014a, 2016). In addition to MDR-TB, drug resistant infections caused by *E. coli* (resistance to cephalosporins and fluoroquinolones), *Klebsiella pneumoniae* (resistance to cephalosporins and carbapenems), *S. aureus* (resistance to methicillin and other β-lactams), *Streptococcus pneumoniae* (resistance to penicillin), *Shigella* spp. (resistance to fluoroquinolones), *N. gonorrhoeae* (resistance to cephalosporins), *Candida* spp. (resistance to azoles) and non-typhoidal *Salmonella* (resistance to fluoroquinolones) are of particular concern in African, Eastern European, Middle Eastern and Asian countries where the proportion of drug

resistant infections can exceed 50% of all cases reported (Ghannoum and Rice 1999; World Health Organization 2014a).

The current climate of wide-spread antimicrobial drug resistance cannot be attributed to a single point source. The WHO highlights the overuse and improper use of antibiotics for both human and animal health, poor surveillance and control of infections, and the lack of commitment by patients to finish treatment as contributing factors to the antimicrobial resistance pandemic (World Health Organization 2014a). It has also been reported that the development of new antimicrobial agents has been vastly outpaced by the rapid onset of antimicrobial drug resistance (Levy and Marshall 2004; Lewis 2013, 2017).

It is evident that we need new antimicrobial therapies. In addition, we also need those that exploit new targets or possess unique modes of action if we are to overcome the global antimicrobial resistance pandemic (Brown and Wright 2016; Davies and Davies 2010; Perfect 2017). Attempts to move away from naturally sourced antibiotics by pharmaceutical companies have largely failed, with fluoroquinolones and oxazolidinones representing the only synthetic antibiotic classes successfully developed in the past 50 years (Lewis 2013, 2017). The reality is that microbial natural products have been, and remain to be, the most important source of new antimicrobial agents (Brown et al. 2014; Newman and Cragg 2007; Newman and Cragg 2012, 2016; Newman et al. 2003). As such, efforts must continue to identify new lead compounds for use as future antimicrobial pharmaceuticals (Brown et al. 2014; Wright 2017).

1.2 Natural products as a source for new antimicrobials

Natural products are organic compounds produced by living organisms that appear to be non-essential to the growth and development of the organism that produces them (Clark 1996; Croteau et al. 2000; Haslam 1986; Mann 1987; Meinwald 2011; Williams et al. 1989). Since the discovery of penicillin (Fleming 1929), natural products have been the most successful source of antimicrobial pharmaceuticals (Bologa et al. 2013; Cassell and Mekalanos 2001; Fischbach and Walsh 2009; Miller and Waldrop 2010), and in the last 30 years alone, more than half of the antibacterial drugs have been derived from a natural product or derivative thereof (Newman and Cragg 2016). Traditional approaches to antimicrobial discovery by screening libraries of natural product extracts or compounds, or by exploiting the physiology, biochemistry and genetics of pathogens have stalled and are no longer effective (Kealey et al. 2017; Wright 2017). In the face of an urgent need for new antimicrobial agents, new strategies are required to overcome the gap in innovation currently existing in the development of anti-infective agents (Genilloud 2014; Lewis 2017). Improved technologies, such as metabolomic and high-throughput profiling methodologies (Bologa et al. 2013; Brown and Wright 2016; Crusemann et al. 2017; Wong et al. 2012), genome mining (Ochi and Hosaka 2013; Onaka 2017; Ziemert et al. 2016), and changes to cultivation strategies to grow previously uncultivable microorganisms (Lewis 2017; Nichols et al. 2010) are currently used to identify new bioactive natural products. It has also been suggested that the exploitation of new or understudied biological reservoirs, such as organisms in the vastly unexplored marine ecosystem, including those microorganisms associated with marine plants (both as

endophytes and epiphytes) and invertebrates, will aide in the discovery of new antimicrobial therapeutics (Kealey et al. 2017; Schinke et al. 2017; Zhang et al. 2016a).

Since the late 1950s and early 1960s, marine plants, invertebrates and microorganisms have been the focus of research efforts to identify marine natural products for the purpose of discovering new pharmaceutical agents (Aly et al. 2011; Bernan et al. 1997; Demain 2006; Fenical 1993 and the annual reviews by Faulkner 1977-2002, and Blunt et al. 2003-2017) . Research into natural products from marine organisms developed, in part, due to the great biodiversity the largely unexplored oceans presented, as high species biodiversity has been suggested to correlate with high chemical diversity among natural products (Haefner 2003; Hughes and Fenical 2010; Imhoff et al. 2011).

Bacteria and fungi are common within the ocean environment, with estimates of their abundance being 10^6 bacterial and 10^3 fungal cells per milliliter of seawater (Hughes and Fenical 2010). In addition to existing free within the ocean seawater, many marine microorganisms colonise the surface or within the tissues of marine invertebrates and plants (Bernan et al. 1997; Jones et al. 2008; Kobayashi and Ishibashi 1993). The relationship between a microorganism and its host, combined with their ability to adapt to unique and extreme environments, are thought to facilitate the production of novel bioactive secondary metabolites (Imhoff et al. 2011; Jones et al. 2008; Kjer et al. 2010; Raghukumar 2008). It is due to these unique interactions, between micro- and macro-organisms and the stresses provided by their environment, that marine-derived fungi and bacteria have become foci as sources for natural products (Jones et al. 2008; Konig et al. 2006; Schulz et al. 2008).

Despite being an area of intense research for 60 years, there is still a great deal to be accomplished in the field of marine natural products. In 2008, Blunt et al. estimated that the discovery of natural products from marine sources is extremely under-developed, as only five phyla, Hemichordata, Cnidaria, Rhodophyta, Chordata and Porifera, have had more than 5% of their estimated species investigated for natural products. In the years since this review, Blunt et al. have documented the rapid rise in studies investigating marine microorganisms belonging to the phyla Ascomycota and Actinobacteria, highlighting the value of these microorganisms in pursuing bioactive marine natural products (Blunt et al. 2015, 2017). Marine microorganisms are excellent sources of new natural products, with many of those isolated having biological activities, such as antimicrobial, anticancer, and antiviral (see the annual reviews by Faulkner 1977-2002, and Blunt et al. 2003-2017).

The research described in this thesis focusses on the isolation and identification of endophytic fungi from marine macroalgae of the Bay of Fundy, New Brunswick, Canada and their potential for producing novel antimicrobial natural products. Furthermore, this thesis discusses the application of an antimicrobial bioactivity profiling technique to assess and prioritise the library of extracts obtained from the marine macroalgal endophytes and the subsequent identification of antimicrobial natural products from promising extracts prioritised using this bioactivity profiling approach.

Chapter 2: Isolation and identification of endophytes from marine macroalgae of the Bay of Fundy, Canada

2.1 Introduction

2.1.1 Endophytic fungi from marine macroalgae

Endophytic fungi live, for either part or all of their life cycle, within the tissues of plants without causing any observable negative effects on their host (Carroll 1988; Strobel et al. 2004; Wilson 1995). The relationships between host and endophyte can vary widely with many relationships being classified as symbiotic, mutualistic or of a balanced antagonism (Muller and Krauss 2005; Redman et al. 2001; Schulz and Boyle 2005; Schulz et al. 1998; Strobel and Daisy 2003; Tan and Zou 2001). As a result of these relationships, endophytic fungi are thought to provide enhanced fitness to their host, through for example, increased salt, drought and cold tolerance (Clay and Schardl 2002; Redman et al. 2011) as well as protection from herbivory (Czarnoleski et al. 2010; Saari et al. 2010).

The possibility of fungi living within marine macroalgae was recognized in the 1890's (Church 1893); however, the detailed life history of the fungus *Mycosphaerella ascophylii* living symbiotically within the brown alga *Ascophyllum nodosum* was not noted until the 1960s (Webber 1967). Although marine brown macroalgae belong to the class Phaeophyceae, of the kingdom Chromista (Guiry 2012), whereas green and red algae are classified under the kingdom Plantae in the phyla Chlorophyta and Rhodophyta, respectively (Lewis and McCourt 2004; Yoon et al. 2006), the term endophyte is used to describe fungi isolated from plant and non-plant sources, such as brown algae and invertebrates (Kjer et al. 2010).

Critical to the successful isolation of endophytic fungi from any plant, especially marine macroalgae, is the development of an effective surface sterilization method for

each host species (Schulz et al. 2008). Some species of macroalgae can be as little as several cells thick, whereas others can be quite robust in thickness (Koeman and van den Hoek 1981; Markager and Sand-Jensen 1996). As such, caution must be used when surface sterilising marine macroalgae, as over-sterilization can kill fungi living within the plant tissues; however under-sterilization will not eliminate all surface dwelling microorganisms (Hollants et al. 2010; Kjer et al. 2010). Also vital to the study of endophytic fungi are the isolation conditions, such as the nutrients available within the isolation medium and the ambient environmental conditions (e.g. light, temperature, humidity etc.). Alteration of any of these conditions may result in isolating and/or culturing only a small fraction of the total fungal population present within the host and this will not accurately reflect the diversity within the selected macroalgae (Kjer et al. 2010).

A wide range of parasitic and saprobic fungi, known as epiphytes, have been reported from marine macroalgae, but few studies have investigated marine macroalgal endophytes (Bugni and Ireland 2004; Jones et al. 2008). The body of work on these endophytes is dominated by the investigation of macroalgae from Chinese coasts (An et al. 2013; Cui et al. 2010a; Cui et al. 2009; Cui et al. 2010b; Du et al. 2017; Du et al. 2012; Du et al. 2014a; Fang et al. 2016; Gao et al. 2009; Gao et al. 2011a; Gao et al. 2011b; Gao et al. 2011c; Ji et al. 2013; Li et al. 2011; Li et al. 2017; Li et al. 2016a; Li et al. 2014a; Li et al. 2015; Li et al. 2014b; Liang et al. 2016a; Liang et al. 2016b; Liu et al. 2016a; Liu et al. 2012; Liu et al. 2013; Liu et al. 2011; Miao et al. 2012a; Miao et al. 2012b; Miao et al. 2014; Nielsen et al. 2000; Qiao et al. 2010a; Qiao et al. 2010b; Qiao et al. 2011; Sun et al. 2012a; Sun et al. 2013a; Sun et al. 2013b; Tang et al. 2014; Wang 2012; Wang et al.

2006; Wu et al. 2013; Xu et al. 2017; Yang et al. 2006; Zhang et al. 2015a; Zhang et al. 2016b; Zhang et al. 2015b, c; Zhang et al. 2015d; Zhang et al. 2014; Zhang et al. 2007a, 2012; Zhang et al. 2010; Zhang et al. 2007b; Zhang et al. 2007c; Zhang et al. 2009; Zhang et al. 2007d; Zhang et al. 2013), although studies have been performed on macroalgae of the North and Baltic seas (Abdel-Lateff et al. 2003a; Abdel-Lateff et al. 2003b; Elsebai et al. 2015; Elsebai et al. 2009, 2010; Elsebai et al. 2013; Elsebai et al. 2011a; Elsebai et al. 2011b; Elsebai et al. 2012; Fries 1988; Fries 1979; Fries and Thorentolling 1978; Klemke et al. 2004; Krohn et al. 2005; Osterhage et al. 2000; Pontius et al. 2008d; Schulz et al. 2008; Seibert et al. 2006; Zuccaro et al. 2008; Zuccaro et al. 2003), the Mediterranean Sea (Abdel-Lateff et al. 2002; Kralj et al. 2006; Osterhage et al. 2002a; Pontius et al. 2008a; Pontius et al. 2008b) and the Indian coasts (Kaushik et al. 2014; Suryanarayanan et al. 2010; Teuscher et al. 2006; Thirunavukkarasu et al. 2015; Venkatachalam et al. 2015). Studies on endophytes from marine macroalgae have also originated from the Red sea (Gamal-Eldeen et al. 2009; Hawas and Al-Farawati 2017; Hawas et al. 2016; Hawas et al. 2012; Hawas et al. 2013) and various locations in the Pacific and Southern oceans (Ariffin et al. 2011; de Silva et al. 2009; Greve et al. 2008a; Greve et al. 2008b; Haroon et al. 2013; Harvey and Goff 2010; Hsiao et al. 2017; Lee et al. 2016; Liao et al. 2015; Sultan et al. 2014; Tarman et al. 2012). Garbary et al. (Deckert and Garbary 2005a; Deckert and Garbary 2005b; Garbary et al. 2006; Garbary et al. 1991; Garbary et al. 2005a; Garbary and Gautam 1989; Garbary et al. 2005b; Garbary and London 1995; Garbary and Macdonald 1995; Lining and Garbary 1992; Toxopeus et al. 2011; Xu et al. 2008), as well as Flewelling et al. (2013a; 2013b), Abdel-Lateff (2008), Dai et al. (2010), Osterhage et al. (2002b), Erbert et al. (2012), Pavao et al. (2016), Pontius et al. (2008c), Kohlmeyer et

al. (1972), Jesus et al. (2017), de Vita-Marques (2008), de Felicio (2015) and Stanley (1992) represent the published reports of endophytic fungi from marine macroalgae of the Atlantic Ocean. The overall body of research covering marine-derived endophytes from algae is therefore relatively small, and research from all regions of the world is required to increase our understanding of these fungi and the natural products they produce (Jones et al. 2008).

Marine-derived endophytes remain relatively unexplored for their natural products when compared to those of terrestrial plants (Jones 2011a, b; Jones et al. 2008; Raghukumar 2008), though it is believed that these endophytic fungi may have the potential to produce different compounds from those of terrestrial sources (Kobayashi and Ishibashi 1993; Raghukumar 2008). Though few studies have been performed, macroalgal-derived endophytes have been shown to be an excellent source of bioactive natural products (Ebel 2010; Flewelling et al. 2015; Rateb and Ebel 2011; Schulz et al. 2002; Suryanarayanan et al. 2010), with the majority of the published bioactivities being primarily antimicrobial and anticancer. As marine-derived endophytes remain relatively unexplored, the potential for identifying new species of fungi from the marine environment that produce previously unknown natural products is significant (Jones et al. 2008; Raghukumar 2008), and it is not until new sources of endophytic fungi are explored, such as the numerous species of algae covering shorelines worldwide, that the true extent of the biological and chemical diversity of these fungi can be realized.

2.1.2 Rationale for investigating marine macroalgae from the Bay of Fundy, New Brunswick, Canada for endophytic fungi

Although marine macroalgae are emerging as excellent sources of endophytic fungi (Ariffin et al. 2011; Flewelling et al. 2013a; Flewelling et al. 2015; Flewelling et al. 2013b; Sarasan et al. 2017; Suryanarayanan 2012; Suryanarayanan and Johnson 2014; Suryanarayanan et al. 2010; Zhang et al. 2016c), less than 1% of the known macroalgal species have been investigated for the endophytes they host (Flewelling et al. 2015). Few survey-based studies attempting to maximize the number of host macroalgae collected and diversity of endophytes isolated have been performed (Ariffin et al. 2011; Flewelling et al. 2013a; Flewelling et al. 2013b; Suryanarayanan et al. 2010; Zhang et al. 2009), with the majority of studies only investigating a single endophyte from a collection for its bioactive natural products. As most of the studies into endophytes from marine macroalgae have been performed from just a handful of locations, there is a dearth of information on the global abundance, distribution and species richness of endophytes from this source (Flewelling et al. 2015). A preliminary investigation into the endophytes of marine macroalgae from the Bay of Fundy has been performed (Flewelling et al. 2013a), where 79 distinct endophytes were isolated from 14 macroalgae hosts, establishing it as an promising location for future studies. The Bay of Fundy supports a diverse number of marine macroalgal species (Bates et al. 2009; South 1984), many of which have not been investigated for their endophytic fungi (Deckert and Garbary 2005a; Deckert and Garbary 2005b; Flewelling et al. 2013a; Flewelling et al. 2015; Garbary et al. 2005a; Garbary and Gautam 1989; Garbary and London 1995; Garbary and Macdonald 1995; Xu et al. 2008). The objective of the research presented in this chapter was to isolate

and identify endophytic fungi from 20 different host species consisting of red, green, and brown algae collected from the Bay of Fundy, Canada.

2.2 Experimental

2.2.1 Media composition

Malt extract agar (MEA) was prepared using Bacto™ malt extract [20 g/L in distilled water (Becton Dickinson, Mississauga, Ontario)] and dehydrated agarose [14 g/L (Fisher Scientific Ltd., Ottawa, Ontario)]. Malt extract agar prepared with artificial seawater (MEA-SW) was made using Bacto™ malt extract (20 g/L), dehydrated agarose (14 g/L) and Instant Ocean® sea salt (24.4 g/L) (Instant Ocean, Cincinnati, OH) in distilled water. Czapek-Dox (49 g/L), potato dextrose (39 g/L), and cornmeal (17 g/L) (Becton Dickinson, Mississauga, Ontario) agars were prepared with distilled water. All culture media was autoclaved (121.1 °C, 15 mins), then transferred to sterile Petri plates (100 × 15 mm, Fisher Scientific Ltd., Ottawa, Ontario), and left to solidify. Media was stored at 4 °C until needed.

2.2.2 Algal collection

Twenty species of marine macroalgae were collected from the shores of Green's Point, L'Etete, New Brunswick, Canada (45°02.363'N, 066°53.483'W), in June and July 2013. Ten species of brown algae were collected: *Alaria esculenta*, *Ascophyllum nodosum*, *Desmarestia viridis*, *Fucus distichus* subsp. *edentatus*, *Fucus distichus* subsp. *evanescens*, *Fucus spiralis*, *Fucus vesiculosus*, *Petalonia fascia*, *Saccharina latissima*,

and *Scytosiphon lomentaria*. Seven species of red algae were collected: *Chondrus crispus*, *Devaleraea ramentacea, Dumontia contorta, Mastocarpus stellatus, Palmaria palmata, Polysiphonia lanosa* and *Porphyra* sp., while three species of green algae were collected: *Spongomorpha arcta*, *Ulva intestinalis* and *Ulva lactuca*. Identification of macroalgae was performed by Thierry Chopin (Phycologist, UNB-SJ Biological Sciences) by a visual examination of the macroalgae. Only algae that were attached to the rocky substrate and showing no signs or symptoms of disease (*i.e.* free of discolouration or signs of herbivory) were used. Geographical coordinates were recorded for each of the individual collection sites.

2.2.3 Surface sterilization of algae

For surface sterilization, a segment of an alga (approximately 5.0 cm in length) was placed in a beaker containing a sterilizing reagent for a specified length of time, followed by immersion in sterile distilled water (Figure 2.1). Sodium hypochlorite (6.0%; Walmart, Mississauga, Ontario) and ethanol (70%; ACS, Fisher Scientific Ltd., Ottawa, Ontario) were used as sterilizing reagents. The location of the segment selected from the thallus of each host species for endophyte isolation could not be standardized as the morphology of each alga tested varied both within and between genera and class.

A surface sterilization method was developed for each algal species prior to the isolation of endophytes to ensure fungi isolated were from the interior of the algae and not from the surface. Seven surface sterilization techniques (Table 2.1) were tested to determine the most appropriate method for each alga and were developed based upon the work of Flewelling et al. (2013a; 2013b). For each technique, algal segments were

immersed in the sterilants for specific lengths of time (Table 2.1, stepwise from left to right as depicted in Figure 2.1) and subsequently blotted on a piece of autoclaved paper towel. Surface sterilized algae were then rubbed across the surface of the media of four Petri plates [two plates of both 2.0% MEA and 2.0% MEA-SW] for use as verification plates to ensure the surface sterilization had been successful (*i.e.* no growth observed on the verification media). Once prepared, verification plates were left to incubate under ambient light and temperature conditions. For each technique tested, the sterilized algae were then cut into either disks (*A. esculenta*, *F. distichus* subsp. *edentatus*, *F. distichus* subsp. *evanescens*, *F. spiralis*, *F. vesiculosus*, *P. fascia*, *P. palmata*, *Porphyra* sp., *S. latissima*, and *U. lactuca*) or segments (*A. nodosum*, *C. crispus*, *D. contorta*, *D. ramentacea*, *D. viridis*, *M. stellatus*, *P. lanosa*, *S. arcta*, *S. lomentaria*, *U. intestinalis*) using either a sterile modified cork borer (0.6 cm in diameter) or a sterile scalpel, with five pieces of algae transferred to one plate each of 2.0% MEA and 2.0% MEA-SW, and were incubated at ambient light and temperature conditions. For each alga, the mildest sterilization technique that resulted in no observed growth of fungi on verification media was used for the isolation of endophytes.

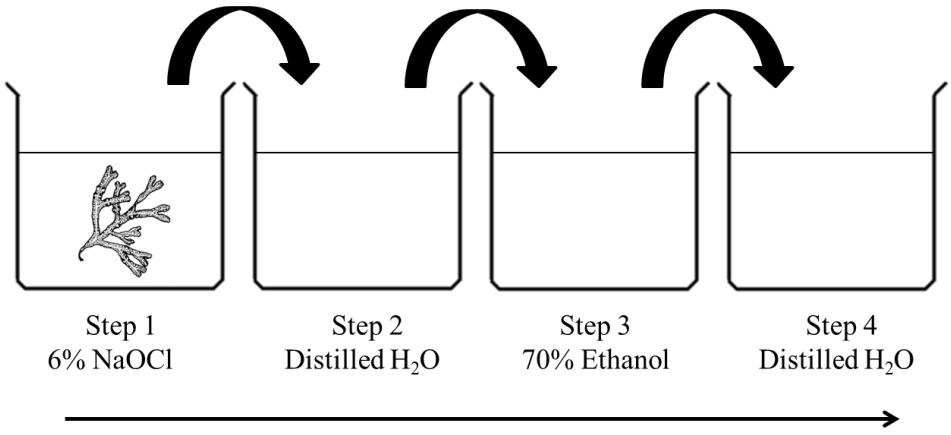


Figure 2.1. General procedure for the surface sterilization of marine macroalgae collected from the Bay of Fundy, New Brunswick, Canada.

Table 2.1. Surface sterilization methods used to determine the appropriate technique for marine algae collected from the Bay of Fundy, New Brunswick.[a]

Technique[b]	Sterilants for surface sterilization[c]			
	Step 1	Step 2	Step 3	Step 4
	NaOCl (6.0%)	Sterile H_2O	Ethanol (70%)	Sterile H_2O
1	5	10	10	10
2	5	10	15	10
3	0	0	15	10
4	0	0	20	10
5	10	10	10	10
6	15	10	25	10
7	10	10	15	10

[a] All sterilizations consisted of four verification plates; (two for each 2.0% MEA and 2.0% MEA-SW)
[b] Surface sterilization proceeded stepwise from left to right, starting with step 1
[c]All sterilization times are measured in seconds

2.2.4 Culture techniques

After establishing the surface sterilization techniques for each macroalgal species, a portion (5 cm in length) of each algal sample was individually surface sterilized using the appropriate conditions (Table 2.2), blotted dry on autoclaved paper towel and rubbed across the surface of the media of six verification plates as described above. Three plates of 2.0% MEA and three plates of 2.0% MEA-SW were used as verification plates for each species of algae. The sterilized algae were then cut into pieces as described above using either a sterile modified cork borer (0.60 cm in diameter) or a sterile scalpel, with five pieces from each sample of sterilized algae placed onto ten plates of 2.0% MEA and ten plates of 2.0% MEA-SW for a total of 50 pieces for each algal species on each of the two media.

Table 2.2. Surface sterilization techniques used on marine macroalgae collected from the Bay of Fundy, New Brunswick.[a]

Algal Species	NaOCl (6.0%)[b]	Ethanol (70%)[b]
Alaria esculenta	10	10
Ascophyllum nodosum	5	15
Chondrus crispus	5	10
Desmarestia viridis[c]	0	0
Devaleraea ramentacea	5	10
Dumontia contorta	5	10
Fucus distichus subsp. *edentatus*	5	10
Fucus distichus subsp. *evanescens*	5	10
Fucus spiralis	15	25
Fucus vesiculosus	0	15
Mastocarpus stellatus	5	10
Palmaria palmata	0	20
Petalonia fascia	10	15
Polysiphonia lanosa	0	15
Porphyra sp.	5	15
Saccharina latissima	10	10
Scytosiphon lomentaria	10	15
Spongomorpha arcta	5	10
Ulva intestinalis	5	10
Ulva lactuca	0	15

[a] All sterilizations consisted of 6 surface verification plates (3 of each 2.0% MEA and 2.0% MEA-SW)
[b] All sterilization times are measured in seconds
[c] Due to the natural acidity of *D. viridis*, no additional surface sterilization was required

2.2.5 Isolation of endophytic fungi

Petri plates containing five algal pieces were monitored for a period of 14 days in ambient lighting and temperature conditions for the presence of endophytic growth from the cut edges of the algal segments. The isolation frequency (IF) of emerging fungal hyphae was determined for each species of algae collected, and is defined as the number of algal pieces from which endophytes were cultured as a percentage of the total number of algal pieces prepared (Petrini et al. 1992):

$$\text{Isolation Frequency (\%)} = \frac{\text{Number of algal pieces showing fungal growth}}{\text{Total number of algal pieces}} \times 100$$

Endophytes were subcultured onto fresh media of either 2.0% MEA or 2.0% MEA-SW, depending on the original isolation medium, whereby sections were cut from the growing edge of the fungal culture and placed at the centre of the fresh medium. This was repeated until a pure fungal isolate was obtained. To aid in morphological identification, fungal isolates were also subcultured onto Czapek-Dox, potato dextrose, and cornmeal agars. Individual isolates within each algal species were then sorted into groups of homogeneous morphotypes, and a representative colony of each distinct isolate was used for the fungal identification, fermentation, and extraction. Distinct fungal isolates were identified through dereplication of fungal isolates showing identical colony morphology (colour, form, margin and elevation) across all four growth media. Specific records detailing the total number of each distinct isolate observed was recorded prior to the dereplication of the fungal isolates.

2.2.6 Cryopreservation of endophytic fungi

Fungal isolates were preserved cryogenically according to the procedure described by Kjer et al. (Kjer et al. 2010). Fungi were grown on slants made of MexA agar [malt extract (20 g), yeast extract (0.10 g), glycerol (50 mL), and dehydrated agar (13 g) in distilled water (1 L)]. Fungal isolates were incubated (25 °C) for one to two weeks until a pure isolate covering the surface of the agar slant was obtained, and then frozen in a stepwise procedure in which isolates were stored at 4 °C (2 h), frozen at -20 °C (2 h) and lastly in permanent storage (-80 °C) in the UNB NPRG fungal repository.

2.2.7 Identification of endophytic fungi

Fungal isolates were identified taxonomically through an examination of the fungal colony and spore morphology. Taxonomic classifications were then confirmed by comparing the internal transcribed spacer region (ITS) DNA regions (White et al. 1990) with the corresponding sequences that are available in the GenBank database (NCBI, US National Library of Medicine, Bethesda, MD, USA).

The genomic DNA of all fungal isolates was extracted using a Qiagen DNeasy® plant mini kit and a Thermo Scientific Pierce Yeast DNA Extraction Reagent Kit according to the manufacturer's guidelines. PCR amplification of the extracted fungal DNA was performed using a Hotstar taq plus master mix (Qiagen, Toronto, Ontario) and ITS 1 and ITS 4 universal fungal primers (Invitrogen, Burlington, Ontario). The reaction mixture for each sample was made as follows: Hotstar taq plus master mix (31.5 µL), coral load concentrate (7.5 µL), ITS 1 and ITS 4 primers (4.5 µL each), distilled water (16.5 µL), and fungal DNA template (3 µL). The mixture was added individually to PCR strip tubes and amplified in a thermal cycler (MyCycler; Bio Rad, Mississauga, Ontario). The protocol for amplification was as follows: one activation step (95 °C, 5 min), followed by 35 cycles of the three step process of denaturation of DNA (95 °C, one min), annealing of primers (56 °C, 30 s), and the extension of new DNA strands (72 °C, one min), and lastly one 10 min extension of DNA strands (72 °C). Amplified DNA was stored at -20 °C until required for gel electrophoresis. Each PCR product was verified through gel electrophoresis for the presence of a DNA band corresponding to the ITS region (approximately 550 base pairs). Electrophoresis (25 min; 120 V) was performed by adding the PCR product (15 µL) and DNA ladder (5 µL; 100 bp ladder, 100-2000 bp) (Invitrogen,

Burlington, Ontario) to an agarose gel [(1% agarose in 1x Tris Borate EDTA (TBE) buffer, 5 µL SYBR®Safe (Invitrogen, Burlington, Ontario)]. Detection of DNA bands was performed through the use of a gel imaging system (Gel Doc™, Bio Rad; Mississauga, Ontario).

Verified PCR products were submitted for sequencing (Genome-Québec; Montreal, Québec, Canada) along with copies of the ITS 1 and ITS 4 primers. An online BLAST search of the GenBank database of the sequenced fungal DNA was performed to identify the fungal isolate. The sequences obtained were manually examined for ambiguity between the isolate in plate culture and the taxa derived from the BLAST search and were submitted to the GenBank database. In cases where electrophoresis indicated that the DNA extraction/PCR procedure had been unsuccessful, the procedure was repeated on a fresh sample of the fungal isolate.

Isolates identified taxonomically to species level had ≥ 99% sequence similarities to entries for conspecifics in GenBank, and isolates identified to the genus level had ≥ 96% sequence similarities with congeneric species. Isolates that could not be unequivocally identified to genus or species level based on morphological observations were only classified to the corresponding genus even when sequence similarities of ≥ 98% were obtained with sequence data for congeneric species in GenBank. If DNA from the ITS region was not isolated after two extraction/amplification attempts for each of the two extraction kits, the corresponding isolate was identified on morphological observations alone (Dobranic et al. 1995; Suryanarayanan et al. 1998; Suryanarayanan et al. 2010).

2.2.8 Statistical analyses

Prior to any dereplication of fungal isolates (see section 2.2.5), the total number of times that each fungal isolate was obtained was recorded to allow rarefaction curves to be plotted using Biodiversity Pro (McAleece et al. 1997). In this case, rarefaction curves estimating the number of species or distinct isolates (S_n) expected in a sample of *n* individuals (fungi from a class of algae) selected at random from a collection containing *N* individuals (the total number of fungi isolated from a host alga) with N_i individuals (the number of times each fungal species or distinct isolate was obtained) of the *i*th species of fungus and were calculated using the following formula (Hurlbert 1971):

$$E\ (S_n) = \sum_{i}^{S} \left[1 - \frac{\left(\frac{N - Ni}{n}\right)}{\left(\frac{N}{n}\right)} \right]$$

2.4 Results and discussion

Two hundred and seventy-four pieces of algae from the 20 host species had endophyte growth, resulting in an overall isolation frequency of 12% (Table 2.3). The isolation frequencies of endophytic fungi from the marine macroalgae varied by host, with the highest isolation frequencies obtained from the two brown algae *Petalonia fascia* and *Scytosiphon lomentaria*, at rates of 49% and 39% respectively (Table 2.3). The lowest isolation frequencies were observed from the brown alga *Saccharina latissima* and the red alga *Chondrus crispus*, each at rates of 1% (Table 2.3). No endophytes were observed growing from the cut edges of the algae segments of *Alaria esculenta*, a brown alga, and of *Palmaria palmata*, a red alga.

Table 2.3. Isolation frequencies and distinct number of isolates of endophytic fungi from marine macroalgae collected from the Bay of Fundy, New Brunswick, Canada.

Macroalgal species	Total algae segments	Segments with endophytic growth	Isolation frequency (%)	Distinct isolates
Alaria esculenta	110	0	0	0
Ascophyllum nodosum	110	14	13	8
Desmarestia viridis	110	13	12	9
Fucus distichus subsp. *edentatus*	110	22	20	6
Fucus distichus subsp. *evanescens*	110	6	5	6
Fucus spiralis	110	12	11	6
Fucus vesiculosus	110	5	5	2
Petalonia fascia	110	54	49	10
Scytosiphon lomentaria	110	43	39	33
Saccharina latissima	110	1	1	1
Spongomorpha arcta	110	21	19	14
Ulva intestinalis	110	17	15	7
Ulva lactuca	110	3	3	3
Chondrus crispus	110	1	1	1
Devaleraea ramentacea	110	10	9	6
Dumontia contorta	110	3	3	3
Mastocarpus stellatus	110	31	28	13
Palmaria palmata	110	0	0	0
Polysiphonia lanosa	110	9	8	7
Porphyra sp.	110	9	8	5
Total	2200	274	12	140

One hundred and forty distinct endophytes were isolated from 20 marine macroalgae collected from the Bay of Fundy, New Brunswick, Canada (Table 2.4). Endophytic fungi have been previously isolated from *Ascophyllum nodosum* (Deckert and Garbary 2005a; Deckert and Garbary 2005b; Flewelling et al. 2013a; Flewelling et al. 2013b; Garbary et al. 1991; Garbary et al. 2005a; Garbary and Gautam 1989; Garbary and London 1995; Garbary and Macdonald 1995), *C. crispus* (Flewelling et al. 2013a), *Devaleraea ramentacea* (Flewelling et al. 2013a), *Fucus spiralis* (Flewelling et al. 2013a; Flewelling et al. 2013b), *Fucus vesiculosus* (Flewelling et al. 2013a; Flewelling et al. 2013b), *Mastocarpus stellatus* (Flewelling et al. 2013a), *P. palmata* (Flewelling et al.

2013a), *Polysiphonia lanosa* (Flewelling et al. 2013a; Flewelling et al. 2013b), *Porphyra* spp. (Flewelling et al. 2013a; Flewelling et al. 2013b), *S. latissima* (Flewelling et al. 2013a), *Spongomorpha arcta* (Flewelling et al. 2013a), *Ulva lactuca* (Flewelling et al. 2013a; Flewelling et al. 2013b) and *Ulva intestinalis* (Flewelling et al. 2013a; Flewelling et al. 2013b). Seven of the host algae, namely, *A. esculenta*, *Desmarestia viridis*, *Dumontia contorta*, *Fucus distichus* subsp. *edentatus*, *Fucus distichus* subsp. *evanescens*, *P. fascia*, and *S. lomentaria*, have not been previously investigated for their endophytic fungi.

Table 2.4. Identification of endophytic fungi isolated from marine algae collected from the Bay of Fundy, Canada.

Host species	Isolate	Species	Accession Number
Ascophyllum nodosum	KP1-045A	*Penicillium* sp. IV	KY054732
	KP1-045B	Orange yeast[a]	
	KP1-045C	*Penicillium* sp. V	KY054733
	KP1-045D	Sterile beige filamentous[a]	
	KP1-045G	Sterile beige V[a]	
	KP1-045I	Sterile white I[a]	
	KP1-045J	Sterile white II[a]	
	KP1-045K	*Dendryphiella* sp. I	KY054734
Desmarestia viridis	KP1-089A	Sterile pigmented ascomycete I[a]	KY054738
	KP2-033A	*Penicillium* sp. XX	
	KP2-033B	*Penicillium* sp. XXI	KY054783
	KP2-033C	Sterile white XXVI[a]	
	KP2-033D	Sterile hyaline ascomycete V[a]	KY054784
	KP2-033E	Sterile pigmented ascomycete III[a]	KY054785
	KP2-033F	Sterile white XXVII[a]	
	KP2-033G	Sterile black filamentous[a]	
	KP2-033H	*Penicillium* sp. XXII	KY054786
Fucus distichus subsp. *edentatus*	KP1-099A	Sterile white VIII[a]	
	KP1-143A	*Metschnikowia* sp.	KY054771
	KP1-143B	Sterile white XII[a]	
	KP1-143C	Sterile white XIII[a]	
	KP1-143D	*Trametes versicolor* I	KY054772
	KP2-017A	Sterile white XXII[a]	
Fucus distichus subsp. *evanescens*	KP1-021A	Sterile beige IV[a]	
	KP1-069A	Sterile beige X[a]	
	KP1-069B	Sterile beige XI[a]	
	KP1-069C	Sterile beige XII[a]	
	KP1-069D	Sterile beige XIII[a]	
	KP1-069E	Sterile white VII[a]	

[a] When initially dereplicated, isolates showed distinctly characteristic morphology, however could not be identified to the genus or species level.

[b] When initially dereplicated, isolates showed distinctly characteristic morphology, however, were identified as the same species through molecular techniques.

Table 2.4. Identification of endophytic fungi isolated from marine algae collected from the Bay of Fundy, Canada, continued.

Host species	Isolate	Species	Accession Number
Fucus spiralis	KP1-013B	*Penicillium* sp. I	KY054728
	KP2-029B	Sterile white XXIV[a]	
	KP2-029C	Sterile beige XXX[a]	
	KP2-029D	Sterile beige XXXI[a]	
	KP2-029E	Sterile white XXV[a]	
	KP2-029F	Sterile beige XXXII[a]	
Fucus vesiculosus	KP1-091A	*Penicillium* sp. VIII	KY054739
	KP2-005A	*Phanerochaete* sp.	KY054778
Petalonia fascia	KP1-135B	*Trichoderma* sp.	KY054767
	KP1-135C	*Penicillium roseopurpureum*	KY054768
	KP1-135D	*Dendryphiella* sp. III	KY054769
	KP1-135E	Sterile white XI[a]	
	KP1-135E2	Sterile beige XVIII[a]	
	KP1-135F	*Penicillium* sp. XIII	KY054770
	KP2-013A	Sterile beige XXVIII[a]	
	KP2-013E	Sterile white XX[a]	
	KP2-013F	Yeast endophyte[a]	KY054781
	KP2-013G	Sterile white XXI[a]	
Scytosiphon lomentaria	KP1-131A	Sterile brown filamentous II[a]	
	KP1-131B	Sterile hyaline ascomycete II[a]	KY054746
	KP1-131C	*Thysanophora* sp. I	KY054748
	KP1-131DA	*Thysanophora* sp. II	KY054749
	KP1-131DB	*Ophiognomonia intermedia*	KY054750
	KP1-131E	Sterile grey filamentous I[a]	
	KP1-131F	*Mollisia* sp. I	KY054751
	KP1-131G	Septate brown filamentous[a]	
	KP1-131H	*Microdochium bolleyi*	KY054752
	KP1-131I	*Dendryphiella* sp. II	KY054753
	KP1-131J	*Myrothecium* sp.	KY054754
	KP1-131K	*Didymella bryoniae*	KY054755
	KP1-131L	*Penicillium* sp. XII	KY054756
	KP1-131M	*Thysanophora penicillioides*	KY054757
	KP1-131N	Sterile pigmented ascomycete II[a]	KY054758

[a] When initially dereplicated, isolates showed distinctly characteristic morphology, however could not be identified to the genus or species level.

[b] When initially dereplicated, isolates showed distinctly characteristic morphology, however, were identified as the same species through molecular techniques.

Table 2.4. Identification of endophytic fungi isolated from marine algae collected from the Bay of Fundy, Canada, continued.

Host species	Isolate	Species	Accession Number
Scytosiphon lomentaria	KP1-131O	Sterile beige XVI[a]	
	KP1-131Q	*Aspergillus fumigatus* III[b]	KY054759
	KP1-131R	*Mollisia* sp. II	KY054760
	KP1-131S	*Mycosphaerella nyssicola*	KY054761
	KP1-131T	*Aspergillus fumigatus* IV[b]	KY054762
	KP1-131U	Sterile green filamentous[a]	
	KP1-131V	Sterile hyaline ascomycete III[a]	KY054763
	KP1-131W	*Diaporthe* sp.	KY054764
	KP1-131Y	*Aspergillus fumigatus* V[b]	KY054765
	KP1-131Z	Sterile hyaline ascomycete IV[a]	KY054766
	KP1-131AA	*Aspergillus fumigatus* II[b]	KY054745
	KP1-131BB	Septate ascomycete[a]	KY054747
	KP1-131CC	*Penicillium* sp. XI	
	KP1-131DD	Sterile grey filamentous II[a]	
	KP1-017A	Sterile hyaline ascomycete I[a]	KY054729
	KP1-017C	*Coprinellus micaceus*	KY054730
	KP1-017D	Sterile beige III[a]	
	KP1-017E	*Penicillium* sp. II	KY054731
Saccharina latissima	KP1-171A	Sterile white XIV[a]	
Spongomorpha arcta	KP1-123A	*Penicillium* sp. IX	KY054742
	KP1-123B	*Penicillium* sp. X	KY054743
	KP1-123C	*Chrysosporium* sp.	KY054744
	KP1-175A	Sterile white XV[a]	
	KP1-175C	Sterile beige XX[a]	
	KP1-175D	Sterile white XVI[a]	
	KP1-175E	*Tolypocladium* sp.	
	KP1-175F	Sterile beige XXI[a]	
	KP1-175G	*Penicillium* sp. XIV	KY054773
	KP1-175H	Sterile white XVII[a]	
	KP1-175J	Septate white filamentous[a]	
	KP1-175K	Septate beige filamentous II[a]	
	KP1-175L	*Penicillium* sp. XV	KY054774
	KP1-175M	*Penicillium* sp. XVI	KY054775

[a] When initially dereplicated, isolates showed distinctly characteristic morphology, however could not be identified to the genus or species level.

[b] When initially dereplicated, isolates showed distinctly characteristic morphology, however, were identified as the same species through molecular techniques.

Table 2.4. Identification of endophytic fungi isolated from marine algae collected from the Bay of Fundy, Canada, continued.

Host species	Isolate	Species	Accession Number
Ulva intestinalis	KP2-009A	*Trametes versicolor* II	KY054779
	KP2-009B	Septate hyaline ascomycete[a]	KY054780
	KP2-025A	Sterile white XXIII[a]	
	KP2-025B	*Penicillium* sp. XVIII	
	KP2-025C	*Penicillium* sp. XIX	
	KP2-025D	*Aspergillus fumigatus* VII[b]	KY054782
	KP2-025E	Sterile beige XXIX[a]	
Ulva lactuca	KP1-095A	Sterile beige XVI[a]	
	KP1-139A	Yellow yeast III[a]	
	KP1-139B	Sterile beige XIX[a]	
Chondrus crispus	KP1-115A	Sterile white IX[a]	
Devaleraea ramentacea	KP1-081A	Sterile beige XV[a]	
	KP1-119A	Yellow yeast II[a]	
	KP1-119B	Sterile white X[a]	
	KP1-119C	*Paraconiothyrium* sp.	KY054740
	KP1-119D	Sterile orange filamentous[a]	
	KP1-119E	*Aureobasidium* sp.	KY054741
Dumontia contorta	KP1-179A	Sterile beige XXII[a]	
	KP1-179B	Sterile beige XXIII[a]	
	KP1-179C	Sterile white XVIII[a]	
Mastocarpus stellatus	KP1-025B	*Penicillium* sp. III	
	KP1-025C	Yellow yeast I[a]	
	KP1-063A	Sterile white III[a]	
	KP1-063B	Septate beige filamentous I[a]	
	KP1-063C	Sterile beige VI[a]	
	KP1-063E	Sterile white IV[a]	
	KP1-063F	Sterile beige VII[a]	
	KP1-063J	*Penicillium* sp. VI	KY054735
	KP1-063L	Sterile beige VIII[a]	
	KP1-063M	Sterile beige IX[a]	

[a] When initially dereplicated, isolates showed distinctly characteristic morphology, however could not be identified to the genus or species level.

[b] When initially dereplicated, isolates showed distinctly characteristic morphology, however, were identified as the same species through molecular techniques.

Table 2.4. Identification of endophytic fungi isolated from marine algae collected from the Bay of Fundy, Canada, continued.

Host species	Isolate	Species	Accession Number
Mastocarpus stellatus	KP1-063N	*Aspergillus fumigatus* I[b]	KY054736
	KP1-063O	Sterile white V[a]	
	KP1-063P	Sterile white VI[a]	
Polysiphonia lanosa	KP2-001A	Sterile beige XXIV[a]	
	KP2-001B	Sterile beige XXV[a]	
	KP2-001C	*Aspergillus fumigatus* VI[b]	KY054776
	KP2-001D	Sterile beige XXVI[a]	
	KP2-001E	Sterile beige XXVII[a]	
	KP2-001F	*Penicillium* sp. XVII	KY054777
	KP2-001G	Sterile white XIX[a]	
Porphyra sp.	KP1-009A	Sterile beige I[a]	
	KP1-009B	Sterile brown filamentous I[a]	
	KP1-009C	Sterile beige II[a]	
	KP1-075A	Sterile beige XIV[a]	
	KP1-075B	*Penicillium* sp. VII	KY054737

[a] When initially dereplicated, isolates showed distinctly characteristic morphology, however could not be identified to the genus or species level.
[b] When initially dereplicated, isolates showed distinctly characteristic morphology, however, were identified as the same species through molecular techniques.

The number of distinct isolates varied by host from as high as 33 endophytic fungal species in *S. lomentaria* to as low as one in both *S. latissima* and *C. crispus* (Table 2.3). Sterile isolates, namely those designated as sterile beige (23%) and sterile white (19%), represented the largest proportion of all distinct fungi isolated (Table 2.4). These sterile isolates were obtained from 17 of the host algae from which endophytes were obtained, with the exception being that of *F. vesiculosus* (Table 2.4). *Penicillium* spp. represented 16% of the distinct isolates obtained, having been obtained from *A. nodosum*, *F. spiralis*, *F. vesiculosus*, *S. lomentaria*, *D. viridis*, *P. fascia*, *U. intestinalis*, *S. arcta*, *P. lanosa*, *Porphyra* sp., and *M. stellatus* (Table 2.4). *Aspergillus fumigatus* was isolated

from *S. lomentaria*, *U. intestinalis*, and *P. lanosa* (Table 2.4). *Dendryphiella* spp. were isolated from three host algae, *A. nodosum*, *S. lomentaria* and *P. fascia* (Table 2.4). *Trametes versicolor* was isolated from both *F. distichus* subsp. *edentatus* and *U. intestinalis* (Table 2.4). *Coprinellus micaceus*, *Paraconiothyrium* sp., *Aureobasidium* sp. *Chrysosporium* sp., *Tolypocladium* sp., *Thysanophora* spp., *Ophiognomonia intermedia*, *Mollisia* spp., *Microdochium bolleyi*, *Myrothecium* sp., *Didymella bryoniae*, *Mycosphaerella nyssicola*, *Diaporthe* sp., *Trichoderma* sp., *Metschnikowia* sp., *Phanerochaete* sp. were isolated from only one host (Table 2.4).

To compare the species diversity between the red, green and brown macroalgae, rarefaction curves (Figure 2.2) were used to estimate the total number of distinct fungal species that would be obtained per class of macroalgae. As few species of macroalgae have been investigated for their fungal endophytes, rarefaction curves of the algal classes (red, green, brown) were plotted in order to compare the fungal diversity of these hosts amongst each other. The rarefaction curves generated (Figure 2.2) estimates the endophyte diversity (*i.e.* species richness) within the host algae when sample size differs, where a plateau on the curve indicates the estimated total species diversity within a given sample (Gotelli and Colwell 2001). These curves suggest that the brown macroalgae possess the highest diversity of endophytic fungi, with the green macroalgae showing the lowest fungal diversity. Trend lines, estimated by using logarithmic functions, have been extrapolated to determine at what point a plateau in the isolation can be achieved. The trend lines for the rarefaction curves of the three classes of algae indicate that continued sampling from the hosts in each class would yield more distinct fungal species and that future work should focus on increasing sampling intensity from these red, green and

brown macroalgae to develop a better understanding of the endophyte diversity within these hosts.

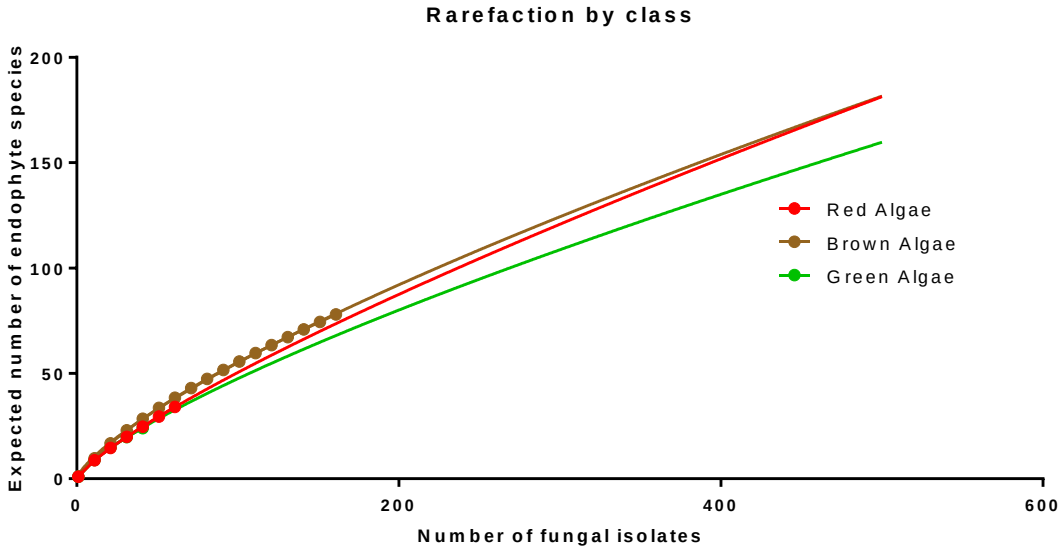


Figure 2.2. Rarefaction curves showing the estimated species richness within the samples of isolates obtained from brown, green and red macroalgae. Curves are based upon the number of distinct isolates obtained from all endophytic fungi isolated from each algal class (brown macroalgae, n = 170, red macroalgae, n = 63, green macroalgae, n = 41). Trend lines calculated by logarithmic regression show the predicted maximum number of endophyte species that could be obtained for each algal class and the total number of fungal isolates that would need to be isolated to achieve this.

Two previous studies have investigated the species richness of endophytes from marine macroalgae by using rarefaction curves. Suryanarayanan and colleagues (2010) screened marine macroalgae from the southern Indian coast for their endophytic fungi and determined that, from a total sample size of 281 fungal isolates, brown macroalgae yielded the highest diversity of endophytic fungal species while the green algae yielded the lowest diversity. These results support the findings of this study, whereby, continued sampling from each of the classes of macroalgae is expected to lead to the isolation of further

endophyte species. However, in contrast to data reported here, Flewelling et al. (2013b) found that the green algae yielded the greatest number of distinct endophytic fungal species while brown algae showed the lowest species diversity of endophytic fungi from macroalgae from the Shetland Islands, UK. This could perhaps be due to differences in the macroalgae investigated, the isolation techniques used, or the seasonal and geographical differences of algae between the two studies.

Several of the host species from the Bay of Fundy investigated in this work have previously been examined for their endophytic fungi (Flewelling et al. 2013a). Similarities exist between the isolation frequencies and number of distinct fungal isolates for *C. crispus*, *M. stellatus*, *F. spiralis*, and *F. vesiculosus* from the two Bay of Fundy studies (Table 2.5); however, much larger differences were seen in the isolation frequencies and number of distinct isolates for *D. ramentacea*, *P. lanosa*, *Porphyra* sp., *A. nodosum*, *S. latissima*, *S. arcta*, *U. intestinalis*, and *U. lactuca* (Table 2.5). Flewelling et al. (2013a) previously found that *P. palmata* (87% IF, 7 distinct isolates) is an excellent source of endophytic fungi; however, no endophytes were isolated here. The differences observed in the isolation frequencies and distinct number of isolates between the two Bay of Fundy studies (Table 2.5) may be attributed to the differences in sample sizes for each host species studied between 2013 and 2016. It may also be due to the difference in collection times for the host macroalgae, as the 2013 study investigated macroalgae from fall 2010 and this work collected the macroalgae in spring and summer 2013. The trend for a greater isolation of endophytic fungi from older plant tissue has been observed from the brown algae *F. vesiculosus* and *F. spiralis* (Flewelling 2012), and has been documented in terrestrial plants, including *Pinus tabulaeformis* and *Picea mariana*

needles (Guo et al. 2008; Johnson and Whitney 1992), and *Bauhinia brevipes* and *Plumeria rubra* leaves (Hilarino et al. 2011; Suryanarayanan and Thennarasan 2004).

Table 2.5. A comparison of the 2013 and 2016 isolation frequencies and distinct number of isolates of endophytic fungi from marine algae collected from the Bay of Fundy, New Brunswick, Canada.

Seaweed species	2013 Isolation frequency (%)	2016 Isolation frequency (%)	2013 Distinct isolates	2016 Distinct isolates
Ascophyllum nodosum	11	13	1	8
Chondrus crispus	0.08	1	2	1
Devaleraea ramentacea	43	9	13	6
Fucus spiralis	8	11	4	6
Fucus vesiculosus	6	5	1	2
Mastocarpus stellatus	33	28	9	13
Palmaria palmata	87	0	7	0
Polysiphonia lanosa	18	8	5	7
Porphyra spp.	57	8	5	5
Saccharina latissima	8	1	13	1
Spongomorpha arcta	27	19	11	14
Ulva intestinalis	21	15	5	7
Ulva lactuca	8	3	3	3

Sixteen endophytes were identified to the species level and 32 were identified to the genus level using a combination of traditional taxonomy and a search of the sequenced fungal DNA in GenBank using the Basic Local Alignment Search Tool (BLAST) (Table 2.4). General criteria exist for sequence based identification of fungal isolates, where a 97% or greater similarity between known and unknown DNA sequences can identify a fungus to the species level and a 93% or greater similarity can lead to identification at only the genus level (Pounder et al. 2007; Raja et al. 2017; Romanelli et al. 2010); although, no universal guidelines currently exist across the literature (Raja et al. 2017). The morphology of the fungal isolates could not be used to further identify the fungi that were identified to the genus level by BLAST, as no defining morphological characteristics could be observed to identify a specific species within the genus. Following a BLAST

search, the DNA sequences of three endophytes (KP1-131N, KP2-013F and KP2-009B), returned a diverse range of possible genera and species, none of which agreed with the morphology of the isolate and were therefore named according to their morphology. Additionally, eight endophytes (KP1-017A, KP1-089A, KP1-131B, KP1-131BB, KP1-131V, KP1-131Z, KP2-033D and KP2-033E), were designated codes according to their morphology, as a BLAST search could not identify any DNA sequences with homology to the DNA sequences of those endophytes. This suggests the potential isolation of new fungal species as endophytes of marine macroalgae; a possibility due to the high estimates of marine-derived fungi and low numbers of investigations performed (Jones 2011a, b; Jones et al. 2008).

Of the 140 distinct isolates obtained, six endophytes (KP1-025B, KP1-131CC, KP1-175E, KP2-025B, KP2-025C, and KP2-033A) were identified to the genus level based upon the presence of characteristic spore morphology. The remaining seventy-five endophytes could not be identified to either the genus or species level despite the use of molecular techniques and growth on four different media types to stimulate the fungi to produce spores. These fungi did not produce any spores or other features that would aid in their identification, and were assigned codes based upon their morphological characteristics, such as colony colour and mycelial characteristics (Dobranic et al. 1995; Flewelling et al. 2013a; Flewelling et al. 2013b; Johnson and Whitney 1989a, b, 1992; Suryanarayanan et al. 1998; Suryanarayanan et al. 2010).

The taxonomic identification of fungi using molecular techniques is a multi-step process (DNA extraction, amplification and sequencing, see section 2.2.7) resulting in a number of possibilities as to why 75 endophytes could not be identified through common

molecular means. It has been reported that some DNA extraction techniques have been shown to be inefficient at lysing fungal cell walls (Maaroufi et al. 2004). Comparisons of multiple methods of DNA extraction from fungi suggests different techniques (including commercial kits and individually designed protocols) led to varying yields and quality of fungal DNA recovered (Fredricks et al. 2005; Löffler et al. 1997; Maaroufi et al. 2004). The two commercial DNA extraction kits and protocols used in this work may not have been sufficient for isolating DNA from these fungi for further amplification and sequencing. Also, many primers have been reported for identifying fungi, such as primers NS1 to NS8, ITS1 to ITS 5, MS1, MS2 and ML 1 to ML 8 (White et al. 1990) and primers NL912, NL209, NL359, NS1, EF3, FR1 (Zuccaro et al. 2008; Zuccaro et al. 2003), and although the ITS region has been reported as the official barcoding marker for identifying fungi to the species level and the most common region used in curated fungal DNA databases (Raja et al. 2017), alternative primers could be used in an attempt to identify the endophytic fungi. In addition to the designation of codes based upon morphological characteristics, some authors have documented non-sporulating fungi as simply "sterile mycelia" or by an isolate code where no identifiable features could lend a taxonomic identification (Guo et al. 2000; Huang et al. 2015; Schulz et al. 2008; Xiang et al. 2016; Zuccaro et al. 2008; Zuccaro et al. 2003). Several groups of fungi were identified by morphological designation: sterile beige yeasts (32 isolates, 13 host algae), sterile white yeasts (27 isolates, 14 host algae), sterile pigmented yeasts (four isolates, four host algae), sterile filamentous fungi (16 isolates, four host algae) and septate filamentous fungi (six isolates, four host algae). These fungi were then differentiated within each group based

upon individual characteristics, such as colony shape and colour, seen by growing these isolates upon Czapek-dox, potato dextrose, and cornmeal agars (Table 2.4).

Twenty-three distinct *Penicillium* spp. were isolated from 11 of the 20 macroalgal hosts investigated (Table 2.4). *Penicillium roseopurpureum*, an isolate from *P. fascia*, was identified to the species level as the morphology of this isolate suggested it to belong to the genus *Penicillium* and a BLAST search of its DNA sequence was found to only return that of *P. roseopurpureum*. *Penicillium* spp. have been found to be halotolerant (Butinar et al. 2011; Gunde-Cimerman et al. 2009), are widespread in the marine environment (Huang et al. 2011; Jones et al. 2015; Kathiresan et al. 2009; Kawahara et al. 2012; Pang et al. 2016; Paz et al. 2010; Proksch et al. 2008), and have been found as common endophytes of marine macroalgae (An et al. 2013; de Silva et al. 2009; Flewelling et al. 2013a; Flewelling et al. 2013b; Gamal-Eldeen et al. 2009; Gao et al. 2011a; Gao et al. 2011b; Li et al. 2014b; Schulz et al. 2008; Suryanarayanan et al. 2010; Zhang et al. 2009; Zuccaro et al. 2008). It is therefore unsurprising that 16% of all isolates obtained in this work belong to the genus *Penicillium*.

Seven isolates of *A. fumigatus* were isolated from *M. stellatus*, *P. lanosa, S. lomentaria*, and *U. intestinalis*. It was previously found that *A. fumigatus* was only isolated from brown algae and not from red or green algae (Flewelling et al. 2013b); however, in this study, *A. fumigatus* was isolated from red, green and brown algae, suggesting these isolates have no host specificity to any one species or class of macroalgae amongst the hosts studied within this work. *Aspergillus* spp. have been found to be common endophytes of marine macroalgae (Cui et al. 2009; Cui et al. 2010b; Flewelling et al. 2013a; Flewelling et al. 2013b; Qiao et al. 2010b; Qiao et al. 2011; Suryanarayanan

et al. 2010; Teuscher et al. 2006; Zhang et al. 2015a; Zhang et al. 2007b; Zhang et al. 2007c; Zhang et al. 2007d; Zuccaro et al. 2008) and similar to that of *Penicillium* spp., *Aspergillus* spp. are commonly found in a variety of niches within the marine environment (Jones et al. 2015; Pang et al. 2016), including isolation from sponges [examples include (Dethoup et al. 2016; Kumar et al. 2012; Peng et al. 2016; Pinheiro et al. 2012; Sun et al. 2012b; Yin et al. 2015)], mangrove sediment and roots [examples include (Huang et al. 2010; Liu et al. 2016b; Song et al. 2011; Wu et al. 2015; Zhou et al. 2011)] and marine sediments [examples include (Choi et al. 2011; Li et al. 2016b; Mathan et al. 2011; Nagano et al. 2016; Wu et al. 2012)] and are also known for their halotolerance (Butinar et al. 2011; Gunde-Cimerman et al. 2009; Kis-Papo et al. 2003).

In addition to *Penicillium* spp. and *Aspergillus* spp., several of the endophytes isolated in this work have been previously isolated from marine macroalgae (Table 2.6), although in some cases from different hosts, and include *Dendryphiella* spp. (Flewelling et al. 2013b; Schulz et al. 2008; Zuccaro et al. 2008), *Metschnikowia* sp. (Flewelling et al. 2013b), *T. versicolor* (Flewelling et al. 2013a), *Myrothecium* sp. (Suryanarayanan et al. 2010), *Trichoderma* sp. (Kaushik et al. 2014; Liang et al. 2016a; Liang et al. 2016b; Miao et al. 2012a; Suryanarayanan et al. 2010; Thirunavukkarasu et al. 2015; Venkatachalam et al. 2015; Zuccaro et al. 2008), *Tolypocladium* sp. (Schulz et al. 2008) and *Aureobasidium* sp. (Flewelling et al. 2013a; Kaushik et al. 2014; Suryanarayanan et al. 2010; Thirunavukkarasu et al. 2015; Venkatachalam et al. 2015). *M. bolleyi* and *M. nyssicola* have not been isolated as endophytes from marine macroalgae, however, isolates belonging to both respective genera have (Flewelling et al. 2013b; Webber 1967).

The majority of endophytic fungi belong to the phylum Ascomycota (Carroll 1988); however, in this work, *T. versicolor*, *Phanerochaete* sp., *C. micaceus*, belonging to the phylum Basidiomycota, were isolated. *Phanerochaete* sp., *O. intermedia*, *Mollisia* sp., *D. bryoniae*, *Thysanophora* spp., *Diaporthe* sp., *C. micaceus*, *Chrysosporium* sp., and *Paraconiothyrium* sp. have all only been previously isolated from terrestrial plants (Table 2.7). The isolation of these endophytes from numerous sources, including those marine and terrestrial, suggests that these endophytic fungi may have low host specificity and could be considered host-generalists (Higgins et al. 2011; Reddy et al. 2016; Suryanarayanan et al. 2010; Suryanarayanan et al. 2003).

Mycosphaerella ascophylli, a common endophyte of *A. nodosum*, was again not isolated from the brown alga *A. nodosum* (Flewelling et al. 2013a; Flewelling et al. 2013b). *Ascophyllum nodosum* and its endophytic fungus *M. ascophylli* has been studied extensively (Deckert and Garbary 2005a; Deckert and Garbary 2005b; Fries 1988; Fries 1979; Fries and Thorentolling 1978; Garbary et al. 2005a; Garbary and Gautam 1989; Garbary and London 1995; Garbary and Macdonald 1995; Kohlmeyer 1972; Webber 1967; Xu et al. 2008), and the fact that *M. ascophylli* was not isolated from *A. nodosum*, despite not typically having been reported without its endophyte, has been suggested to be a result of differing isolation protocols (Flewelling et al. 2013b) as small changes in the isolation conditions, such as a change in pH, have been shown to have an impact on the isolation of endophytic fungi (Kjer et al. 2010; Qi et al. 2012). This indicates that the use of any condition for isolating endophytic fungi may limit the isolation of some fungi and also shows that the endophytes isolated from plant tissues may not represent the true diversity within the host (Kjer et al. 2010; Qi et al. 2012).

Table 2.6. Endophytic fungi isolated from marine macroalgae, collected in the Bay of Fundy, New Brunswick, Canada, that have been previously isolated from marine macroalgae, terrestrial plants or sediment.

Endophyte	Algal host	Previous source	Environment	Reference
Dendryphiella sp.	*A. nodosum*	*Ulva intestinalis*	Marine	Schulz et al. 2008
	P. fascia	*Fucus* spp.	Marine	
	S. lomentaria	*A. nodosum*	Marine	Flewelling et al. 2013b
		Zostera marina	Marine	Newell 1981
		Rhizophora mangle	Marine	Ukoima et al. 2010
		Sediment	Marine	dela Cruz et al. 2006, 2006a
Metschnikowia sp.	*F. distichus* subsp. *edentatus*	*Plocamium cartilagineum*	Marine	Flewelling et al. 2013b
Trametes versicolor	*F. distichus* subsp. *edentatus*	*D. ramentacea*	Marine	Flewelling et al. 2013a
	U. intestinalis			
Myrothecium sp.	*S. lomentaria*	*Caulerpa sertuilariodes*	Marine	Suryanarayanan et al. 2010
Trichoderma sp.	*P. fascia*	*Halimeda macroloba*	Marine	Suryanarayanan et al. 2010
		Dictyota dichotoma	Marine	
		Padina gymnospora	Marine	
		Gracilaria crassa	Marine	
		Sargassum wightii	Marine	Thirunavukkarasu et al. 2015, Venkatachalam et al. 2015
Tolypocladium sp.	*S. arcta*	*Fucus vesiculosus*	Marine	Schulz et al. 2008
Aureobasidium sp.	*D. ramentacea*	*D. ramentacea*	Marine	Flewelling et al. 2013a
		P. lanosa	Marine	

Table 2.7. Endophytic fungi isolated from marine macroalgae, collected in the Bay of Fundy, New Brunswick, Canada, that have not been previously reported as an endophyte of marine macroalgae.

Endophyte	Algal host	Previous source	Environment	Reference
Phanerochaete sp.	*F. vesiculosus*	*Platanus acerifolia*	Terrestrial	Robles et al. 2015
Ophiognomonia intermedia	*S. lomentaria*	*Quercus emoryi*	Terrestrial	Faeth & Hammon 1997
		Quercus emoryi	Terrestrial	Wilson et al. 1997
		Soybean cultivar Conquista	Terrestrial	de Souza Leite et al. 2013
		Coffea arabica	Terrestrial	Bongiorno et al. 2016
Mollisia sp.	*S. lomentaria*	*Picea abies*	Terrestrial	Barklund & Kowalski 1996
		Empetrum nigrum	Terrestrial	Tejesvi et al. 2010
		Vaccinium vitis-idaea	Terrestrial	
		Deschampsia antarctica	Terrestrial	Upson et al. 2009
Microdochium bolleyi	*S. lomentaria*	*Ammophila breviligulata*	Terrestrial	David et al. 2016
		Fagonia cretica	Terrestrial	Zhang et al. 2008
Didymella bryoniae	*S. lomentaria*	Soybean cultivar Monsoy	Terrestrial	de Souza Leite et al. 2013
		Soybean cultivar Conquista	Terrestrial	
Thysanophora penicillioides	*S. lomentaria*	*Picea abies*	Terrestrial	Muller & Hallaksela 1998
		Picea abies	Terrestrial	Muller & Hallaksela 1998b
		Abies alba	Terrestrial	Sieber-Canavesi & Sieber 1993
Mycosphaerella nyssicola	*S. lomentaria*	*Coffea arabica*	Terrestrial	Bongiorno et al. 2016
Diaporthe sp.	*S. lomentaria*	Soybean cultivar Monsoy	Terrestrial	de Souza Leite et al. 2013
		Soybean cultivar Conquista	Terrestrial	

Table 2.7. Endophytic fungi isolated from marine macroalgae, collected in the Bay of Fundy, New Brunswick, Canada, that have not been previously reported as an endophyte of marine macroalgae, continued.

Endophyte	Algal host	Previous source	Environment	Reference
Coprinellus micaceus	*S. lomentaria*	*Broussonetia papyrifera*	Terrestrial	de Errasti et al. 2010
		Celtis occidentalis	Terrestrial	
Chrysosporium sp.	*S. arcta*	Soybean cultivar Hwangkeumkong	Terrestrial	Hamayun et al. 2009
Paraconiothyrium sp.	*D. ramentacea*	*Acer truncatum*	Terrestrial	Guo et al. 2015
		Soybean cultivar Conquista	Terrestrial	de Souza Leite et al. 2013
		Soybean cultivar Conquista	Terrestrial	
		Acer truncatum	Terrestrial	Liu et al. 2015
		Taxus baccata	Terrestrial	Somjaipeng et al. 2015
		Taxus baccata	Terrestrial	Somjaipeng et al. 2016
		Cephalotaxus harringtonia	Terrestrial	Tian et al. 2014
		Acer truncatum	Terrestrial	Zhang et al. 2016
		Cephalotaxus harringtonia	Terrestrial	Prado et al. 2013

2.5 Conclusions

The objective of this research was to isolate and identify endophytic fungi from marine macroalgae from the Bay of Fundy, Canada. One hundred and forty distinct fungal isolates, representing 20 genera and 75 unidentified isolates designated by morphology, were obtained from 18 marine macroalgae. This supports the notion that marine macroalgae are indeed an excellent source of fungal biodiversity. As so few species of marine macroalgae have been investigated, further studies into the biodiversity of endophytic fungi from marine macroalgae are warranted to better characterize the extent of the fungal diversity from this relatively untapped source. In addition to sterile isolates, *Penicillium* spp. and *Aspergillus fumigatus* were repeatedly isolated from numerous hosts, with several of the fungi isolated in this work not previously found as endophytes of marine macroalgae. Eight endophytes obtained in this work could not be identified taxonomically due to low homology with entries in GenBank, suggesting the isolation of new fungal species as endophytes of marine macroalgae; however, further work would be required to confirm this observation.

Chapter 3: Development of a simple bioactivity profiling technique for the prioritization of a library of extracts from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada

3.1 Introduction

In the face of an urgent need to find new antimicrobial drugs, natural products remain the most successful source of these pharmaceuticals in the last 30 years (Newman and Cragg 2007; Newman and Cragg 2012, 2016; Newman et al. 2003). To discover new antimicrobial natural products, innovative techniques are required to rapidly recognize redundancies in their discovery and prioritise natural product libraries for efficient, yet, thorough investigation; a technique which in this field is commonly referred to as dereplication (Kealey et al. 2017; Rakshith et al. 2016; Wong et al. 2012; Yang et al. 2013). Over and above the need for new antimicrobial drugs, new targets and modes of action are needed to circumvent and outpace the ever growing drug resistance that has been reported worldwide for decades (Brown and Wright 2016; Fischbach and Walsh 2009; Lewis 2013, 2017). In short, we need new antimicrobials that work differently than the current regime as well as better, more rapid approaches for finding them.

Bioactivity profiling techniques allow for the prioritisation of natural product libraries in an effort to identify unique bioactive metabolites (Wong et al. 2012). The concept of bioactivity profiling has existed for decades, such as in the case of the structure-activity profiling algorithm used by the National Cancer Institute with their 60 tumor cell line screening (Bates et al. 1995; Covell et al. 2007; Park et al. 2010; Sausville and Johnson 2000; Su et al. 2011). Previous work by the Linington research group (Wong et al. 2012) has led to the concept of antibiotic mode of action profiling (BioMAP) in an effort to not only identify new antibiotic natural products, but also those with modes of action different to those of current antibiotic drugs (Figure 3.1).

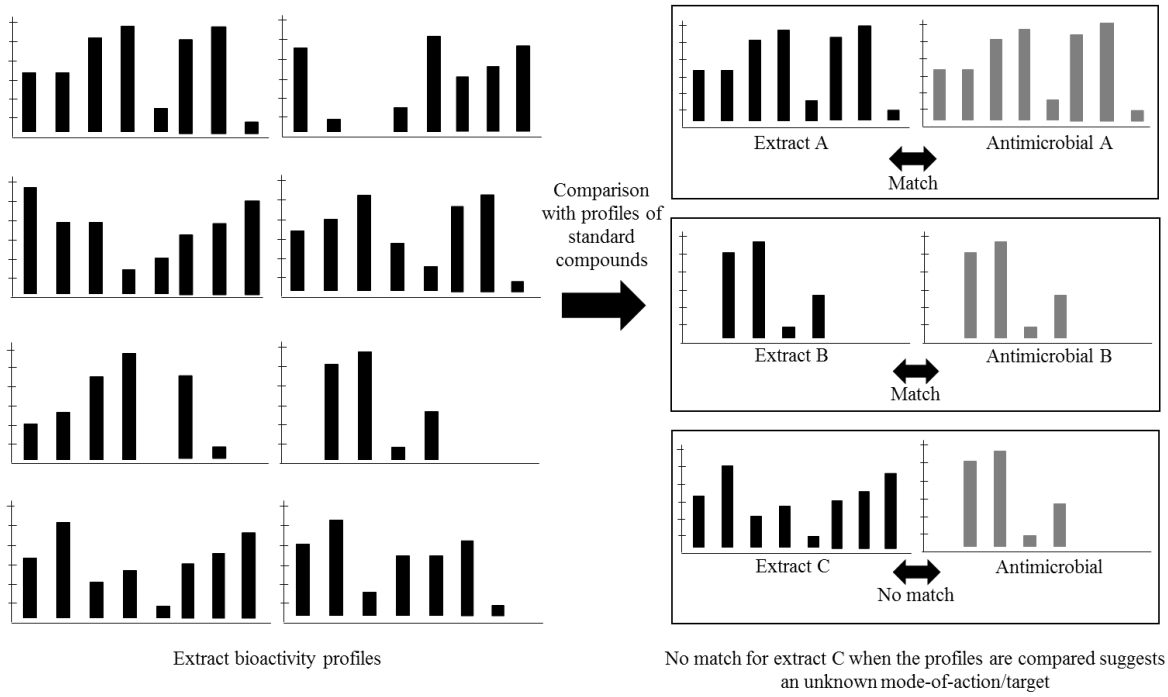


Figure 3.1. A conceptual representation of bioactivity profiling. This figure highlights the goal of not only identifying new natural products, but also those that may have unknown mode-of-action against pathogenic microorganisms. Conceptually, bioactivity profiling involves a comparison between the profiles of extracts and the profiles of antimicrobial drugs, where similarities between the profiles suggests the extract may contain natural products with known modes of action. Bioactivity profiles of extracts with no similarity to those of the antimicrobial drugs screened, suggests the extract may contain antimicrobial natural products with unknown modes of action and are therefore of interest for further investigation using bioassay-guided fractionation.

The BioMAP technique presented by the Linington group was successful in identifying the new antibiotic natural product arromycin from an initial screen of over 3000 prefractions against 15 pathogens. However, the extent of the work put into analysing their library of extracts, facilitated by the use of specialised high-throughput screening robotics, limits the scale of analysis that can be replicated in the absence of these sophisticated tools.

The objective of the research presented in this chapter was therefore to obtain extracts for each of the 140 endophytes isolated from marine macroalgae of the Bay of

Fundy and to screen these extracts for antimicrobial activity. Furthermore, this research aims to develop a simplified, though efficient, bioactivity profiling technique to subsequently prioritise the extracts for bioassay-guided fractionation.

3.2 Experimental

3.2.1 Liquid culture fermentation

For each fungal isolate, a portion of the fungal colony (5 mm × 5 mm) was transferred to 2.0% Bacto™ malt extract broth [MEB, 100 mL (Becton Dickinson, Mississauga, Ontario)] in an Erlenmeyer flask (250 mL). The tops of the flasks were covered with aluminum foil and shaken (150 rpm) at room temperature and ambient light for two weeks. Following the fermentation, cultures were immediately extracted.

3.2.2 Preparation of extracts

All solvents for extraction were ACS certified (Fisher Scientific Ltd., Ottawa, Ontario). Following sonication of the fermentation culture (5 min), mycelia and cell debris were removed by filtration through cotton wool, with the filtered culture broth extracted with EtOAc (3 × 50 mL) and the combined organic extracts concentrated *in vacuo* to give an extract. All extracts were stored at -20 °C until required for biological assays. Prior to biological screening, stock solutions of the extracts (5 mg/mL) were prepared with sterile-filtered DMSO and stored at 4 °C.

3.2.3 Preparation of antimicrobial standards for bioactivity profiling data set

All antimicrobial standards, with the exception of rifampin, were obtained from Sigma Aldrich (Oakville, Ontario). Rifampin was obtained from Fisher Scientific (Ottawa, Ontario). Stock solutions for each antimicrobial (50 µg/mL) were prepared with sterile-filtered DMSO and stored at 4 °C.

3.2.4 Antifungal activity assay

Antifungal activity against *Candida albicans* (ATCC 14053) and *Saccharomyces cerevisiae* (ATCC 9763) was evaluated using a microbroth dilution antimicrobial assay. All procedures of the antimicrobial susceptibility tests, except for plate absorbance measurements, were performed in a Class II biological safety cabinet (Labconco, Kansas City, Missouri, USA). Immediately prior to use, stock solutions of each crude extract or antibiotic (40 µL) were diluted with either Difco™ Sabouraud dextrose broth (*C. albicans*, 960 µL) or yeast mold broth (*S. cerevisiae*, 960 µL) (Becton Dickinson, Mississauga, Ontario) and the resulting test solutions (100 µL; 4% DMSO) were transferred to the non-peripheral wells of a clear, non-tissue cultured 96-well microtitre plate, in triplicate (BD Falcon™, Becton Dickinson, Mississauga, Ontario). Each plate contained three positive control wells (*C. albicans*: nystatin, 2.7 µM; *S. cerevisiae*: amphotericin B, 2.7 µM, 100 µL per well) in triplicate. Wells were then inoculated with suspensions of either *C. albicans* or *S. cerevisiae* (100 µL; 1×10^6 CFU/mL), to obtain a cell density of 5×10^5 CFU/mL. To reduce evaporation from the plates, sterile water (200 µL) was added to all perimeter wells. Each plate contained three negative control wells [4% DMSO in broth (100 µL) inoculated with suspensions of the appropriate organism

(100 µL)] and three untreated blank control wells [2% DMSO in broth (200 µL)]. Initial absorbance measurements (600 nm) were recorded prior to a 24 h incubation period (37 °C) where a final absorbance measurement was then taken for percentage inhibition determination. Optical densities (OD) were measured using a Molecular Devices Emax microplate reader.

Final absorbance readings were subtracted from the initial readings to obtain the change in optical density (ΔOD). The change in optical density values were corrected for background absorbance of the media by subtracting the mean ΔOD readings of the blanks from the mean ΔOD readings of the control and crude extracts wells, while the percentage inhibition of fungal growth was defined as:

$$[1 - (\text{mean test or positive control } \Delta OD / \text{mean negative control } \Delta OD)] \times 100$$

3.2.5 Antibacterial activity assay

Antibacterial activity against *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), and *Enterococcus faecium* (ATCC 35667) was evaluated in the same manner as described for the antifungal activity assay. The growth medium for *P. aeruginosa*, *E. coli* and *S. aureus* was BBL™ Mueller Hinton II cation adjusted broth (Becton Dickinson, Mississauga, Ontario), whereas BBL™ Brain Heart Infusion broth (Becton Dickinson, Mississauga, Ontario) was used for *E. faecium*. Positive controls consisted of a triplicate concentration of antibiotic (21 µM & 2.6 µM, gentamicin for *P. aeruginosa* and *E. coli*, respectively;

0.85 μM erythromycin for *S. aureus*; 1.4 μM tetracycline for *E. faecium*; 100 μL per well) inoculated with suspensions of the appropriate pathogen (100 μL).

3.2.6 Antimycobacterial activity assay

Antimycobacterial activity against *Mycobacterium tuberculosis* strain H37Ra (ATCC 25177), *Mycobacterium avium* (ATCC 25291) and *Mycobacterium smegmatis* (ATCC 700084) was evaluated using a microplate resazurin assay according to O'Neill et al. (2014). Immediately prior to use, stock solutions of crude extracts and antimicrobials (40 μL) were diluted with modified Middlebrook 7H9 broth (960 μL; BBL™ MGIT™, Becton Dickinson, Mississauga, Ontario) and the resulting crude extracts (100 μL) were transferred to the non-peripheral wells of a black, non-tissue culture treated, low-binding, 96-well microtitre plate (VWR, Mississauga, Ontario) in triplicate and inoculated with suspensions of the appropriate organism (100 μL) of cell density 2.0×10^6 cells/ mL. To reduce evaporation from the plates, sterile water (200 μL) was added to all perimeter wells. The positive control consisted of rifampin (*M. tuberculosis*, 0.012 μM; *M. avium* 12 μM) or ciprofloxacin (*M. smegmatis*, 3.8 μM) in triplicate. In addition to the positive controls, negative controls [4% DMSO in modified Middlebrook 7H9 broth (100 μL) inoculated with suspensions of the appropriate organism (100 μL)], blanks [2% DMSO in modified Middlebrook 7H9 broth (200 μL), and test solutions (100 μL) with modified Middlebrook 7H9 broth (100 μL)] were included in triplicate in each plate. *M. tuberculosis* and *M. avium* plates were incubated (37 °C; 5% CO_2) for three and four days, respectively, in a humid environment and *M. smegmatis* plates were incubated for one day in a standard incubator. Following incubation, a solution of resazurin (0.0625 mg/mL)

in 5% aqueous Tween 80 (50 µL) was added to all non-peripheral wells. Plates were then incubated for a further 24 h, sealed with an adhesive polyester film (50 µm; VWR, Mississauga, Ontario), and mycobacterial growth was assessed fluorometrically at 37 °C (Molecular Devices Gemini EM dual-scanning microplate spectrofluorometer with a 530 nm excitation filter and a 590 nm emission filter operating in top-scan mode). Fluorescence values were corrected for any background fluorescence of the media and crude extracts by subtracting the mean fluorescence readings of the appropriate blanks from the mean fluorescence readings of the control and crude extract wells. The percentage inhibition of mycobacterial growth was then defined as:

[1 − (mean test or positive control fluorescence/mean negative control fluorescence)] × 100.

3.2.7 Creation of bioactivity profiles for a library of marine macroalgal endophyte extracts

The percentage inhibition values obtained for each extract or antimicrobial standard against the nine test microorganisms were compiled and subsequently normalized as follows:

1. All negative percentage inhibition values were converted to zero.
2. The maximum percentage inhibition value for an extract or antimicrobial standard against the nine test pathogens was identified.

3. All percentage inhibition values for each extract and antimicrobial standard were divided by the maximum value of that extract or antimicrobial standard to create a concentration independent ratio of activities between 0 and 1.

The bioactivity of the extracts was categorized into three levels based on the normalized inhibition values: high activity (> 0.5), moderate activity (0.25 – 0.5) and low activity (< 0.25).

3.2.8 Identification of unique bioactivity profiles within a library of marine macroalgal endophyte extracts

Principal component analysis (PCA) was performed on the bioactivity profiles of all extracts to identify the unique bioactivity profiles within the data set (Unscrambler 10.3, CAMO Software). The data was mean centred and nine principal components (one for each test microorganism) were created for the analysis, with every combination of principal component plotted. Unique bioactivity profiles were identified as those located outside of the 95% confidence interval of each PCA plot.

3.2.9 Comparison of unique extract bioactivity profiles to those of commercially available antimicrobials

Hierarchical cluster analysis using Cluster 3.0 (de Hoon et al. 2004) with a Euclidean distance measure and average linkage clustering method was performed to compare the bioactivity profiles of 17 commercially available antimicrobials with the bioactivity profiles identified using PCA. The hierarchical cluster analysis dendrogram

was visualised using Java Treeview (Saldanha 2004) whereby clusters were identified where bioactivity profiles of extracts and/or antimicrobial standards possessed correlations ≥ 0.80.

3.3 Results and discussion

3.3.1 Bioactivity screening of endophyte extracts

One hundred and forty fungal endophytes isolated from marine macroalgae of the Bay of Fundy, Canada were individually fermented in 2% Bacto™ malt extract broth for two weeks and subsequently extracted with EtOAc to obtain an extract for each fungal isolate. Endophytic fungi from marine macroalgae have recently been highlighted as excellent sources of antimicrobial natural products (Flewelling et al. 2015; Sarasan et al. 2017; Zhang et al. 2016c) and as such, the extracts obtained in this work were screened against nine microorganisms at a concentration of 100 µg/mL in an effort to identify their antimicrobial potential.

3.3.2 Development of bioactivity profiles

The aim of antimicrobial bioactivity profiling is to prioritise extracts in a library that not only possess unique profiles of antimicrobial activity when compared between extracts, but also to identify those extracts that possess unique profiles of antimicrobial activity when compared to standard antimicrobial drugs covering a range of biological targets and/or modes of action. To select extracts for fractionation that have a unique profile of antimicrobial activity, extracts were screened against nine microorganisms in

standard microbroth dilution assays. This suite of microorganisms included two Gram positive bacteria (*Staphylococcus aureus* and *Enterococcus faecium*), two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), two fungi (*Candida albicans* and *Saccharomyces cerevisiae*) and three mycobacteria (*Mycobacterium tuberculosis*, *Mycobacterium avium* and *Mycobacterium smegmatis*). While previous methods of bioactivity profiling only included Gram positive and Gram negative bacteria (Wong et al. 2012), this approach broadens the scope of the antimicrobial activity of each extract and allows for the inclusion of fungi and mycobacteria.

Seventeen antimicrobial standards covering a range of modes of action were selected for use in the bioactivity profiling technique (Table 3.1). These antimicrobials included cell wall inhibitors (amoxicillin, ethambutol, isoniazid, and vancomycin) (Johnsson et al. 1995; Kohanski et al. 2010; Mikusova et al. 1995), fungal cell membrane inhibitors (amphotericin B, miconazole, and nystatin) (Georgopapadakou and Walsh 1996; Hammond et al. 1974), protein synthesis inhibitors (chloramphenicol, erythromycin, gentamicin, kanamycin, streptomycin, and tetracycline) (Kohanski et al. 2010), DNA transcription and synthesis inhibitors (actinomycin D, ciprofloxacin), a RNA synthesis inhibitor (rifampin) (Kohanski et al. 2010), and a metabolism inhibitor (sulfamethazine) (McCullough and Maren 1973). These compounds were selected for these experiments as they represent examples for each of the various biological pathways targeted for antimicrobial chemotherapy. To allow for comparison between the panel of antimicrobial standards and the library of extracts, the antimicrobials were screened at 1% of the extract screening concentration (1 µg/mL) to approximate the concentration of a given bioactive natural product within an extract.

Table 3.1. Antimicrobial standards used in the bioactivity profiling of extracts of endophytes isolated from marine macroalgae collect from the Bay of Fundy, New Brunswick, Canada.

Biological pathway	Antibiotic	Primary target
Cell wall synthesis	Amoxicillin	Penicillin binding proteins[a]
	Ethambutol	Arabinogalactan synthesis[b]
	Isoniazid	InhA enzyme[c]
	Vancomycin	Peptidoglycan units (terminal D-Ala-D-Ala dipeptide)[a]
Fungal cell membrane synthesis	Amphotericin B	Ergosterol[d, e]
	Miconazole	Lanosterol 14 α-demethylase[e]
	Nystatin	Ergosterol[d, e]
Protein synthesis	Chloramphenicol	50S ribosome (inhibits elongation step)[a]
	Erythromycin	50S ribosome (dissociation of the peptidyl-tRNA molecule from the ribosomes during elongation)[a]
	Gentamicin	30S ribosome (mistranslation by tRNA mismatching)[a]
	Kanamycin	30S ribosome (mistranslation by tRNA mismatching)[a]
	Streptomycin	30S ribosome (mistranslation by tRNA mismatching)[a]
	Tetracycline	30S ribosome (inhibits aminoacyl tRNA binding to the ribosome)[a]
DNA transcription and synthesis	Actinomycin D	Binds to DNA, thus interfering with growing RNA chains[f]
	Ciprofloxacin	Topoisomerase II (DNA gyrase)[a]
RNA synthesis	Rifampin	DNA-dependent RNA polymerase[a]
Metabolism	Sulfamethazine	Competitive inhibitor for DHPS, involved in folate synthesis[g]

[a] Kohanski et al. 2010; [b] Mikusova et al. 1995; [c] Johnsson et al. 1995; [d] Hammond et al. 1974; [e] Georgopapadakou & Walsh 1996; [f] Sobell 1985; [g] McCullough & Maren 1973

Extracts are typically complex mixtures of metabolites, therefore, in an attempt to overcome any potential concentration differences of bioactive natural products that may be present in any given extract, the percentage inhibition bioactivity data for each extract was normalised to the highest percentage inhibition obtained. This ultimately gave an activity range of zero (not active) to one (most active). As this technique uses unpurified extracts for analysis, this normalization step allowed for a concentration independent comparison of the bioactivity profile of each extract. In order to enable a comparison between all bioactivity profiles, this normalisation step was also performed on the bioactivity profiles of the antimicrobial standards.

The bioactivity profiles developed for each of the 17 antimicrobials standards (Figure 3.3) highlight the individual characteristics these drugs possess. Many of the antimicrobial drugs (ciprofloxacin, erythromycin, tetracycline and gentamicin) possessed broad-spectrum activity towards Gram positive bacteria, Gram negative bacteria and mycobacteria. Other antimicrobials, such as isoniazid, nystatin and amphotericin B, possessed narrow-spectrum activity towards particular organisms (*M. tuberculosis* for isoniazid and *C. albicans* for nystatin and amphotericin B). It should be noted that the expected activity of ethambutol towards *M. tuberculosis* was not observed and instead, this drug possessed activity towards *E. faecium*. This may be due to the concentration of the antimicrobial drug (1 µg/mL) used in the creation of the bioactivity profiles.

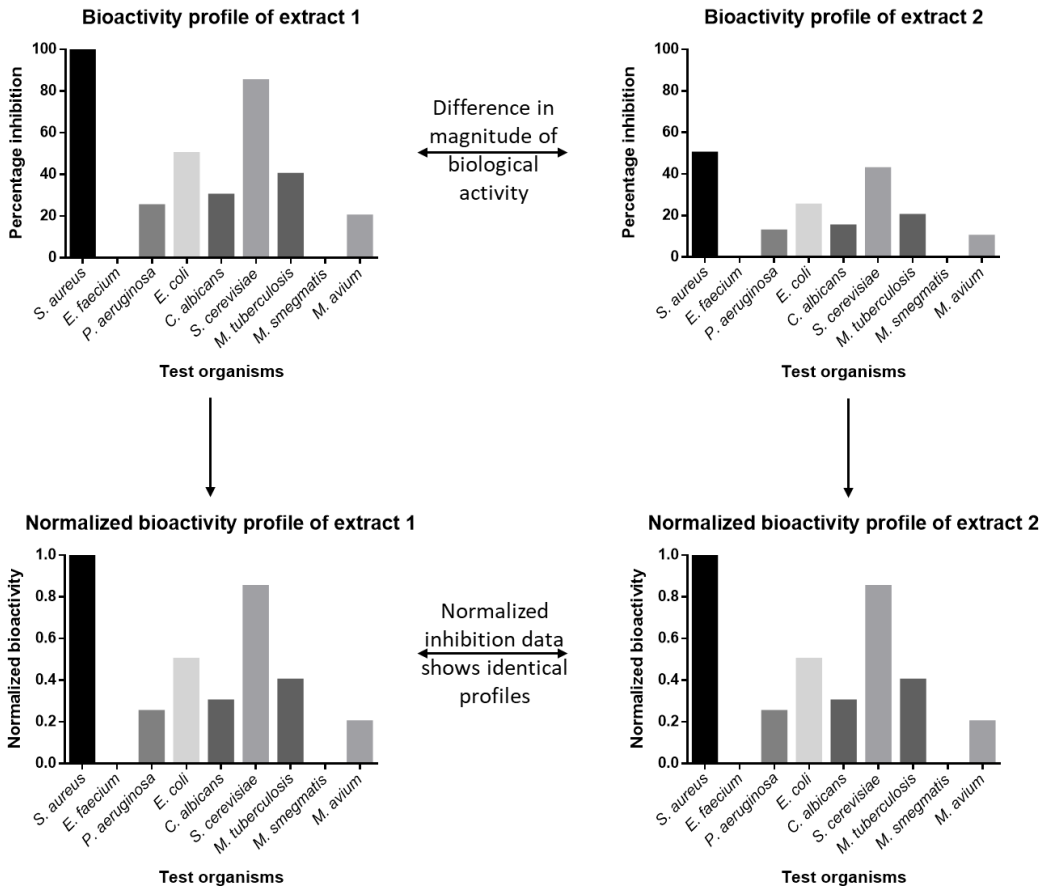


Figure 3.2. Example of the normalization of percentage inhibition values for fungal extracts allowing for direct comparison of extracts. Normalization was conducted in an attempt to eliminate concentration differences of antimicrobial natural products that may be present in extracts from endophytic fungi. This normalization step was also applied to the bioactivity profiles of the antimicrobial standards.

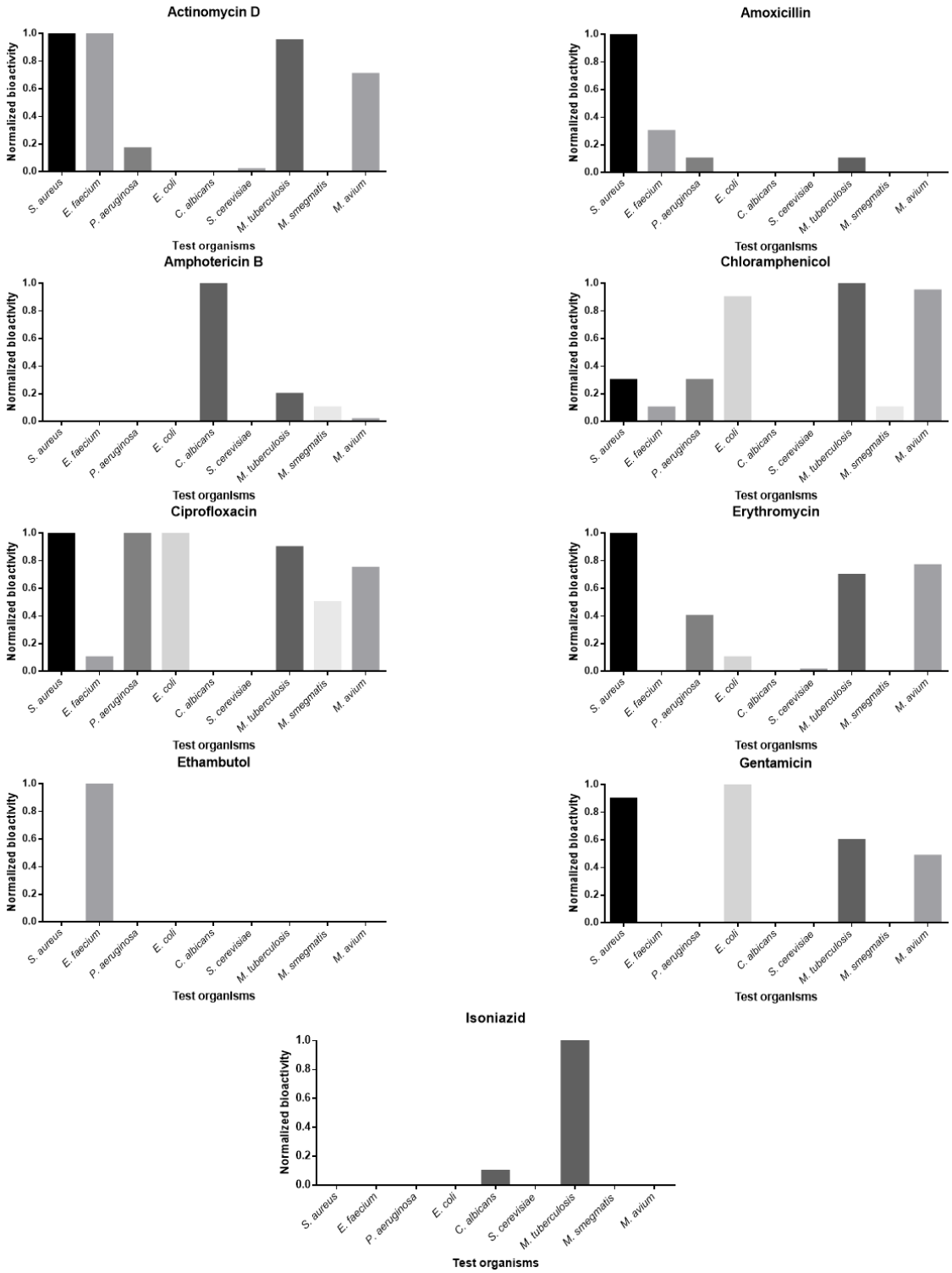


Figure 3.3. Bioactivity profiles of antimicrobial standards.

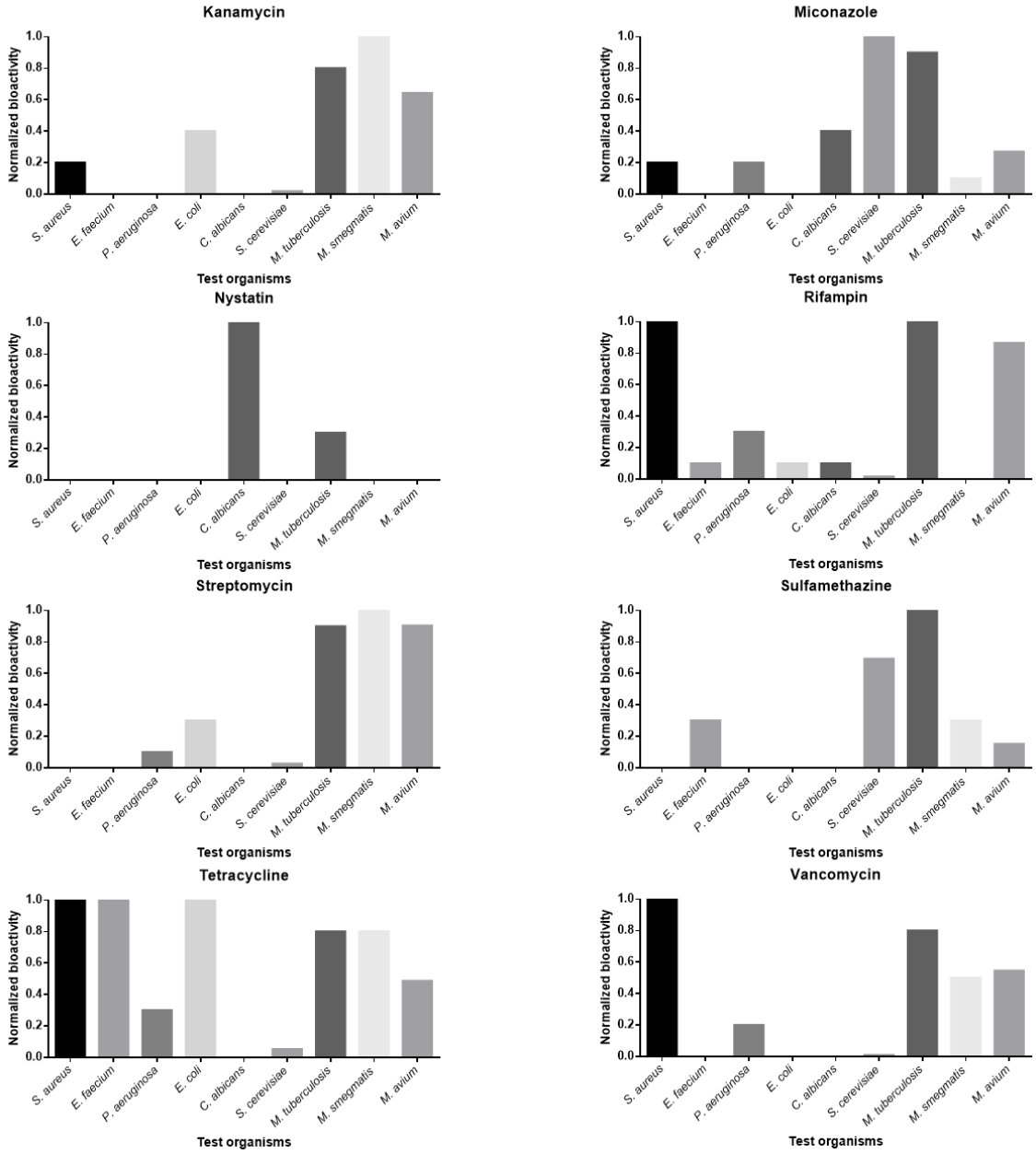


Figure 3.3. Bioactivity profiles of antimicrobial standards, continued.

3.3.3 Principal component analysis of bioactivity profiles derived from fungal endophyte extracts

To identify extracts within the library that displayed unique profiles, principal component analysis (PCA) was performed. Principal component analysis allows for comparison of complex data sets (Bro and Smilde 2014; Lavine 2006; Ringnér 2008; Wold et al. 1987) and is also an appropriate tool for identifying outliers within a data set (Bro and Smilde 2014; Wold et al. 1987). As the bioactivity profiles contain nine variables (one for each pathogen), a technique such as PCA can be used to simplify the data in order to identify the bioactivity profiles that are unique to the library of extracts assessed for their antimicrobial activity and to ensure that only unique profiles in the library are then further analysed against the profiles of the antimicrobial standards. The use of PCA also eliminates the need to use the magnitude of the biological activity as a determinant for prioritisation. Previous work by Higginbotham et al. (2014) using bioactivity profiling simply relied on the magnitude or strength of the bioactivity to determine which profiles of extracts to further analyse against the antimicrobial standards.

Upon investigation of the principal components (PCs) obtained through PCA (PC1 vs. PC2 through PC8 vs. PC9; example PCA plot Figure 3.4; all PCA plots can be found in appendix 9), 37 extracts were identified as outliers in the data set as the profiles of these extracts were located outside of the 95% confidence circle determined for the data set within the PCA plots. Many of the extracts in the PCA were outliers only in particular PCs, a result due to the bioactivity towards certain test organisms contributing to the variance observed in these principal components (as seen in loadings plots of the PCA; appendix 9). KP1-131DD and KP2-033A were found to be outliers in plots with

PC3 as they possessed low activity towards *M. tuberculosis* but high activity towards *S. aureus* or *C. albicans*, whereas KP1-009B, KP1-017C, KP1-131C, KP1-131H and KP1-175M were only outliers in plots with PC4, due to the presence or absence of high activity towards *S. aureus* and *M. tuberculosis*. Extracts from KP1-175D and KP1-175E were outliers in PC5 as they possessed high activity against *C. albicans*, but showed no activity towards *S. aureus*, with KP1-045I, KP1-063J, KP1-131O, KP1-175K and KP2-025B outliers in PC6 as they all possessed high activity towards *P. aeruginosa*. Extracts KP1-045A, KP1-075B, KP1-131K, KP1-131T and KP2-001D were outliers in PC7 as they all showed activity towards *S. cerevisiae*, KP1-131G and KP2-033B were outliers in PC8 (activity towards *E. coli* and *M. tuberculosis*) and KP1-017A, KP1-091A, KP1-123C, KP1-131E, KP2-009B and KP2-033E were outliers in PC9 (activity towards *E. faecium*). Several extracts (KP1-123A, KP1-123B, KP1-131A, KP1-131S, KP1-175L and KP2-033D) were not constrained to certain PCs and were found to be outliers in numerous PC plots, while KP1-017E (PC1 vs. PC4), KP1-131DA (PC1 vs. PC4), KP1-069D (PC3 vs. PC5) and KP1-131Q (PC4 vs. PC8) were only outliers in one PC plot.

This analysis suggests that these extracts have the most unique profiles within the library of 140 extracts tested and warrant further analysis. It is known that, when screening natural product libraries, it is common to encounter redundancy in the compounds tested or in the extracts from which there were derived (Caicedo et al. 2016; Covington et al. 2017; Gaudencio and Pereira 2015; Hou et al. 2012; Kurita et al. 2015; Liu et al. 2010) and therefore it is unsurprising to find the majority of extracts in this library to not be identified by PCA as outliers within the dataset. This is due to the fact that many of the

extracts possessed similar bioactivity profiles, showing activity towards *M. tuberculosis* or a combination of activity towards *M. tuberculosis* and *M. smegmatis*.

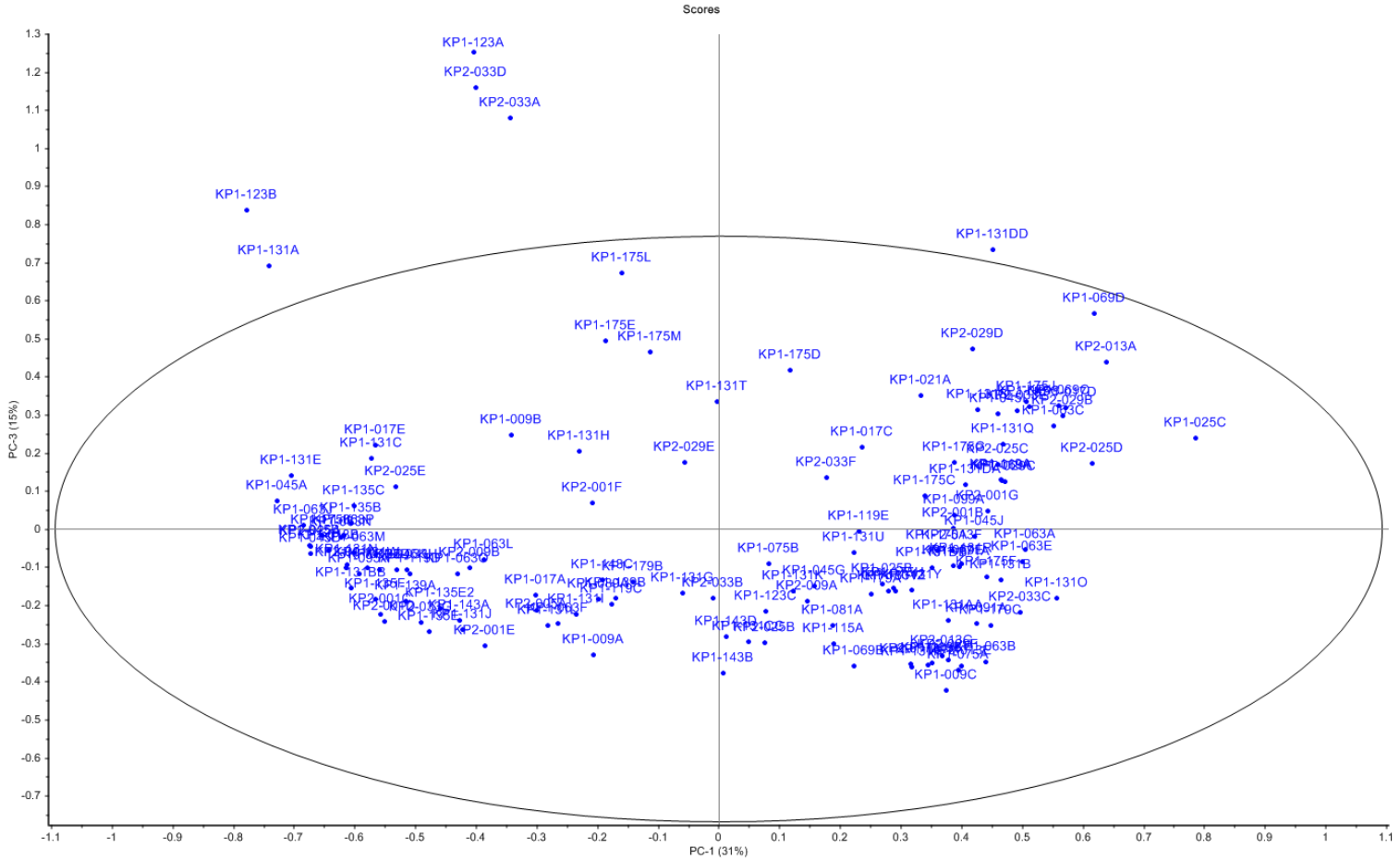


Figure 3.4. Example of output obtained from the principal component analysis (PCA) of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles considered outliers were identified as those found outside of the 95% confidence circle for each PCA plot. All PCA plots produced (PC1 vs. PC2 through PC8 vs. PC9) were investigated for outliers. See appendix 9 for PCA plots.

3.3.4 Cluster analysis of bioactivity profiles derived from fungal endophyte extracts and antimicrobial standards

Hierarchical cluster analysis (Figure 3.5; Euclidean distance measure with average linkage) was used to assess the similarity of the extract bioactivity profiles identified as outliers in the principal component analysis to bioactivity profiles obtained for 17 antimicrobial therapeutics. Although numerous distance measures could have been applied to the data set when using a hierarchical cluster analysis (*e.g.* Correlation, Manhattan, Kendall's Tau, etc.), the Euclidean distance measure allows for the most intuitive and direct comparison of the data set, is the best measure for continuous data (*i.e.* the normalized percentage inhibition data from 0 to 1), and is appropriate for assessing data measured on the same scale (Almeida et al. 2007; Lavine 2006). Using the Euclidean distance measure also eliminated any further normalization of the data that may have skewed the outcome or may have ignored any variability that exists within the data set following the initial normalization of the percentage inhibition data.

In order to determine which extracts possessed bioactivity profiles different than those of the antimicrobial standards, clusters were identified in the dendrogram where bioactivity profiles of extracts and/or antimicrobial standards possessed correlations $\geq$ 0.80. Correlation coefficient values ranging from 0.7 to 0.9 have been used to describe data with very strong correlations and therefore a cut-off point half way between the limits of this range ($\geq$ 0.80) was chosen for forming clusters of extracts or antimicrobials from the hierarchical cluster analysis (Chan 2003; Kozak 2009; Mukaka 2012; Ratner 2009). Through the use of this clustering method, 26 clusters were formed, whereby 13 of the

clusters formed contained the bioactivity profiles of the antimicrobial standards (Figure 3.5).

Several of the antimicrobial standards included in the dataset clustered together (Figure 3.5). Amphotericin B and nystatin showed highly similar bioactivity profiles, a result consistent with their similar mode of action towards ergosterol in fungi (Georgopapadakou and Walsh 1996; Hammond et al. 1974). Kanamycin and streptomycin also showed highly similar bioactivity profiles, as is consistent with their spectra of activity and their targeting of the 30s ribosomal subunit and interruption of protein synthesis in bacteria (Kohanski et al. 2010). The clustering of antimicrobial standards with similar modes of action, as seen in this analysis, gives confidence that the bioactivity profiling technique presented in this work can not only identify antimicrobial standards with similar modes of action, but also supports the concept that bioactivity profiling can identify extracts with natural products possessing modes of action different to those of the antimicrobials tested.

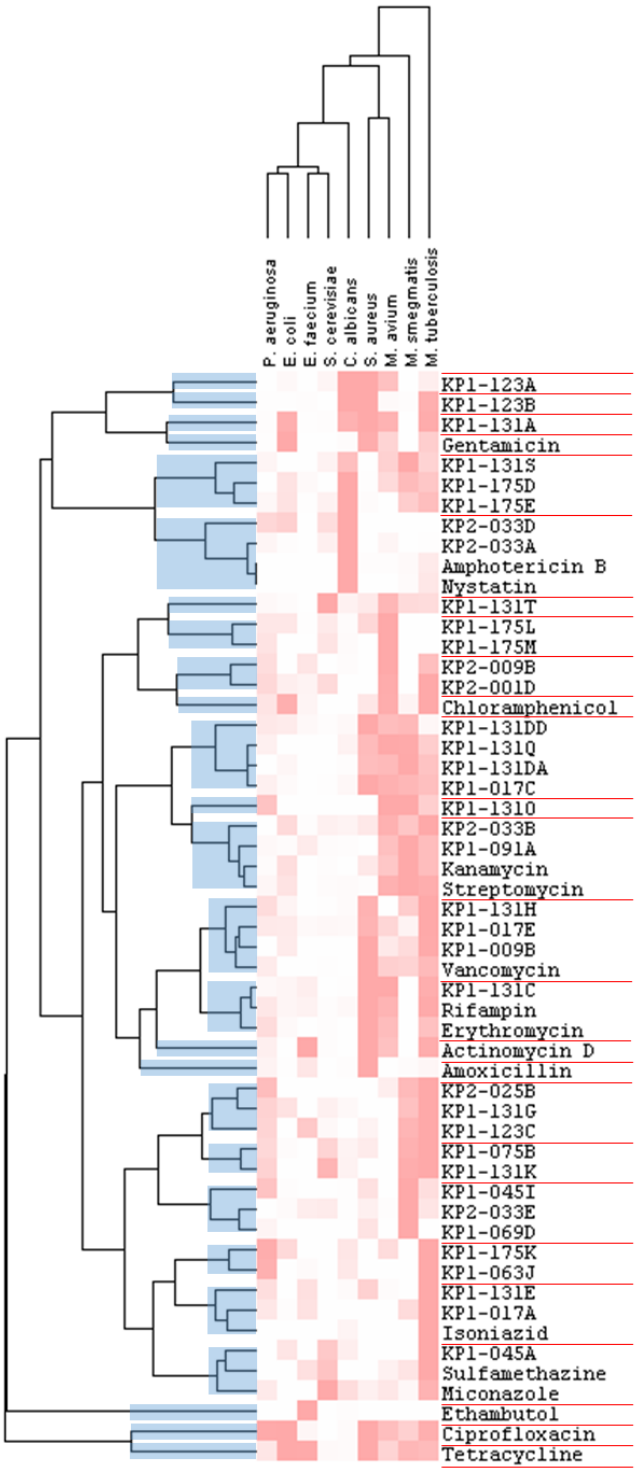


Figure 3.5. Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of antimicrobial standards and extracts identified as outliers using principal component analysis. Twenty-six clusters were formed (identified by red lines separating the labels and blue shading around dendrogram), as the bioactivity profiles of the extracts or antimicrobial standards within the cluster showed correlations of ≥ 0.80.

As shown in figures 3.5 and 3.6, the profiles of 11 extracts clustered with the profiles of the antimicrobial standards used within this analysis. The profiles of KP2-033A and KP2-033D showed similarities to the antifungal drugs amphotericin B and nystatin, though KP2-033D also showed activity against Gram negative bacteria (Figure 3.6). The profiles of KP1-091A and KP2-033B showed similarities to the profiles of the aminoglycosides kanamycin and streptomycin (Figure 3.6), displaying activity towards the three mycobacteria. It was also revealed that KP1-017E, KP1-131H and KP1-009B showed similarities to vancomycin (Figure 3.6) as they primarily showed activity towards *S. aureus* and *M. tuberculosis*. KP1-131C resembled the profile of rifampin as it showed activity towards *S. aureus*, *M. tuberculosis* and *M. avium* (Figure 3.6). KP1-131E and KP1-017A clustered with isoniazid due to their activity towards *M. tuberculosis* (Figure 3.6). KP1-045A and sulfamethazine clustered together as their normalized bioactivity profiles both showed activity towards *S. cerevisiae* and *M. tuberculosis* (Figure 3.6). As the profiles of these extracts showed similarity to the profiles of the antimicrobial standards, their priority for fractionation would rate lower than extracts that did not cluster in the hierarchical cluster analysis with the antimicrobial standards. This is suggestive that these 11 extracts possess antimicrobial natural products with biological targets similar to those of their nearest antimicrobial standard or combinations of antimicrobial natural products that when screened together in an extract possess activity similar to that of the antimicrobial standards.

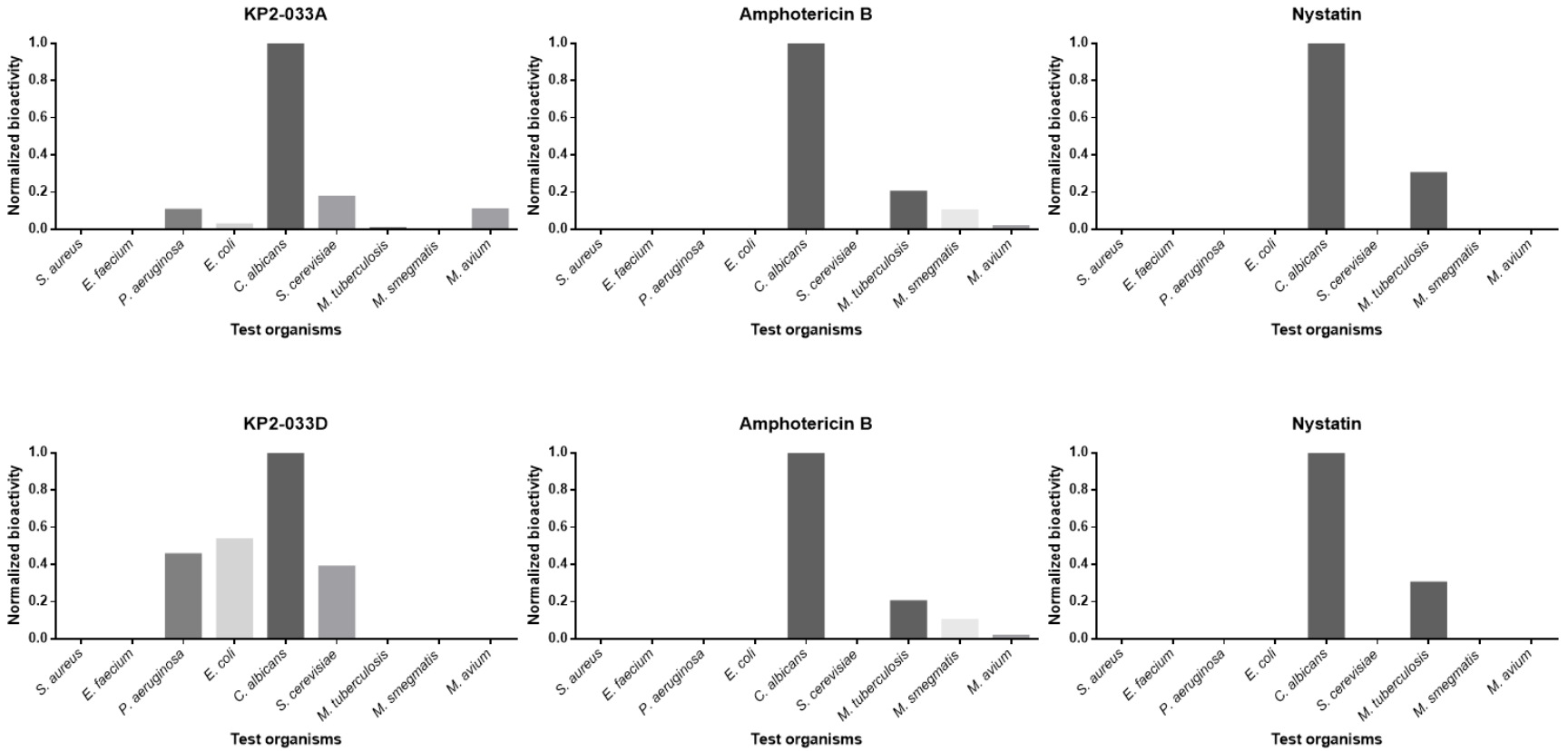


Figure 3.6. Profiles of extracts considered similar to the profiles of the nearest antimicrobial standard.

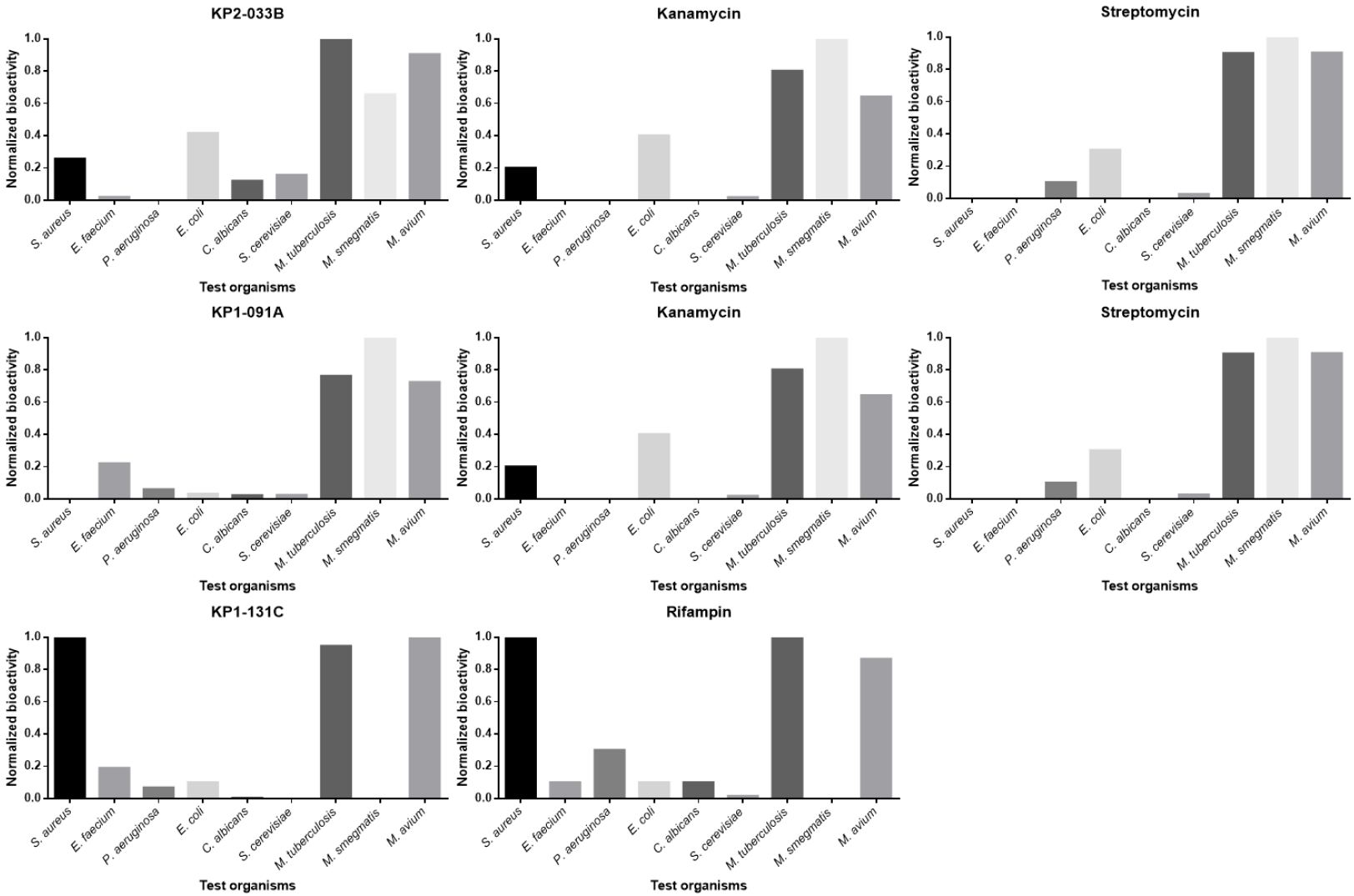


Figure 3.6. Profiles of extracts considered similar to the profiles of the nearest antimicrobial standard, continued.

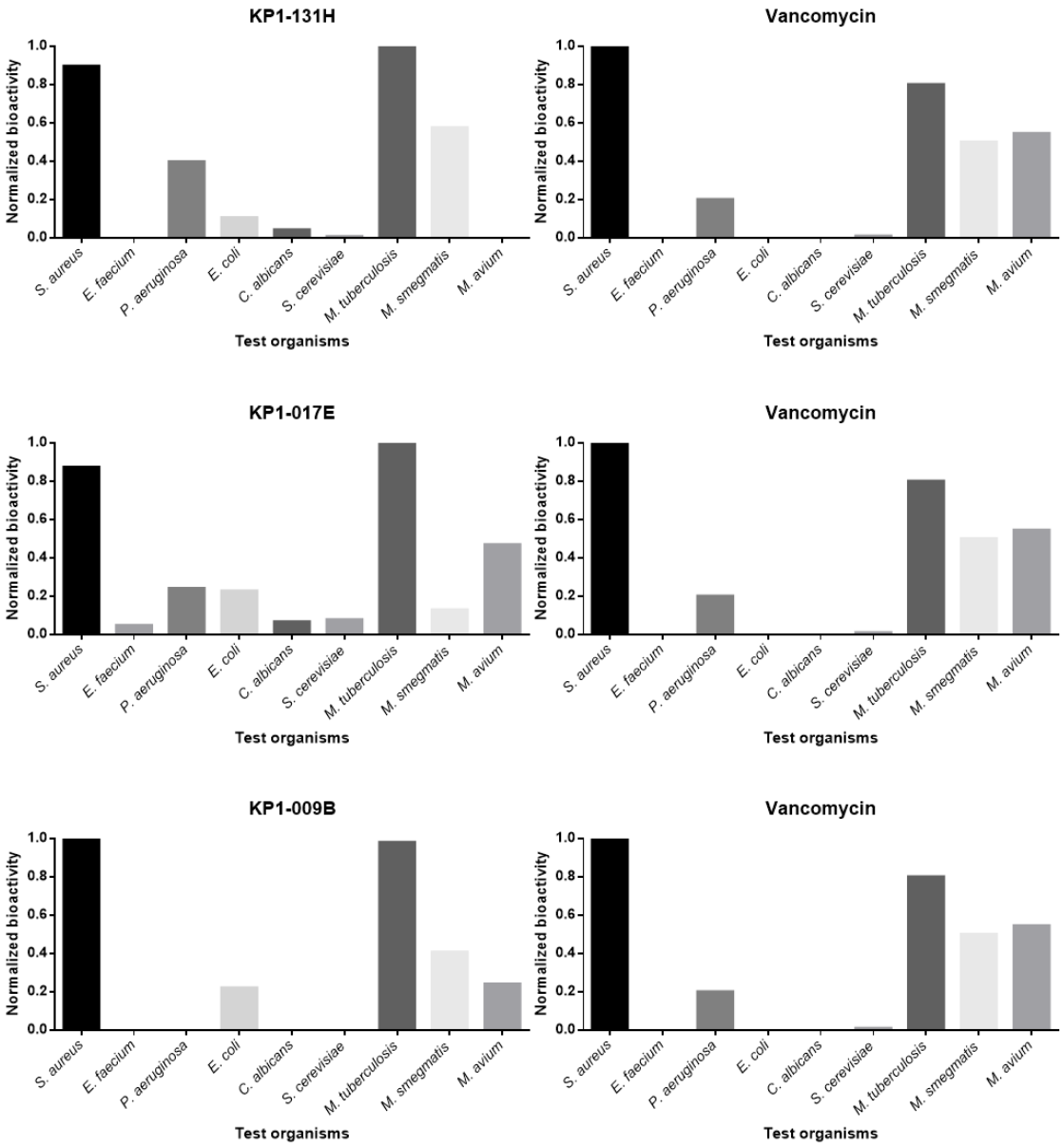


Figure 3.6. Profiles of extracts considered similar to the profiles of the nearest antimicrobial standard, continued.

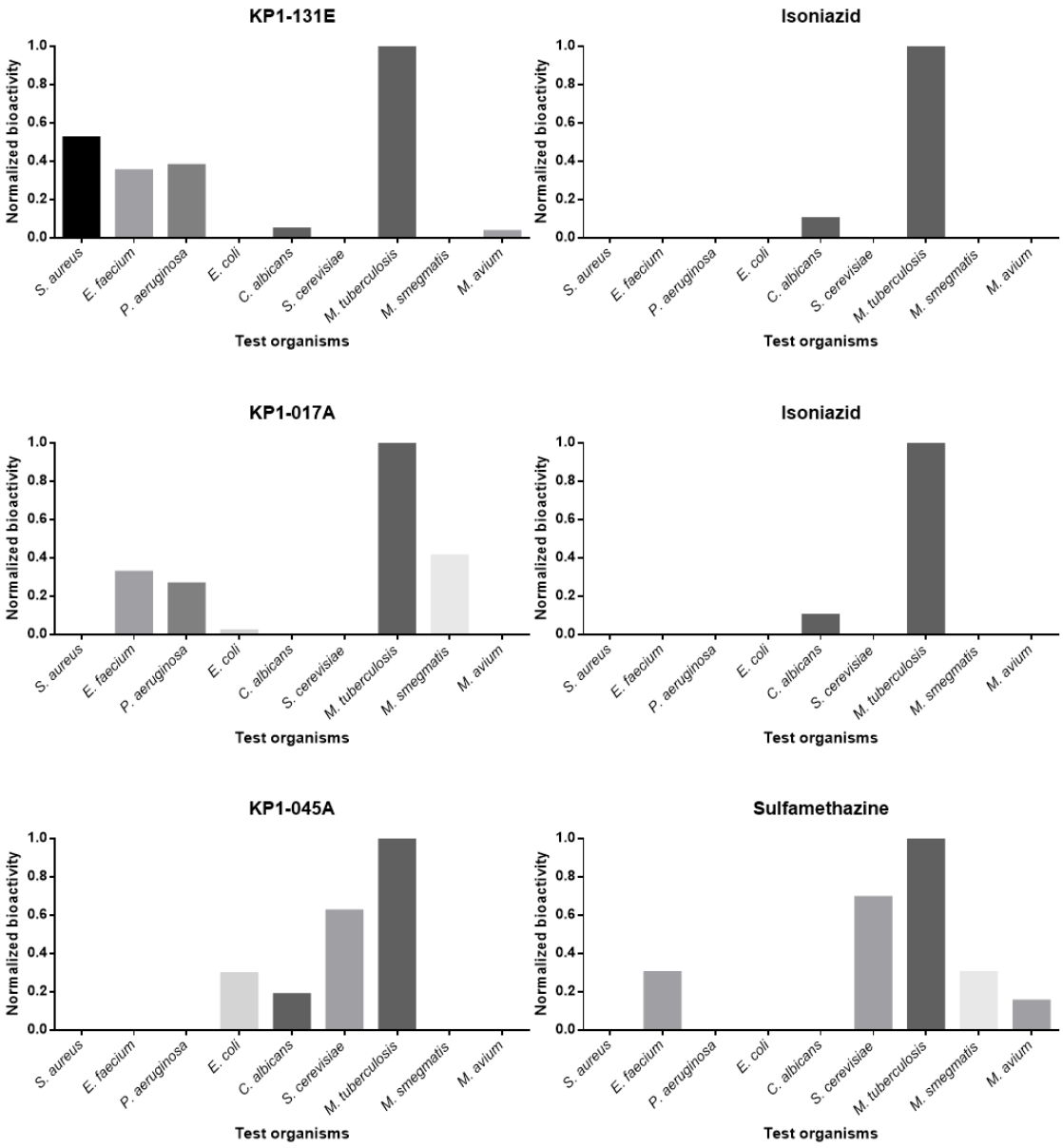


Figure 3.6. Profiles of extracts considered similar to the profiles of the nearest antimicrobial standard, continued.

Using this clustering technique, it was revealed that the profiles of 26 extracts did not cluster with the profiles of the antimicrobial standards (Figure 3.5 for cluster analysis & Figure 3.7 for bioactivity profiles of extracts). The profiles of many of these extracts clustered together, namely KP1-131S, KP1-175D and KP1-175E; KP1-175L and KP1-175M; KP2-001D and KP2-009B; KP1-131DD, KP1-131Q, KP1-131DA and KP1-017C; KP2-025B, KP1-131G, and KP1-123C; KP1-075B, and KP1-131K; KP1-045I, KP2-033E and KP1-069D; KP1-175K and KP1-063J (Figure 3.7), suggesting that these extracts possess natural products with similar antimicrobial targets.

The unique combinations of high broad-spectrum activity exhibited in the profiles of many of the extracts influenced the fact that they did not cluster with the antimicrobial standards. It should be noted though, that broad-spectrum activity observed from an extract may be caused by individual compounds possessing the broad spectrum activity observed, or, the presence of multiple bioactive constituents with differing antimicrobial activity. In the case of KP1-131A, the extract possessed Gram positive and Gram negative antibacterial activity, antifungal activity and antimycobacterial activity, a feature not present in any of the antimicrobial standards assessed here. The profiles of KP1-131DD, KP1-131Q, KP1-131DA, and KP1-017C showed activity towards at least two *Mycobacterium* spp., similar to that of kanamycin and streptomycin, but also showed high activity towards *S. aureus*, a feature not observed for these antimicrobials. KP1-123A and KP1-123B showed high activity towards *S. aureus* and *C. albicans*, features not observed in the antimicrobials. KP1-131S, KP1-175D, KP1-175E primarily showed activity towards *C. albicans* and *Mycobacterium* spp. and KP1-131T primarily possessed activity towards *S. cerevisiae* and *M. avium*, whereby combinations of high antifungal and

antimycobacterial activities were only observed in miconazole and sulfamethazine, however towards *S. cerevisiae* and *M. tuberculosis*. KP1-175L and KP1-175M primarily possessed activity towards *M. avium* and no antimicrobial standard screened showed just activity towards this pathogen. KP2-001D and KP2-009B showed activity towards *M. tuberculosis* and *M. avium* with low or moderate *E. faecium* and *P. aeruginosa* activity, KP2-025B and KP1-131G possessed *M. tuberculosis* and *M. smegmatis* activity with high Gram negative activity and KP1-123C revealed activity towards *E. faecium*, *P. aeruginosa*, *M. tuberculosis* and *M. smegmatis*.

While combinations of antimycobacterial activity and Gram positive and Gram negative antibacterial activity were observed in the profiles for many of the antimicrobial standards included in the bioactivity profiling, the combinations for the profiles of KP2-001D, KP2-009B, KP2-025B, KP1-131G and KP1-123C were not seen. KP1-075B and KP1-131K primarily had activity against *P. aeruginosa*, *S. cerevisiae*, *M. tuberculosis* and *M. smegmatis*, while KP1-063J and KP1-175K showed high activity towards *P. aeruginosa* and *M. tuberculosis* in the normalized bioactivity profiles (Figure 3.7). The profiles of these extracts likely did not cluster with those of the antimicrobials tested as few of the antimicrobials tested possessed activity towards *S. cerevisiae* or only showed high activity towards *P. aeruginosa* and *M. tuberculosis*. KP1-045I, KP2-033E, KP1-069D primarily showed high *M. smegmatis* activity, a feature of the bioactivity profile not seen in the antimicrobial standards.

There were 26 extracts (Figure 3.7) that did not cluster with the bioactivity profiles of the antimicrobials, and these are the extracts of top priority for bioassay-guided

fractionation studies to determine the biologically active constituents that are responsible for the bioactivity observed in the profile.

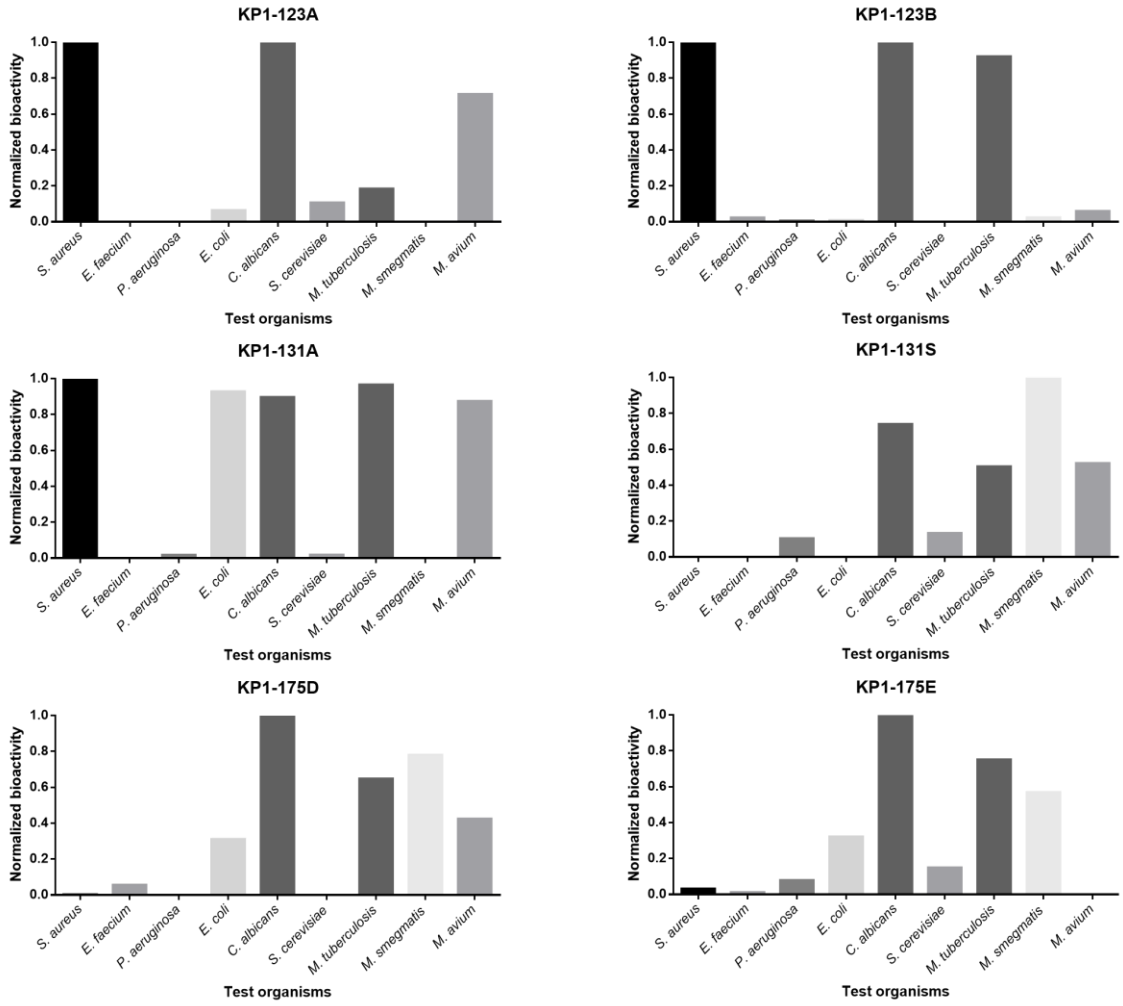


Figure 3.7. Bioactivity profiles of extracts not found to cluster with the bioactivity profiles of antimicrobial standards covering a range of cell targets and modes of action.

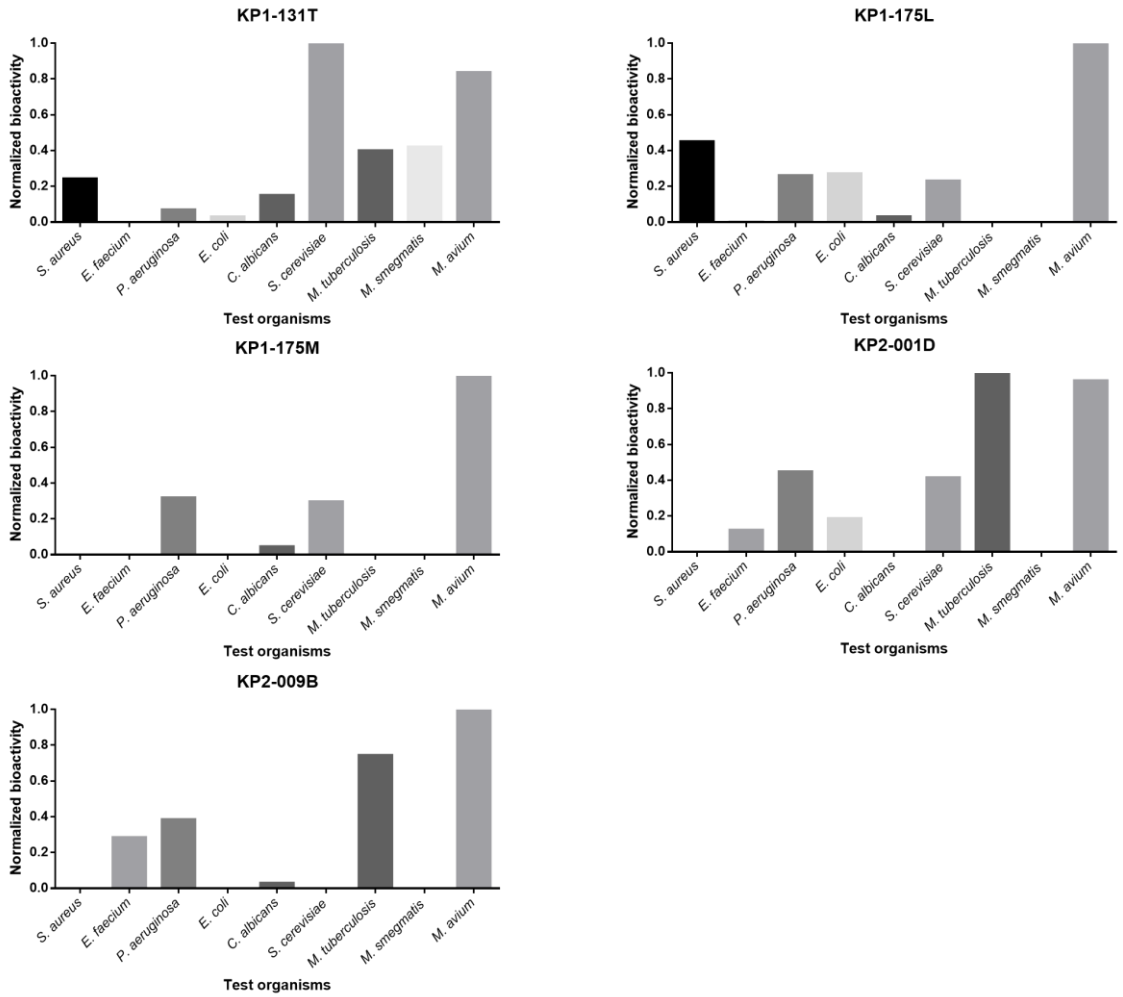


Figure 3.7. Bioactivity profiles of extracts not found to cluster with the bioactivity profiles of antimicrobial standards covering a range of cell targets and modes of action, continued.

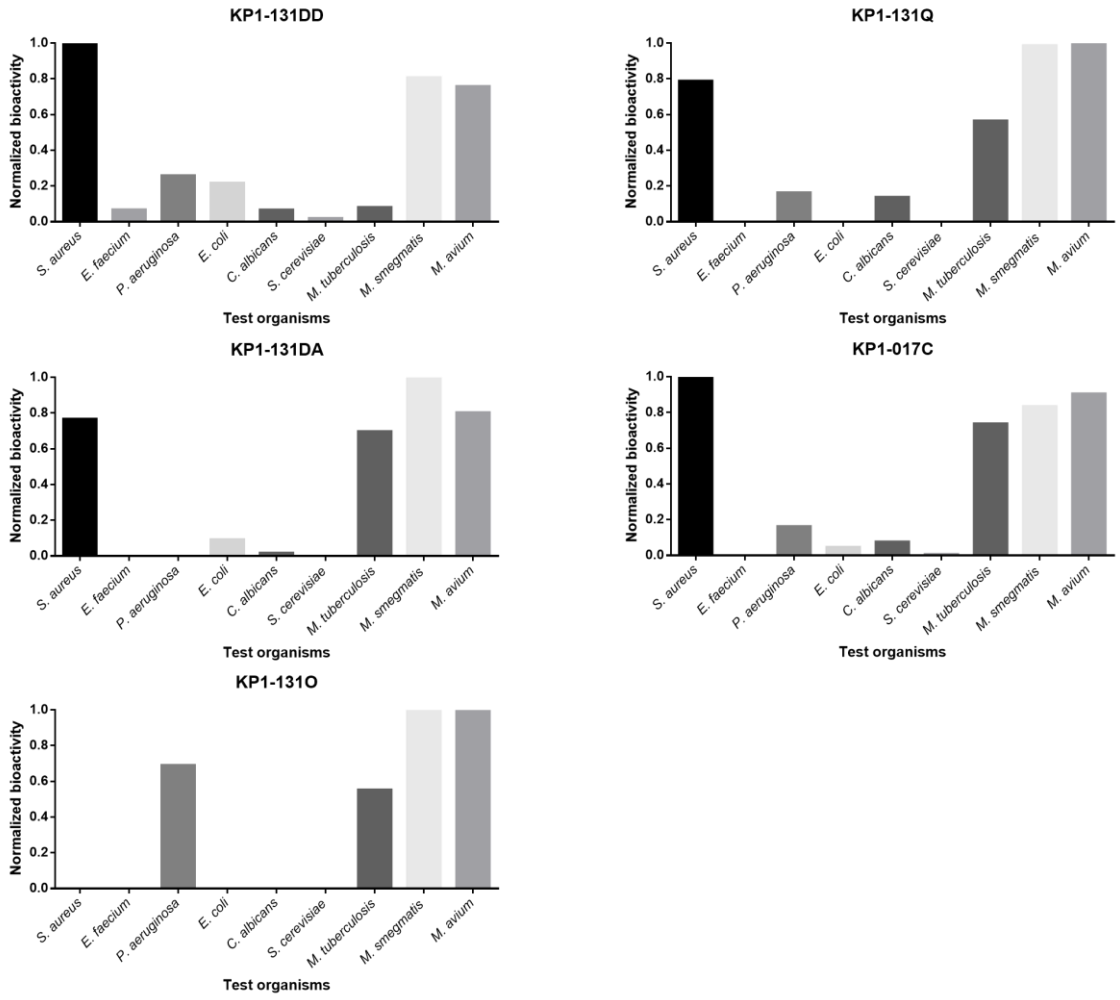


Figure 3.7. Bioactivity profiles of extracts not found to cluster with the bioactivity profiles of antimicrobial standards covering a range of cell targets and modes of action, continued.

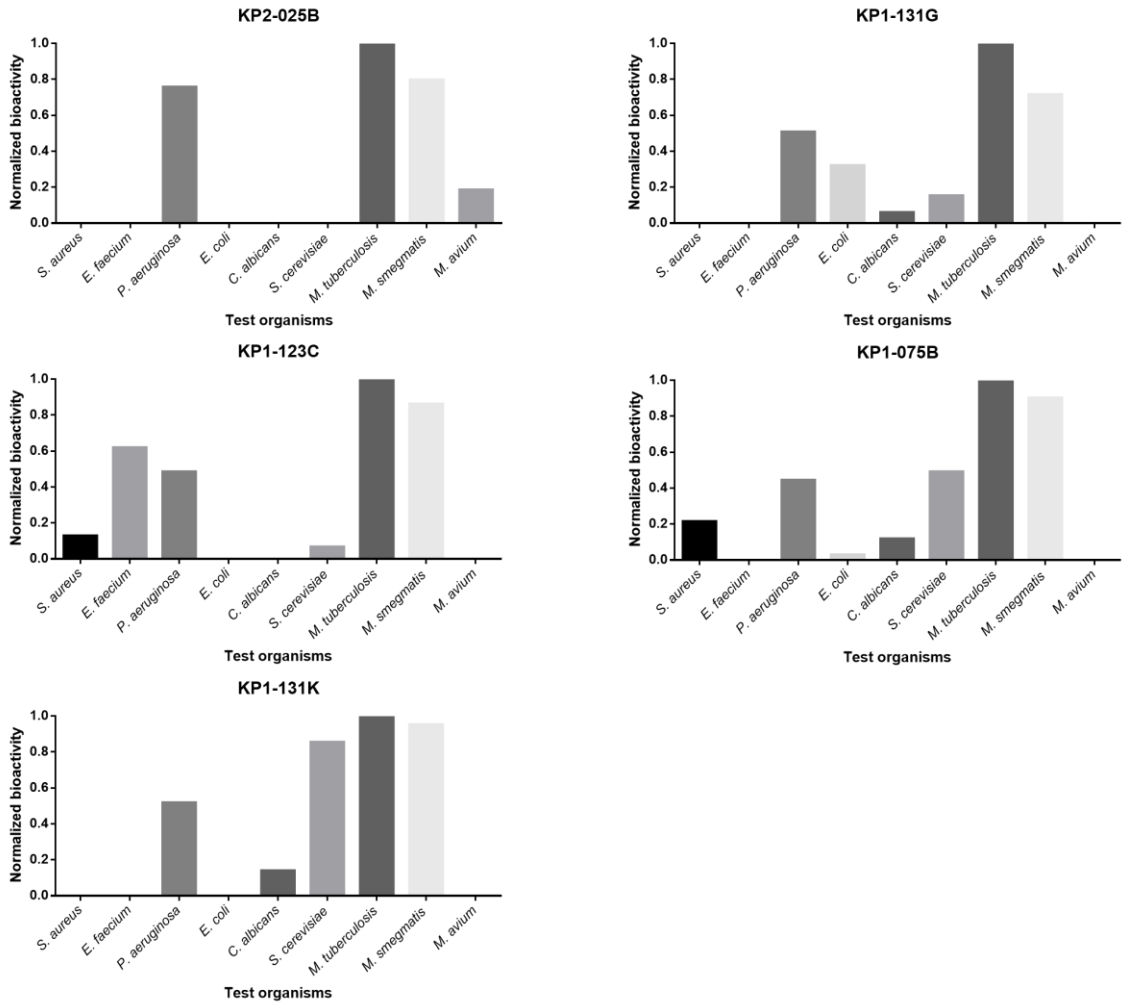


Figure 3.7. Bioactivity profiles of extracts not found to cluster with the bioactivity profiles of antimicrobial standards covering a range of cell targets and modes of action, continued.

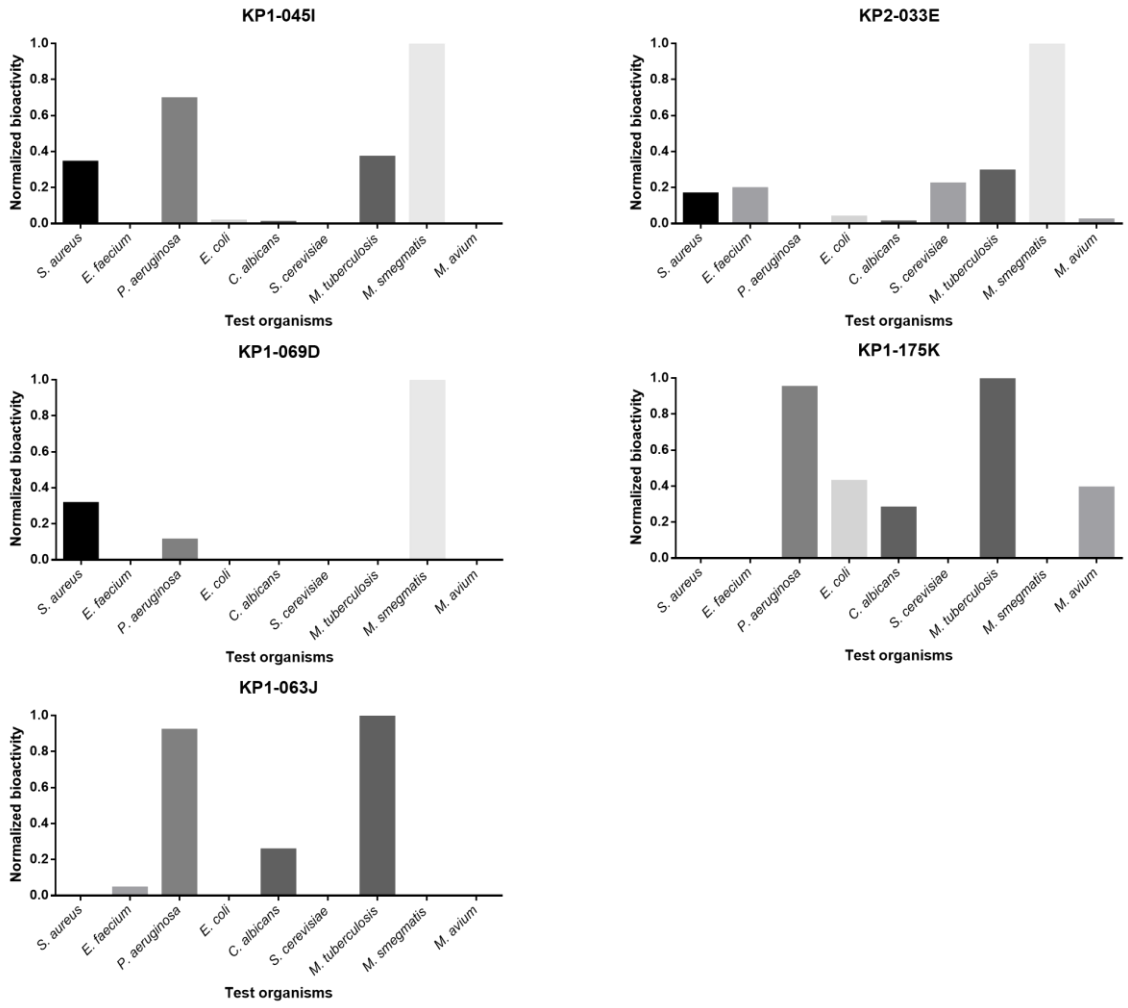


Figure 3.7. Bioactivity profiles of extracts not found to cluster with the bioactivity profiles of antimicrobial standards covering a range of cell targets and modes of action, continued.

3.4 Conclusions and future considerations

One hundred and forty fungal extracts were screened against nine pathogens for their antimicrobial activity. In order to prioritise the extracts for further investigation into their bioactive natural products, a bioactivity profiling technique was applied following the normalization of the percentage inhibition data obtained by screening the extracts at 100 µg/mL. The use of principal component analysis facilitated the prioritisation of the 37 most unique bioactivity profiles within the library of extracts for further analysis against a panel of 17 antimicrobial standards. Hierarchical cluster analysis revealed that the bioactivity profiles of 26 extracts did not cluster with the bioactivity profiles of the 17 antimicrobial standards analysed. These 26 extracts warrant further investigation into their biologically active chemical constituents responsible for the bioactivity seen in their bioactivity profiles. The bioactivity profiling approach applied here was successful in identifying extracts for fractionation, and provides a basis for further development. This technique can be applied to extracts from any biological source and can be used for other discovery platforms focussed on biological targets other than pathogenic microorganisms, such as cancerous cell lines. The use of bioactivity profiling can also be applied to specific, narrow spectrum antimicrobial targets, such as various mycobacteria, Gram positive bacteria, Gram negative bacteria or pathogenic fungi to find new antimicrobial drugs that specifically target those organisms.

Future work for this technique can also include the prefractionation or clean-up of extracts, a step which may eliminate any confounding clustering in the analyses that may be caused by nuisance compounds, such as fatty acids (known for their antimycobacterial activity (Kanetsuna 1985). This could prevent a selection bias in the PCA for extracts that

do not exhibit significant antimycobacterial activity by eliminating unwanted portions of the extract that are known inhibitors of certain microorganisms.

The 17 antimicrobial standards included in this work broadly covered the key classes of antimicrobial drugs; however, increasing the number of antimicrobial standards used within the bioactivity profiling analysis would provide a greater basis for comparison with the extracts. As the different antimicrobials within each of the classes can show different spectra of activity, as well as different potency towards various pathogens, the inclusion of multiple examples of each drug class could provide a more robust analysis when comparing the bioactivity profiles of the extracts to those of the standards.

Changing the concentration of the extracts and/or the standards may result in a different outcome for the clustering analysis. Originally, the method employed by the Linington research group used minimum inhibitory concentrations (MICs) calculated from a dose response rather than percentage inhibitions at a single concentration. While the method used in this work was found to be effective, the use of a single concentration at 100 µg/mL for the extracts and 1 µg/mL for the antimicrobial standards does not take into account samples showing 100% inhibitory activity at the concentration tested. With 100% inhibitory activity observed when using a single test concentration, it is not possible to determine the true shape of the bioactivity profile, as screening extracts or standards above their MIC may artificially inflate the bioactivity profile and skew the PCA and cluster analyses. The aim of this technique, though, was to create a simplified bioactivity profiling strategy and therefore, the effort, time, and expense of rescreening extracts at additional concentrations to eliminate 100% inhibitory activity (in this case 9 extracts to be retested against 9 microorganisms) may not be beneficial to the overall goal of

efficiently prioritising extracts for fractionation studies, considering that 26 extracts from the library were prioritised for further investigation.

In summary, the use of bioactivity profiling successfully prioritised the extracts from endophytic fungi of marine macroalgae of the Bay of Fundy, Canada. While further work can be performed to refine the technique, 26 extracts have been identified for further investigation to identify the natural products responsible for the biological activity.

Chapter 4: Isolation of antimicrobial natural products from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada

4.1 Introduction

Endophytic fungi from marine macroalgae are becoming recognized as an important source of bioactive natural products despite being underrepresented in the literature (Flewelling et al. 2015; Ji and Wang 2016; Sarasan et al. 2017; Zhang et al. 2016c). Recent reviews on the bioactivity of marine macroalgal endophytes highlight the vast chemical diversity of natural products isolated, and the breadth of biological activity they possess (Flewelling et al. 2015; Ji and Wang 2016; Sarasan et al. 2017; Zhang et al. 2016c). Approximately 150 new natural products have been reported from endophytes of marine macroalgae with nearly half having been reported with anticancer, antimicrobial and immunosuppressant activities, to name but a few (Flewelling et al. 2015). As the natural products of endophytes from marine macroalgae are underrepresented in the literature, they represent an untapped resource in the search for new antimicrobial molecules.

One hundred and forty extracts were screened using a bioactivity profiling technique, with twenty-six extracts identified through hierarchical cluster analysis as warranting further investigation as they did not cluster with the bioactivity profiles of known antimicrobial drugs. The objective of the experiments presented in this chapter was therefore to isolate and identify the natural products responsible for the antimicrobial activity observed in the bioactivity profiles for four endophytic fungi from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

4.2 Experimental

4.2.1 General experimental

All solvents for extraction and isolation of natural products were ACS certified or HPLC grade. NMR spectra were recorded on an Agilent 400-MR DD2 instrument at 400 MHz for ^{1}H and 100 MHz for ^{13}C using standard 1D and 2D pulse programs for samples dissolved in $CDCl_3$, CD_3OD, and $(CD_3)_2SO$ (Sigma Aldrich, Oakville, Ontario), where all NMR spectra were referenced to the residual protonated solvent signal [δ_H 7.26 ppm, δ_C 77.16 for $CDCl_3$; δ_H 3.31 ppm, δ_C 49.00 ppm for CD_3OD; δ_H 2.50 ppm, δ_C 39.52 ppm for $(CD_3)_2SO$]. High resolution electrospray ionization mass spectrometry (HRESIMS) was performed on a Thermo LTQ Exactive Orbitrap LC-MS and on a Thermo LTQ Orbitrap Velos MS. Infrared spectra were recorded on a Perkin Elmer Spectrum Two FT-IR with samples as thin films on NaCl disks. Optical rotations were determined using an Optical Activity Ltd. AA-10 polarimeter. Flash column chromatography was performed using a Biotage Flash + chromatography system and Silicycle silica gel (25 g, 230-400 mesh, 40-63 µm) and Silicycle C18 (17%, 25 g, 230-400 mesh, 40-63 µm) cartridges for normal phase and reversed phase chromatography, respectively. Sephadex® LH-20 (25-100 µm bead size; MeOH used to swell stationary phase) was used for size exclusion chromatography and semi-preparative normal and reversed phase high performance liquid chromatography (NP-HPLC & RP-HPLC, respectively) was performed using a Waters 600 pump, a Phenomenex Luna silica column (NP-HPLC; 10 µm, 100 Å, 250 × 10 mm), a Phenomenex C18 Luna silica column (RP-HPLC; 10 µm, 100 Å, 250 × 10 mm) and a Waters 2485 dual wavelength detector (190 nm & 254 nm) at a flow rate of 4 mL/min.

Normal phase TLC (thin layer chromatography) was performed on SiliaPlate aluminum backed TLC plates (200 μm thickness, Silicycle, Quebec City, Quebec), with reversed phase TLC performed on SiliaPlate aluminum backed C18 TLC plates (200 μm thickness, Silicycle, Quebec City, Quebec). TLC plates were visualized under UV (254 nm) and by dipping plates in a solution of cerium molybdate followed by charring. Experimental conditions for antimicrobial bioassays were the same as reported in Chapter 3.

4.2.2 *Penicillium* sp. IX (KP1-123A)

4.2.2.1 Fermentation and extraction

Penicillium sp. IX (KP1-123A) was fermented with shaking (150 rpm) in 2.0% Bacto™ malt extract broth (4 L: 40 × 100 mL batches in 250 mL Erlenmeyer flasks covered with aluminum foil) at ambient room temperature and ambient light for 14 days. After fermentation, the culture was sonicated for 5 min and the fungal material was separated from the culture broth by filtration over cotton wool. The broth was extracted with EtOAc (3 × 300 mL EtOAc per litre of broth) and concentrated *in vacuo* to give an extract (439 mg).

4.2.2.2 Bioassay guided fractionation

A portion of the crude extract (430 mg) was dissolved in 9:1 MeOH/H_2O (50 mL) and extracted with hexanes (3 × 25 mL) before being diluted with H_2O (25 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The aqueous fraction was then concentrated *in vacuo*, dissolved in H_2O (50 mL) and extracted with EtOAc (3 × 25 mL) and *n*-BuOH (3 × 25

mL). The resulting five fractions were concentrated *in vacuo* and only the CH_2Cl_2 fraction (248 mg) showed significant antimicrobial activity. A portion of the CH_2Cl_2 fraction (237 mg) was further separated by size exclusion chromatography over Sephadex® LH-20 in MeOH to give six fractions. These fractions were determined by combining the contents of the test tubes according to the presence or absence of bioactivity using a bioautographic overlay assay with methicillin resistant *Staphylococcus aureus* and the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Fraction 3 (115 mg) showed antimicrobial activity in the bioautographic overlay assay and was further purified by reverse phase HPLC (30 mg, 45:55 CH_3CN/H_2O buffered with 0.1% TFA) to give **1** (penicillic acid, 3 mg) and **2** (methylenolactocin, 15 mg).

4.2.2.3 Biological assays

Bioautographic assays were performed using NP-TLC plates spotted with test fractions. Molten BBL™ Mueller Hinton II cation adjusted agar (25 mL of approximately 45 °C agar) was inoculated (300 µL) with an 18 h bacterial culture and the resulting mixture was poured over the NP-TLC plates in sterile square Petri plates (90 mm × 15 mm; Fisher Scientific Ltd., Ottawa, Ontario). After the agar had solidified, Petri plates were incubated for 24 h at 37 °C. After incubation, the agar surface was sprayed with a thin layer of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), whereby bioactive fractions were visualized as clear spots against a purple background.

The antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and the antibacterial activity against *S. aureus* (ATCC 29213) was evaluated as previously described in Chapter 3, with MIC and IC_{50} determination

performed as described previously on two-fold serial dilutions in triplicate (Patterson et al. 2015). Compounds were tested at 10 concentrations (*S. aureus*, 400 – 0.78 µg/mL; *M. tuberculosis*, 100 – 0.20 µg/mL) obtained from one dilution series. Positive controls for the antimycobacterial and antibacterial assays consisted of a triplicate concentration of rifampin for *M. tuberculosis* (0.012 µM), and erythromycin for *S. aureus* (0.85 µM). General cytotoxicity against the human embryonic kidney 293 cell line was assessed according to the work performed by Carpenter et al. (2012) on two-fold serial dilutions in triplicate (250 – 0.49 µg/mL). The bioactivity of the compounds was categorized into three levels based on the IC_{50}: strong activity (< 1 µM), moderate activity (1 – 100 µM) and weak activity (> 100 µM). Therapeutic indices were calculated as a ratio of the general cytotoxicity IC_{50} to the antimicrobial IC_{50}. Bioactivity profiles were created as described in Chapter 3.

Penicillic acid (**1**): white solid; $[\alpha]^{20}_{D}$ 0 (*c* 0.22, MeOH); IR (thin film) ν_{max} 3285, 2942, 1747, 1639, 1421, 1347, 1213, 1160, 913 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ_H 5.41 (1H, s, H-3), 5.29 (1H, s, H-8b), 5.11 (1H, s, H-8a), 3.85 (3H, s, H-9), 1.65 (3H, s, H-7); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ_C 179.4 (C, C-4), 170.0 (C, C-2), 140.1 (C, C-6), 115.2 (CH_2, C-8), 102.4 (C, C-5), 89.3 (CH, C-3), 59.8 (CH_3, C-9), 17.1 (CH_3, C-7); HRESIMS m/z [M + H^+] 171.0653 (calcd for $C_8H_{11}O_4$ 171.0652).

Methylenolactocin (**2**): white solid; $[\alpha]^{20}_{D}$ -12 (*c* 0.83, MeOH); IR (thin film) ν_{max} 3119, 2962, 2933, 2862, 1769, 1716, 1400, 1211, 1155, 952 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 6.47 (1H, d, *J* = 1.8 Hz, H-10b), 6.03 (1H, d, *J* = 1.8 Hz, H-10a), 4.81 (1H, m, H-4), 3.64 (1H, brs, H-3), 1.74 (2H, m, H-5), 1.32 (6H, m, H-6, H-7, H-8), 0.89 (3H, t, *J* = 5.8 Hz, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 173.7 (C, C-11), 168.3 (C, C-1), 132.5 (C, C-2), 126.1 (CH_2, C-10), 78.9 (CH, C-4), 49.5 (CH, C-3), 35.8 (CH_2, C-5), 31.5 (CH_2, C-8), 24.6 (CH_2, C-7), 22.6 (CH_2, C-6), 14.1 (CH_3, C-9); HRESIMS m/z [M + H^+] 213.1123 (calcd for $C_{11}H_{17}O_4$ 213.1121).

4.2.3 *Aspergillus fumigatus* III (KP1-131Q)

4.2.3.1 Fermentation and extraction

Aspergillus fumigatus III (KP1-131Q) was fermented with shaking (150 rpm) in 2.0% Bacto™ malt extract broth (9.1 L: 91 × 100 mL batches in 250mL Erlenmeyer flasks covered with aluminum foil) at ambient room temperature and ambient light for 14 days. After fermentation, the culture was sonicated for 5 min and the fungal material was separated from the culture broth by filtration over cotton wool. The broth was extracted with EtOAc (3 × 300 mL EtOAc per litre of broth) and concentrated *in vacuo* to give an extract (1.24 g).

4.2.3.2 Bioassay guided fractionation

A portion of the crude extract (1.17 g) was dissolved in 9:1 MeOH/H_2O (200 mL) and extracted with hexanes (3 × 100 mL) before being diluted with H_2O (200 mL) and

extracted with CH_2Cl_2 (3 × 100 mL). The aqueous fraction was then concentrated *in vacuo*, dissolved in H_2O (200 mL) and extracted with EtOAc (3 × 100 mL) and *n*-BuOH (3 × 100 mL). The resulting five fractions were concentrated *in vacuo* and only the CH_2Cl_2 fraction (701 mg) showed significant antimicrobial activity. A portion of the CH_2Cl_2 fraction (633 mg) was further separated by size exclusion chromatography over Sephadex® LH-20 in MeOH to give nine fractions. These fractions were determined by combining the contents of the test tubes according to the presence or absence of bioactivity using a bioautographic overlay assay with *Staphylococcus aureus* and the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Fraction 3 (213 mg) showed antimicrobial activity in the bioautographic overlay assay and was further fractionated by reversed phase flash column chromatography (column eluted with 100% H_2O to 100% CH_3CN in 10% increments, 1:1 CH_3CN/EtOAc, 100% EtOAc, and 100% MeOH). A portion of fraction 7 (12 mg) was further purified by reversed phase HPLC (1:1 CH_3CN/H_2O) to give **3** (6 mg).

The fifth size exclusion fraction (240 mg) also showed antimicrobial activity in the bioautographic overlay assay and was further purified by reversed phase HPLC (15 mg; 20:80 CH_3CN/H_2O) to give **4** (8 mg).

4.2.3.3 Biological assays

Bioautographic assays were performed using NP-TLC plates spotted with test fractions. Molten BBL™ Mueller Hinton II cation adjusted agar (25 mL of approximately 45 °C agar) was inoculated (300 µL) with an 18 h bacterial culture and the resulting mixture was poured over the NP-TLC plates in sterile square Petri plates (90 mm × 15

mm; Fisher Scientific Ltd., Ottawa, Ontario). After the agar had solidified, Petri plates were incubated for 24 h at 37 °C. After incubation, the agar surface was sprayed with a thin layer of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), whereby bioactive fractions were visualized as clear spots against a purple background.

The antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177), the antibacterial activity against *S. aureus* (ATCC 29213) and the antifungal activity against *Candida albicans* (ATCC 14053) was evaluated as previously described in Chapter 3, with MIC and IC_{50} determination performed as described previously on two-fold serial dilutions in triplicate (Patterson et al. 2015). Fumagillin (**3**) was tested at a minimum of 12 and a maximum of 14 concentrations (*S. aureus,* 400 – 0.20 and 300 – 150 µg/mL; *M. tuberculosis*, 400 – 0.20 µg/mL). Fumigatin oxide (**4**) was tested at a minimum of 12 and a maximum of 14 concentrations (*C. albicans*, 400 – 0.20 and 300 – 150 µg/mL; *S. aureus,* 400 – 0.40 and 300 – 75 µg/mL; *M. tuberculosis*, 200 – 0.10 µg/mL). Positive controls for the antimycobacterial, antibacterial and antifungal assays consisted of a triplicate concentration of rifampin for *M. tuberculosis* (0.012 µM), erythromycin for *S. aureus* (0.85 µM), and nystatin for *C. albicans* (2.7 µM). General cytotoxicity against the human embryonic kidney 293 cell line was assessed according to the work performed by Carpenter et al. (2012) on two-fold serial dilutions in triplicate (250 – 0.49 µg/mL). The bioactivity of the compounds was categorized into three levels based on the IC_{50}: strong activity (< 1 µM), moderate activity (1 – 100 µM) and weak activity (> 100 µM). Therapeutic indices were calculated as a ratio of the general cytotoxicity IC_{50} to the antimicrobial IC_{50}. Bioactivity profiles were created as described in Chapter 3.

Fumagillin (**3**): yellow oil; $[\alpha]^{22}_{D}$ -34 (*c* 0.58, MeOH); IR (thin film) ν_{max} 3418, 2928, 1708, 1624, 1451, 1387, 1243, 1133, 1011, 757 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.36 (1H, m, H-8′), 7.36 (1H, m, H-3′), 6.78 (1H, m, H-6′), 6.78 (1H, m, H-5′), 6.62 (1H, m, H-7′), 6.62 (1H, m, H-4′), 6.05 (1H, d, *J* = 15.2 Hz, H-9′), 5.98 (1H, d, *J* = 15.3 Hz, H-2′), 5.70 (1H, m, H-5), 5.25 (1H, brs, H-11), 3.74 (1H, dd, *J* = 11.2, 2.7 Hz, H-6), 3.41 (3H, s, H-16), 2.99 (1H, d, *J* = 4.2 Hz, H-1b), 2.69 (1H, m, H-9), 2.58 (1H, d, *J* = 4.2 Hz, H-1a), 2.22 (2H, m, H-10), 2.13 (1H, m, H-3a), 1.98 (1H, d, *J* = 11.2 Hz, H-7), 1.91 (2H, m, H-4), 1.76 (3H, s, H-13), 1.68 (3H, s, H-14), 1.21 (3H, s, H-15), 1.09 (1H, m, H-3b); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 170.2 (C, C-1′), 167.7 (C, C-10′), 145.5 (CH, C-8′), 145.4 (CH, C-3′), 141.0 (C, C-6′), 140.5 (CH, C-5′), 135.9 (CH, C-7′), 135.1 (C, C-12), 134.8 (CH, C-4′), 124.2 (CH, C-2′), 123.6 (CH, C-9′), 119.8 (CH, C-11), 80.8 (CH, C-6), 68.1 (CH, C-5), 62.4 (CH, C-9), 60.6 (C, C-2), 60.5 (C, C-8), 56.9 (CH_3, C-16), 51.7 (CH_2, C-1), 49.8 (CH, C-7), 30.3 (CH_2, C-3), 28.3 (CH_2, C-10), 26.6 (CH_2, C-4), 25.9 (CH_3, C-13), 18.1 (CH_3, C-14), 14.2 (CH_3, C-15); HRESIMS m/z 481.2206 [M + Na^+] (calcd for $C_{26}H_{34}O_7Na$, 481.2197).

Fumigatin oxide (**4**): yellow oil; $[\alpha]^{23}_{D}$ +24 (*c* 0.82, 95% EtOH); IR (thin film) ν_{max} 3351, 2995, 2944, 2851, 1675, 1628, 1335, 1252, 1066, 977 cm^{-1}; ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ_H 3.82 (1H, s, H-7), 3.74 (3H, s, H-6), 1.49 (3H, s, H-8); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ_C 189.4 (C, C-4), 188.0 (C, C-1), 146.5 (C, C-2), 140.0 (C, C-3), 59.5 (CH_3, C-6), 58.6 (CH, C-7), 58.2 (C, C-5), 13.9 (CH_3, C-8); HRESIMS m/z 207.0262 [M + Na^+] (calcd for $C_8H_8O_5Na$, 207.0264).

4.2.4 Sterile grey filamentous II (KP1-131DD)

4.2.4.1 Fermentation and extraction

Sterile grey filamentous II (KP1-131DD) was fermented with shaking (150 rpm) in 2% Bacto™ malt extract broth (24.9 L: 249 × 100 mL batches in 250 mL Erlenmeyer flasks covered with aluminum foil) at ambient room temperature and ambient light for 14 days. After fermentation, the culture was sonicated for 5 min and the fungal material was separated from the culture broth by filtration over cotton wool. The broth was extracted with EtOAc (3 × 300 mL EtOAc per litre of broth) and concentrated *in vacuo* to give an extract (2.75 g).

4.2.4.2 Bioassay guided fractionation

A portion of the crude extract (2.70 g) was dissolved in 9:1 MeOH/H_2O (200 mL) and extracted with hexanes (3 × 100 mL) before being diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The aqueous fraction was then concentrated *in vacuo*, dissolved in H_2O (200 mL) and extracted with EtOAc (3 × 100 mL) and *n*-BuOH (3 × 100 mL). The resulting five fractions were concentrated *in vacuo* with the CH_2Cl_2 fraction (1.39 g) showing antimicrobial activity. A portion of the CH_2Cl_2 fraction (1.13 g) was further separated by normal phase flash column chromatography (column eluted with 100% hexanes to 100% EtOAc in 10% increments, 1:1 MeOH/EtOAc, and 100% MeOH) with the 12th fraction of the column (1:1 MeOH/EtOAc) giving **5** (579 mg). Normal phase HPLC of the 5th flash column fraction (20 mg; 65:35 hexanes/EtOAc) led to the isolation of **8** (1 mg) and RP-HPLC of the 7th (16 mg; buffered 72:28 H_2O/CH_3CN

with 0.05% TFA) and 8th fractions (22 mg; buffered 70:30 H_2O/CH_3CN with 0.05% TFA) led to the isolation of **9** (1 mg)

4.2.4.3 Biological assays

Bioautographic assays were performed using NP-TLC plates spotted with test fractions. Molten BBL™ Mueller Hinton II cation adjusted agar (25 mL of approximately 45 °C agar) was inoculated (300 µL) with an 18 h bacterial culture and the resulting mixture was poured over the NP-TLC plates in sterile square Petri plates (90 mm × 15 mm; Fisher Scientific Ltd., Ottawa, Ontario). After the agar had solidified, Petri plates were incubated for 24 h at 37 °C. After incubation, the agar surface was sprayed with a thin layer of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), whereby bioactive fractions were visualized as clear spots against a purple background.

The antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and the antibacterial activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 10145) was evaluated as previously described in Chapter 3, with MIC and IC_{50} determination performed as described previously on two-fold serial dilutions in triplicate (Patterson et al. 2015). Poly(3*R*,5*R*-dihydroxyhexanoic acid) (**5**) was tested at a minimum of 12 and a maximum of 14 concentrations (*S. aureus*, *E. coli* and *P. aeruginosa*, 400 – 0.40 and 300 – 75 µg/mL; *M. tuberculosis*, 400 – 0.20 µg/mL). Positive controls for the antimycobacterial and antibacterial assays consisted of a triplicate concentration of rifampin for *M. tuberculosis* (0.012 µM), gentamicin for *E. coli* (2.6 µM), gentamicin for *P. aeruginosa* (21 µM), and erythromycin for *S. aureus* (0.85 µM). General cytotoxicity

against the human embryonic kidney 293 cell line was assessed according to the work performed by Carpenter et al. (2012) on two-fold serial dilutions in triplicate (250 – 0.49 µg/mL). The bioactivity of the compounds was categorized into three levels based on the IC_{50}: strong activity (< 1 µg/mL), moderate activity (1 – 100 µg/mL) and weak activity (> 100 µg/mL). Therapeutic indices were calculated as a ratio of the general cytotoxicity IC_{50} to the antimicrobial IC_{50}. Bioactivity profiles were created as described in Chapter 3.

4.2.4.4 Attempted cyclization of poly(3,5-dihydroxyhexanoic acid) (5)

1,3-Dicyclohexylcarbodiimide (DCC, 33 mg), and DMAP (1.6 mg) were added to a solution of **5** (20 mg) in anhydrous CH_2Cl_2 (2 mL). The solution was stirred overnight at room temperature, adsorbed onto silica (2.2 g) and subjected to vacuum liquid chromatography (2.1 g silica; column eluted with 100% hexanes to 100% EtOAc in 10% increments, 1:1 MeOH/EtOAc, 100% MeOH and 100% acetone) and collected in tubes. The contents of the test tubes were combined according to their TLC profiles to create 5 fractions.

4.2.4.5 Attempted acetylation of poly(3,5-dihydroxyhexanoic acid) (5)

Compound **5** (25 mg) was dissolved in anhydrous pyridine (0.5 mL) and Ac_2O (0.5 mL) and stirred at room temperature overnight. Excess pyridine and Ac_2O was removed under reduced pressure and the resulting solid was adsorbed onto silica (2.3 g) and subjected to vacuum liquid chromatography (2.1 g silica; column eluted with 100% hexanes to 100% EtOAc in 10% increments, 1:1 MeOH/EtOAc, 100% MeOH and 100%

acetone) and collected in tubes. The contents of the test tubes were combined according to their TLC profiles to create 5 fractions.

4.2.4.6 Attempted methylation of poly(3,5-dihydroxyhexanoic acid) (5)

Compound **5** (20 mg) was dissolved in anhydrous MeOH (2.0 mL) and AcCl (drop) and stirred at room temperature overnight. The solution was adsorbed onto silica (2.2 g) and subjected to vacuum liquid chromatography (2.1 g silica; column eluted with 100% hexanes to 100% EtOAc in 10% increments, 1:1 MeOH/EtOAc, 100% MeOH and 100% acetone) and collected in tubes. The contents of the test tubes were combined according to their TLC profiles to create 5 fractions.

4.2.4.7 Hydrolysis of poly(3,5-dihydroxyhexanoic acid) (5)

Compound **5** (10 mg) was dissolved in an aqueous solution of KOH (0.5 mL) and MeOH (2.0 mL) and stirred at room temperature for 75 h. The solution was acidified by adding HCl (2.5 mL), extracted with EtOAc (3 × 1.0 mL) and subsequently subjected to normal phase column chromatography (5 g silica; column eluted with 100% EtOAc and washed with 100% MeOH) and collect in tubes. The contents of the test tubes were combined according to their TLC profiles to create 3 fractions

Poly(3R,5R-dihydroxyhexanoic acid) (**5**): brown solid; IR (thin film) ν_{max} 3426, 2987, 2932, 1723, 1387, 1273, 1176, 753 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ_H 5.10 (1H, m, H-5), 4.09 (1H, m, H-3), 2.51 (1H, dd, *J* = 15.2, 4.7 Hz, H-2a), 2.41 (1H, dd, *J* = 15.2, 8.1 Hz, H-2b), 1.86 (1H, ddd, *J* = 14.0, 7.9, 7.0 Hz, H-4a), 1.68 (1H, ddd, *J* = 13.8, 6.5, 5.1 Hz, H-4b), 1.28 (3H, d, *J* = 6.3 Hz, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 172.8 (C, C-1), 70.1 (CH, C-5), 66.6 (CH, C-3), 43.9 (CH_2, C-2), 43.7 (CH_2, C-4), 20.2 (CH_3, C-6); HRESIMS See Table 4.3.3.4.

4R-hydroxy-6R-methyltetrahydropyran-2-one (**6**): brown oil; $[\alpha]^{23}_D$ +19 (*c* 0.27, $CHCl_3$); IR (thin film) ν_{max} 3419, 2919, 2856, 1641, 1463, 1379, 1252, 1184 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 4.84 (1H, ddq, *J* = 11.2, 6.5, 3.1 Hz, H-6), 4.39, (1H, m, H-4), 2.74 (1H, dd, *J* = 17.7, 5.0 Hz, H-3a), 2.62 (1H, ddd, *J* = 17.6, 3.7, 1.8 Hz, H-3b), 1.98 (1H, dddd, *J* = 14.5, 3.8, 3.4, 1.7 Hz, H-5a), 1.74 (1H, ddd, *J* = 14.5, 11.2, 3.3 Hz, H-5b), 1.41 (3H, d, *J* = 6.4 Hz, H-7). ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 170.4 (C, C-2), 72.3 (CH, C-6), 63.0 (CH, C-4), 38.5 (CH_2, C-5), 37.8 (CH_2, C-3), 21.5 (CH_3, C-7). HRESIMS m/z 131.0704 [$M+H^+$] (calcd for $C_6H_{11}O_3$, 131.0703).

Isosclerone (**8**): brown oil; $[\alpha]^{22}_D$ +125 (*c* 0.16, MeOH); IR (thin film) ν_{max} 3418, 2932, 2856, 1713, 1637, 1459, 1243, 808, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 12.42 (1H, s, 8-OH), 7.50 (1H, dd, *J* = 8.4, 7.5 Hz, H-6), 7.02 (1H, d, *J* = 7.5 Hz, H-5), 6.93 (1H, d, *J* = 8.4 Hz, H-7), 4.92 (1H, dd, *J* = 7.4, 3.5 Hz, H-4), 3.01 (1H, ddd, *J* = 17.9, 8.4, 4.8 Hz, H-2a), 2.65 (1H, ddd, *J* = 17.9, 8.4, 4.8 Hz, H-2b), 2.35 (1H, m, H-3a), 2.19 (1H, m, H-3b). HRESIMS m/z 179.0704 [$M+H^+$] (calcd for $C_{10}H_{11}O_3$, 179.0703).

Scytalone (**9**): brown oil; $[\alpha]^{22}_{D}$ 0 (*c* 0.1, 95% EtOH); IR (thin film) ν_{max} 3346, 2932, 2856, 1628, 1366, 1273, 1167, 841 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ_H 6.22 (1H, brs, H-5), 6.10 (1H, brd, H-7), 4.25 (1H, m, H-3), 3.08 (1H, d, *J* = 16.0, 3.6 Hz, H-4a), 2.84 (1H, d, *J* = 16.8 Hz, H-4b), 2.84 (1H, dd, *J* = 16.8, 3.6 Hz, H-2a), 2.61 (1H, dd, *J* = 17.3, 7.8 Hz, H-2b). ^{13}C NMR (CD_3OD, 100 MHz) δ_C 202.3 (C, C-1), 166.6 (C, C-6), 166.6 (C, C-8), 145.9 (C, C-10), 111.5 (C, C-9), 109.6 (CH, C-5), 101.7 (CH, C-7), 66.9 (CH, C-3), 47.4 (CH_2, C-2), 39.2 (CH_2, C-4). HRESIMS m/z 195.0654 $[M+H^+]$ (calcd for $C_{10}H_{11}O_4$, 195.0652).

4.2.5 *Tolypocladium* sp. (KP1-175E)

4.2.5.1 Fermentation and extraction

Tolypocladium sp. (KP1-175E) was fermented with shaking (150 rpm) in 2% Bacto™ malt extract broth (4 L: 40 × 100 mL batches in 250 mL Erlenmeyer flasks covered with aluminum foil) at ambient room temperature and ambient light for 14 days. After fermentation, the culture was sonicated for 5 min and the fungal material was separated from the culture broth by filtration over cotton wool. The broth was extracted with EtOAc (3 × 300 mL EtOAc per litre of broth) and concentrated *in vacuo* to give an extract (323 mg).

4.2.5.2 Bioassay guided fractionation

A portion of the crude extract (310 mg) was dissolved in 9:1 MeOH/H_2O (50 mL) and extracted with hexanes (3 × 25 mL) before being diluted with H_2O (25 mL) and

extracted with CH_2Cl_2 (3 × 25 mL). The aqueous fraction was then concentrated *in vacuo*, dissolved in H_2O (50 mL) and extracted with EtOAc (3 × 25 mL) and *n*-BuOH (3 × 25 mL). The resulting five fractions were concentrated *in vacuo* and only the CH_2Cl_2 fraction (193 mg) showed significant antimicrobial activity. A portion of the CH_2Cl_2 fraction (187 mg) was further separated by size exclusion chromatography over Sephadex® LH-20 in MeOH to give five fractions. These fractions were determined by combining the contents of the test tubes according to the presence or absence of bioactivity using a bioautographic overlay assay with *Staphylococcus aureus* and the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Fraction 3 (112 mg) showed antimicrobial activity in the bioautographic overlay assay and was further purified by reversed phase HPLC (25 mg, 45:55 CH_3CN/H_2O buffered with 0.1% TFA) to give **10** (5 mg).

4.2.5.3 Biological assays

Bioautographic assays were performed using NP-TLC plates spotted with test fractions. Molten BBL™ Mueller Hinton II cation adjusted agar (25 mL of approximately 45 °C agar) was inoculated (300 µL) with an 18 h bacterial culture and the resulting mixture was poured over the NP-TLC plates in sterile square Petri plates (90 mm × 15 mm; Fisher Scientific Ltd., Ottawa, Ontario). After the agar had solidified, Petri plates were incubated for 24 h at 37 °C. After incubation, the agar surface was sprayed with a thin layer of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), whereby bioactive fractions were visualized as clear spots against a purple background.

The antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and the antibacterial activity against *S. aureus* (ATCC 29213), methicillin resistant *S. aureus* (MRSA; ATCC 33591), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 10145) was evaluated as previously described in Chapter 3, with MIC and IC_{50} determination performed as described previously on two-fold serial dilutions in triplicate (Patterson et al. 2015). (*P*/*M*)-Maximiscin (**10**) was tested at a minimum of 10 and a maximum of 12 concentrations (*E. coli* and *P. aeruginosa*, MRSA 200 – 0.40 µg/mL; *S. aureus*, 100- 0.05 µg/mL; *M. tuberculosis*, 400 – 0.20 µg/mL). Positive controls for the antimycobacterial and antibacterial assays consisted of a triplicate concentration of rifampin for *M. tuberculosis* (0.012 µM), gentamicin for *E. coli* (2.6 µM), gentamicin for *P. aeruginosa* (21 µM), erythromycin for *S. aureus* (0.85 µM), and vancomycin for MRSA (3.5 µM). General cytotoxicity against the human embryonic kidney 293 cell line was assessed according to the work performed by Carpenter et al. (2012) on two-fold serial dilutions in triplicate (250 – 0.49 µg/mL). The bioactivity of the compounds was categorized into three levels based on the IC_{50}: strong activity (< 1 µM), moderate activity (1 – 100 µM) and weak activity (> 100 µM). Therapeutic indices were calculated as a ratio of the general cytotoxicity IC_{50} to the antimicrobial IC_{50}. Bioactivity profiles were created as described in Chapter 3.

(*P/M*)-*maximiscin* (**10**): brown solid; $[\alpha]^{20}_{D}$ -64 (*c* 0.47, MeOH); IR (thin film) ν_{max} 3290, 2951, 1721, 1639, 1552, 1455, 1388, 1253, 1189, 1044, 908, 758 cm^{-1};

P-(**10**): ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ_H 10.32 (1H, brs, OH-4), 7.53 (1H, d, *J* = 7.9, H-6), 6.72 (1H, m, H-2′), 6.08 (1H, brs, OH-5′), 5.95 (1H, d, *J* = 7.9 Hz, H-5), 5.46 (1H, m, H-13), 5.02 (1H, brs, OH-4′), 4.89 (1H, ddd, *J* = 6.3, 1.1, 0.8 Hz, H-6′), 4.76 (1H, dd, *J* = 16.9, 1.8, H-14a), 4.63 (1H, dd, *J* = 10.3, 2.3 Hz, H-14b), 4.26 (1H, brs, H-3′), 3.97 (1H, dd, *J* = 6.5, 1.9 Hz, H-5′), 3.92 (1H, brs, H-4′), 3.69 (3H, s, H-8′), 2.93 (1H, m, H-8), 2.35 (1H, t, *J* = 10.3, H-7), 2.33 (1H, m, H-12), 1.67 (1H, m, H-11a), 1.67 (1H, m, H-9a), 1.54 (1H, m, H-10), 0.89 (3H, d, *J* = 6.5 Hz, H-15), 0.85 (1H, m, H-9b), 0.69 (1H, m, H-11b), 0.66 (3H, d, *J* = 6.0 Hz, H-16); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ_C 166.0 (C, C-7′), 162.3 (C, C-4), 159.3 (C, C-2), 145.7 (CH, C-2′), 143.3 (CH, C-13), 134.2 (CH, C-6), 126.9 (C, C-1′), 113.2 (C, C-3), 112.5 (CH_2, C-14), 98.3 (CH, C-5), 86.5 (CH, C-6′), 71.1 (CH, C-4′), 71.0 (CH, C-5′), 66.9 (CH, C-3′), 51.2 (CH_3, C-8′), 45.8 (CH, C-7), 44.1 (CH_2, C-11), 42.0 (CH, C-8), 41.7 (CH_2, C-9), 31.4 (CH, C-10), 31.3 (CH, C-12), 22.7 (CH_3, C-15), 20.4 (CH_3, C-16).

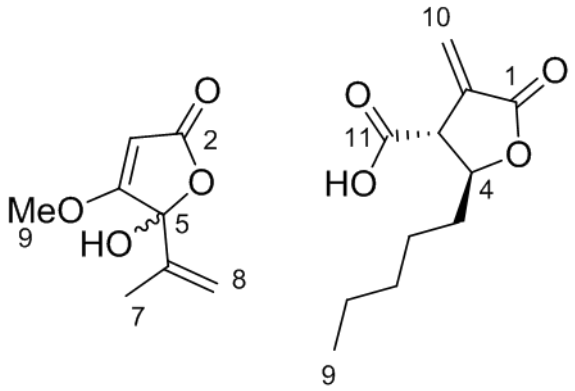
M-(**10**): ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ_H 10.36 (1H, brs, OH-4), 7.53 (1H, d, *J* = 7.9, H-6), 6.69 (1H, m, H-2′), 6.15 (1H, brs, OH-5′), 5.92 (1H, d, *J* = 7.9 Hz, H-5), 5.53 (1H, m, H-13), 4.98 (1H, brs, OH-4′), 4.86 (1H, m, H-6′), 4.77 (1H, dd, *J* = 16.8, 1.8, H-14a), 4.67 (1H, dd, *J* = 10.3, 2.3 Hz, H-14b), 4.26 (1H, brs, H-3′), 3.97 (1H, dd, *J* = 6.5, 1.9 Hz, H-5′), 3.92 (1H, brs, H-4′), 3.66 (3H, s, H-8′), 2.75 (1H, m, H-8), 2.59 (1H, t, *J* = 11.0, H-7), 2.13 (1H, m, H-12), 1.70 (1H, m, H-9a), 1.67 (1H, m, H-11a), 1.54 (1H, m, H-10), 0.89 (3H, d, *J* = 6.5 Hz, H-15), 0.81 (1H, m, H-9b), 0.69 (1H, m, H-11b), 0.63 (3H, d, *J* = 6.6 Hz, H-16); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ_C 166.0 (C, C-7′), 161.5 (C, C-2), 161.3 (C, C-4), 145.5 (CH, C-2′), 143.1 (CH, C-13), 134.0 (CH, C-6), 127.1 (C, C-1′), 113.6 (C, C-3), 112.6 (CH_2, C-14), 98.9 (CH, C-5), 86.7 (CH, C-6′), 71.3 (CH, C-5′), 71.2 (CH, C-4′), 67.0 (CH, C-3′), 52.1 (CH_3, C-8′), 45.8 (CH, C-7), 44.3 (CH_2, C-11), 42.9 (CH, C-8), 42.0 (CH_2, C-9), 32.3 (CH, C-12), 31.4 (CH, C-10), 22.6 (CH_3, C-15), 20.4 (CH_3, C-16). HRESIMS m/z 450.2125 [M + H^+] (calcd for $C_{23}H_{32}NO_8$, 450.2122).

4.3 Results and discussion

4.3.1 *Penicillium* sp. IX (KP1-123A)

The EtOAc extract of *Penicillium* sp. IX (KP1-123A) obtained from a two-week bench-scale (4L) fermentation was fractionated using a modified Kupchan liquid-liquid partition, with the CH_2Cl_2 fraction showing significant antimicrobial activity. Size exclusion chromatography of the CH_2Cl_2 fraction over Sephadex® LH-20 followed by reverse-phase high performance liquid chromatography (RP-HPLC) of the subsequent bioactive fraction led to the isolation of penicillic acid (**1**) and methylenolactocin (**2**) (Figure 4.3.1.1). Penicillic acid [**1**; (Bladt et al. 2013; Sharma et al. 2016; Vansteelandt et al. 2013)] and methylenolactocin [**2**; (Park et al. 1987, 1988)] were identified through a comparison of the mass spectrometry, NMR spectroscopy (for **1** see Table 4.3.1.1; for **2** see Table 4.3.1.2), infrared spectroscopy and polarimetry data for each compound with literature values. Due to limited material, assessing the bioactivity of **1** and **2** against *Mycobacterium tuberculosis* and *Staphylococcus aureus* was prioritized, whereby, moderate activity was observed for both **1** and **2** against *M. tuberculosis* H37Ra, while there was weak activity observed for **1** and **2** against *S. aureus* (Tables 4.3.1.3 & 4.3.1.4). When compared to **1**, **2** was found to be less cytotoxic towards the human embryonic kidney cell line HEK-293 (Tables 4.3.1.3 & 4.3.1.4) and when coupled with the antimicrobial activity, the therapeutic index, a ratio of the therapeutic effect to cytotoxicity, of **2** was an order of magnitude greater than **1** (*M. tuberculosis* therapeutic index: 41 for **2**, and 4.7 for **1**; *S. aureus* therapeutic index: 2.0 for **2**, and 0.15 for **1**), indicating **2** to have greater therapeutic potential than **1**.

The bioactivity profiles for penicillic acid and methylenolactocin (25 µg/mL test concentration; methodology as reported in Chapter 3) showed similar activity towards *M. tuberculosis* and *S. aureus* (Figure 4.3.1.2); however, the natural products on their own did not possess the same activity towards *S. aureus* as that of the extract from which they were derived (Figure 4.3.1.2). Also, neither of the isolated compounds showed the antifungal activity observed in the extract.



1 **2**

Figure 4.3.1.1. The structures of penicillic acid (**1**) and methylenolactocin (**2**).

Penicillic acid was first isolated from *Penicillium puberulum* (Birkinshaw et al. 1936) and subsequently isolated from several *Aspergillus* spp. (Kimura et al. 2014; Lindenfelser and Ciegler 1977; Namikoshi et al. 2003) and *Penicillium* spp. (Betina et al. 1968; Lindenfelser and Ciegler 1977; Olivigni and Bullerman 1978b; Van Eijk 1969; Vansteelandt et al. 2013; Wirth et al. 1956). Penicillic acid, a mycotoxin commonly reported for its toxicity and carcinogenicity in eukaryotic organisms (Dickens and Jones 1961; Frisvad and Thrane 1987; Murnaghan 1946; Stetina 1986) has shown broad

spectrum antimicrobial activity against Gram positive bacteria, Gram negative bacteria, fungi and mycobacteria (Geiger and Conn 1945; Kang and Kim 2004; Kavanagh 1947; Nguyen et al. 2016; Olivigni and Bullerman 1978a; Phainuphong et al. 2017). The antimicrobial mode of action of penicillic acid has been investigated, whereby it was only broadly described as acting on sulfhydryl groups of bacterial enzymes (Geiger and Conn 1945) but has not been elaborated further in regards to specific cellular targets.

Methylenolactocin (**2**) is an α-methylene-γ-lactone previously isolated from *Penicillium* sp. (Park et al. 1987, 1988). The focus of numerous syntheses in the literature for use as a building block in the syntheses of complex natural products (Ariza et al. 2001; Chandrasekharam and Liu 1998; De Azevedo et al. 1992; Fernandes and Chowdhury 2011; Forbes et al. 1999; Ghatak et al. 1997; Ghosh et al. 2009; Hajra et al. 2008; Hon et al. 2005; Jongkol et al. 2009; Kongsaeree et al. 2001; Loh and Lye 2001; Mawson and Weavers 1995; Nallasivam and Fernandes 2017; Saha and Roy 2010; Sarkar and Ghosh 1996; Sibi et al. 1996; Takahata et al. 1995; Vaupel and Knochel 1995; Zeller et al. 2014; Zhu and Lu 1995a, b), **2** has shown antimicrobial activity against Gram positive bacteria, Gram negative bacteria and fungi (Chakrabarty et al. 2015; Park et al. 1987, 1988) and has also shown to be an effective *in vivo* antitumor agent for mice inoculated with Ehrlich carcinoma (Park et al. 1987, 1988). The antimycobacterial activity of **2** has not been previously reported.

Table 4.3.1.1. A comparison of the experimental and reported proton and carbon NMR data obtained for penicillic acid (**1**).

No.	Experimental data in $(CD_3)_2SO$		Reported data in $(CD_3)_2SO$	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm[a]	δH ppm[b] int., mult., (*J* in Hz)
2	170.0, C	-	170.1	-
3	89.3, CH	5.41, 1H, s	89.3	5.39, 1H, s
4	179.4, C	-	179.4	-
5	102.4, C	-	102.4	-
6	140.1, C	-	140.1	-
7	17.1, CH_3	1.65, 3H, s	17.1	1.65, 3H, s
8	115.2, CH_2	5.11, 1H, s 5.29, 1H, s	115.3	5.16, 1H, s 5.33, 1H, brs
9	59.8, CH_3	3.85, 3H, s	59.9	3.84, 3H, s
5-OH	-	not observed	-	3.33, 1H, brs

[a] Sharma et al. 2016; [b] Bladt et al. 2013.

Table 4.3.1.2. A comparison of the experimental and reported proton and carbon NMR data obtained for methylenolactocin (**2**)[a].

No.	Experimental data in $CDCl_3$		Reported data in $CDCl_3$[b]		Experimental data in CD_3OD
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)	δ_H ppm int., mult., (*J* in Hz)
1	168.3, C	-	168.6	-	-
2	132.5, C	-	132.5	-	-
3	49.5, CH	3.64, 1H, brs	49.6	3.65, 1H, dt (*J* = 5.6, 2.9 Hz)	3.72, 1H, m
4	78.9, CH	4.81, 1H, m	79.2	4.83, 1H, q (*J* = 5.6, 5.4 Hz)	4.79, 1H, q (*J* = 6.1 Hz)
5	35.8, CH_2	1.74, 2H, m	35.7	1.72, 2H, m	1.74, 2H, q (*J* = 7.3 Hz)
6	22.6, CH_2	1.32, 2H, m	22.4	1.36, 2H, m	1.36, 2H, m
7	24.6, CH_2	1.32, 2H, m	24.4	1.36, 2H, m	1.36, 2H, m
8	31.5, CH_2	1.32, 2H, m	31.3	1.36, 2H, m	1.36, 2H, m
9	14.1, CH_3	0.89, 3H, t (*J* = 5.8 Hz)	13.9	0.90, 3H, t (*J* = 5.9 Hz)	0.93, 3H, t (*J* = 7.0 Hz)
10	126.1, CH_2	6.03, 1H, d (*J* = 1.8 Hz) 6.47, 1H, d (*J* = 1.8 Hz)	126.1	6.04, 1H, d (*J* = 2.9 Hz) 6.47, 1H, d (*J* = 2.9 Hz)	5.99, 1H, d (*J* = 2.9 Hz) 6.32, 1H, d (*J* = 2.9 Hz)
11	173.7, C	-	174.3		-
OH	-	not observed	-	10.77, s	not observed

[a] Repeated attempts to obtain ^{1}H NMR of **2** in $CDCl_3$ resulted in unresolved peaks and undistinguishable multiplicity due to poor solubility. Data in CD_3OD confirmed the expected multiplicity for **2**, though no reported data in CD_3OD could be obtained.
[b] Park et al. 1987, 1988.

Table 4.3.1.3. Biological activity (MIC and IC_{50} in µM) of penicillic acid (**1**) isolated from *Penicillium* sp. IX, an endophyte of *Spongomorpha arcta*.

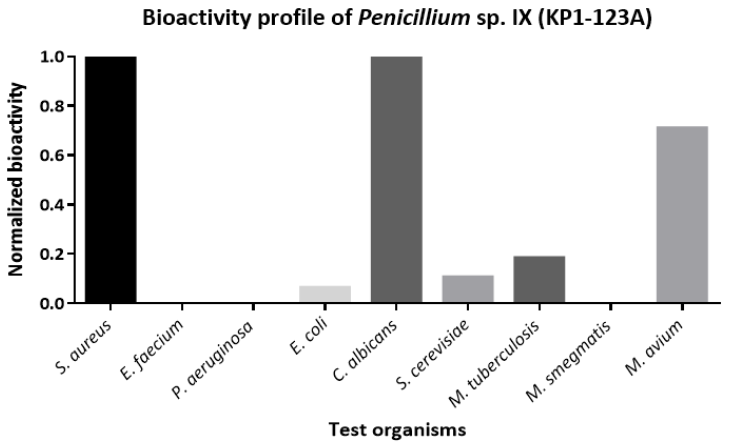
Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	300	25 (22-28)
Staphylococcus aureus	> 1000	777 (701-862)
Human embryonic kidney 293 cells	400	117 (98-140)

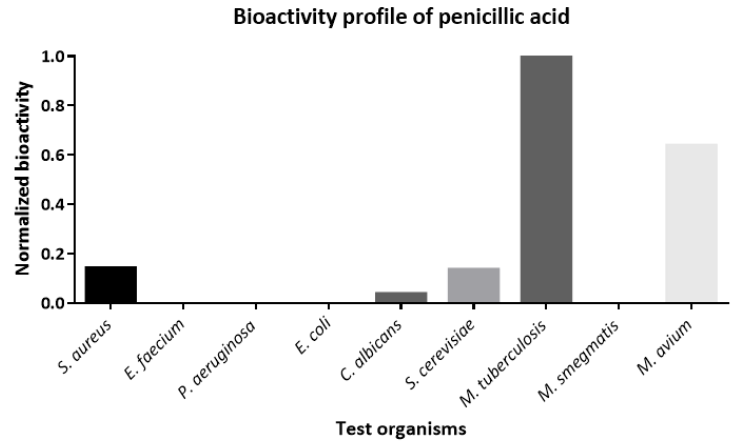
[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.

Table 4.3.1.4. Biological activity (MIC and IC_{50} in µM) of methylenolactocin (**2**) isolated from *Penicillium* sp. IX, an endophyte of *Spongomorpha arcta*.

Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	250	21 (15-30)
Staphylococcus aureus	> 1000	426 (352-516)
Human embryonic kidney 293 cells	> 1000	865 (655-1140)

[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.





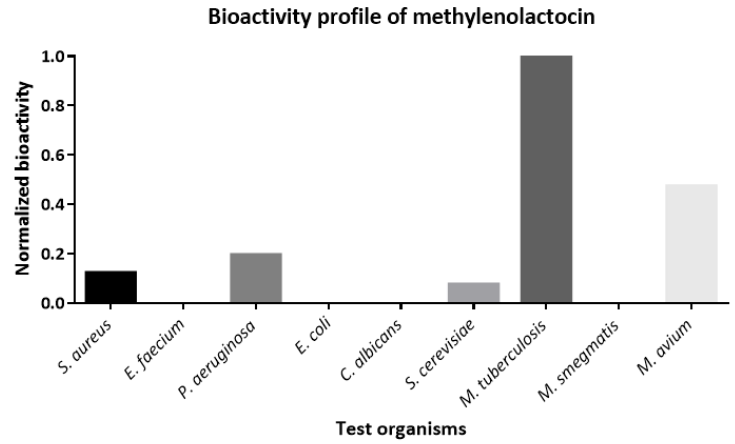
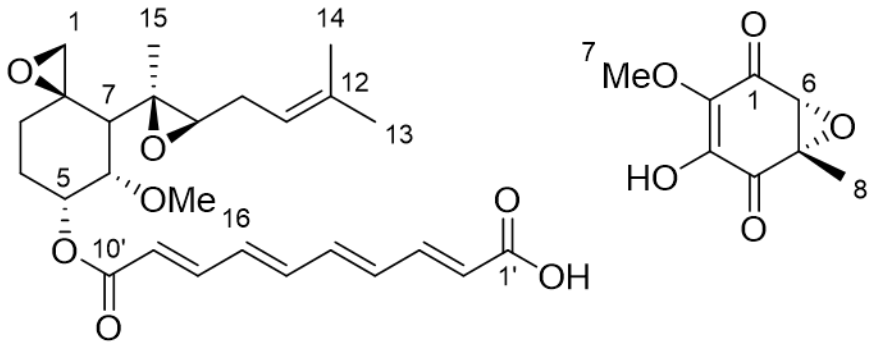


Figure 4.3.1.2. Bioactivity profiles obtained for the extract of *Penicillium* sp. IX, penicillic acid and methylenolactocin.

4.3.2 *Aspergillus fumigatus* III (KP1-131Q)

The EtOAc extract of *Aspergillus fumigatus* III (KP1-131Q) obtained from a two-week bench-scale (9.1L) fermentation was fractionated using a modified Kupchan liquid-liquid partition, with the CH_2Cl_2 fraction showing significant antimicrobial activity. Size exclusion chromatography of the CH_2Cl_2 fraction over Sephadex® LH-20 followed by reverse-phase flash column chromatography and reverse-phase high performance liquid chromatography (RP-HPLC) of the subsequent bioactive fractions led to the isolation of fumagillin [**3**; (Eble and Hanson 1951; Wiemann et al. 2013); Figure 4.3.2.1] and fumigatin oxide [**4**; (Cole and Cox 1981; Yamamoto et al. 1965), Figure 4.3.2.1], with a comparison of the experimental mass spectrometry, NMR spectroscopy (for **3** see Table 4.3.2.1; for **4** see Table 4.3.2.2), infrared spectroscopy and polarimetry data with literature values confirming the structures. It should be noted that discrepancies were seen between the experimental and reported ^{13}C chemical shifts of the carbonyl carbons of **4** (carbons 1 and 4; Table 4.3.2.2). The structure elucidation of **4** was achieved through an analysis of the 2D NMR data, and ultimately confirmed the structure of **4** as fumigatin oxide despite these chemical shift differences. Unfortunately, despite the fact that the optical rotation **4** has been reported (Yamamoto et al. 1965), no reports since have detailed the absolute stereochemistry of this compound. The relative stereochemistry of **4** is therefore reported here.



3 **4**

Figure 4.3.2.1. The structures of fumagillin (**3**) and fumigatin oxide (**4**).

Fumagillin (**3**) is commonly isolated from the extracts of *A. fumigatus* (Chu et al. 2001; Eble and Hanson 1951; Hanson and Eble 1949; Ingber et al. 1990; Lamrani et al. 2008; Wiemann et al. 2013). Originally reported for its antimicrobial and antiviral activity (Hanson and Eble 1949), **3** has since been found to have excellent activity as an antiangiogenesis molecule (Griffith et al. 1998; Ingber et al. 1990; Liu et al. 1998; Sin et al. 1997; Zhang et al. 2006). Antiangiogenic natural products, molecules that inhibit the formation of new blood vessels from existing blood vessels, have developed interest due to their therapeutic potential in the treatment of cancer (Khalid et al. 2016; Lu et al. 2016; Vasudev and Reynolds 2014). Fumagillin showed moderate activity against *Staphylococcus aureus* and weak activity towards *Mycobacterium tuberculosis* (Table 4.3.2.3) and has previously shown activity against *Enterococcus faecalis* (originally reported by the authors as *Streptococcus faecalis*), *Bacillus subtilis* and *S. aureus* (Hanson and Eble 1949). It has also previously been reported to possess antiviral activity against a *S. aureus* bacteriophage (Hanson and Eble 1949; Mills 1955), antifungal activity against microsporidiosis causing fungi (Didier 1997; Didier et al. 2006; Molina et al. 2000;

Molina et al. 2002; Shadduck 1980), antimalarial activity (Chen et al. 2009), and activity against the parasitic amoeba *Endamoeba histolytica* (Anderson 1952; Hrenoff and Nakamuraj 1951; Killough et al. 1952). Fumagillin has not been previously reported to possess activity against *M. tuberculosis* H37Ra.

Fumigatin oxide (**4**) was originally isolated from *A. fumigatus* in 1965 (Yamamoto et al. 1965) but has only since been reported from *Ramichloridium apiculatum* in 1991 (Nozawa et al. 1991). This is the first report of **4** being isolated from an endophytic fungus. Fumigatin oxide was found to be weakly bioactive against *Candida albicans* (Table 4.3.2.4) and has only previously been reported to inhibit the growth of *B. subtilis* and *Escherichia coli* (Nozawa et al. 1991). As a bioautographic overlay assay with *S. aureus* was used to guide the fractionation of this extract towards **4**, it is interesting that it showed no discernable bioactivity against *S. aureus* in a microbroth dilution assay. This may be a result of the instability of **4** when in alkaline solutions (Yamamoto et al. 1967), leading to inconsistent results when evaluating the biological activity of this compound. Both fumagillin and fumigatin oxide showed less than 50% activity towards the human embryonic kidney 293 cell line at 250 µg/mL and therefore a median lethal concentration could not be determined.

It is suspected that in the case of fumagillin and fumigatin oxide that it was their combined biological activity that ultimately led to the formation of the extract profile, as neither compound individually possessed the activity seen in the extract from which they were derived (Figure 4.3.2.2; 25 µg/mL test concentration; methodology as reported in Chapter 3). Also, neither compound possessed the activity towards *Mycobacterium smegmatis* that was observed in the extract (Figure 4.3.2.2).

Table 4.3.2.1. A comparison of the experimental and reported proton and carbon NMR data obtained for fumagillin (**3**).

No.	Experimental data in CD_3OD		Reported data in CD_3OD[a]	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
1	51.7, CH_2	2.58, 1H, d (*J* = 4.2 Hz)	51.8	2.59, 1H, d (*J* = 4.4 Hz)
		2.99, 1H, d (*J* = 4.2 Hz)		2.99, 1H, d (*J* = 4.0 Hz)
2	60.6, C	-	60.8	-
3	30.3, CH_2	1.09, 1H, m	30.4	1.09, 1H, m
		2.13, 1H, m		2.15, 1H, m
4	26.6, CH_2	1.91, 2H, m	26.8	1.91, 2H, m
5	68.1, CH	5.70, 1H, m	68.2	5.71, 1H, m
6	80.8, CH	3.74, 1H, dd (*J* = 11.2, 2.7 Hz)	80.7	3.74, 1H, dd (*J* = 11.2, 1.6 Hz)
7	49.8, CH	1.98, 1H, d (*J* = 11.2 Hz)	50.0	1.99, 1H, d (*J* = 10.8 Hz)
8	60.5, C	-	60.6	-
9	62.4, CH	2.69, 1H, m	62.6	2.70, 1H, m
10	28.3, CH_2	2.22, 2H, m	28.4	2.24, 2H, m
11	119.8, CH	5.25, 1H, brs	120.0	5.25, 1H, brs
12	135.1, C	-	135.0	-
13	25.9, CH_3	1.76, 3H, s	26.1	1.76, 3H, s
14	18.1, CH_3	1.68, 3H, s	18.2	1.68, 3H, s
15	14.2, CH_3	1.21, 3H, s	14.4	1.20, 3H, s
16	56.9, CH_3	3.41, 3H, s	57.0	3.41, 3H, s
1′	170.2, C	-	170.4	-
2′	124.2, CH	5.98, 1H, d (*J* = 15.3 Hz)	124.2	5.99, 1H, d (*J* = 15.2 Hz)
3′	145.4, CH	7.36, 1H, m	145.6	7.35, 1H, m
4′	134.8, CH	6.62, 1H, m	135.0	6.65, 1H, m
5′	140.5, CH	6.78, 1H, m	140.7	6.76, 1H, m
6′	141.0, CH	6.78, 1H, m	141.1	6.79, 1H, m
7′	135.9, CH	6.62, 1H, m	135.3	6.65, 1H, m
8′	145.5, CH	7.36, 1H, m	145.7	7.39, 1H, brd (*J* = 16.4 Hz)
9′	123.6, CH	6.05, 1H, d (*J* = 15.2 Hz)	123.7	6.05, 1H, d (*J* = 15.2 Hz)
10′	167.7, C	-	167.8	-

[a] Wiemann et al. 2013.

Table 4.3.2.2. A comparison of the experimental and reported proton and carbon NMR data obtained for fumigatin oxide (**4**).

No.	Experimental data in $(CD_3)_2SO$			Reported data in $(CD_3)_2SO$[a]	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	HMBC to	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
1	188.0, C	-		183.4	-
2	146.5, C	-		145.8	-
3	140.0, C	-		139.6	-
4	189.4, C	-		184.7	-
5	58.2, C	-		57.9	-
6	59.5, CH	3.74, 1H, s	140.0	58.4	3.75, 1H, s
7	58.6, CH_3	3.82, 3H, s	140.0, 58.2, 188.0, 13.9	59.2	3.82, 3H, s
8	13.9, CH_3	1.49, 3H, s	58.2, 189.4	13.7	1.50, 3H, s

[a] Cole & Cox 1981.

Table 4.3.2.3. Biological activity (MIC and IC_{50} in µM) of fumagillin (**3**) isolated from KP1-131Q, an endophyte of *Scytosiphon lomentaria*.

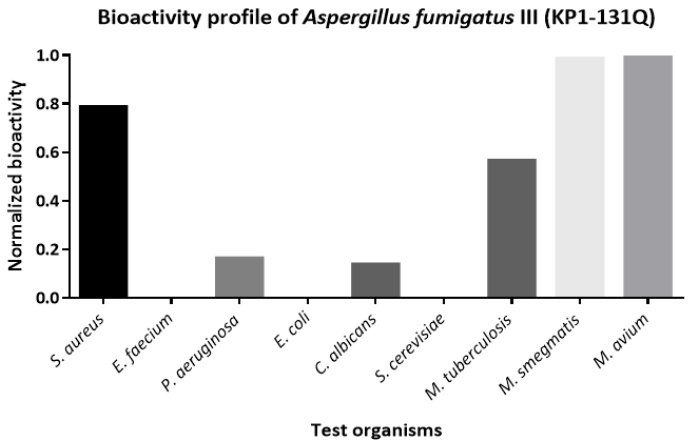
Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	900	141 (121-164)
Staphylococcus aureus	350	26 (22-31)

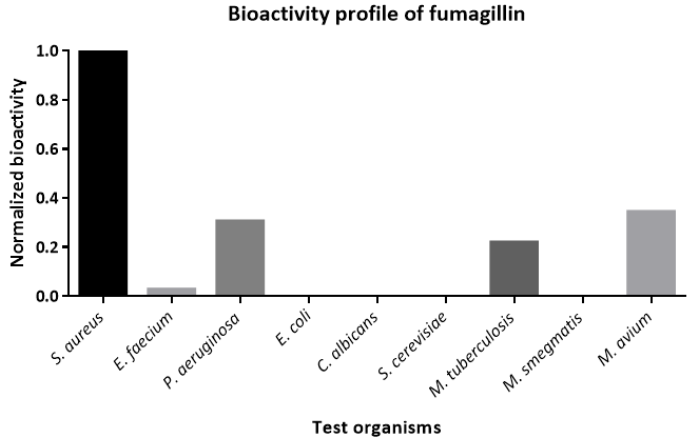
[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.

Table 4.3.2.4. Biological activity (MIC and IC_{50} in µM) of fumigatin oxide (**4**) isolated from KP1-131Q, an endophyte of *Scytosiphon lomentaria*.

Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	> 1000	> 1000
Candida albicans	> 1000	879 (840-919)
Staphylococcus aureus	> 1000	> 1000

[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.





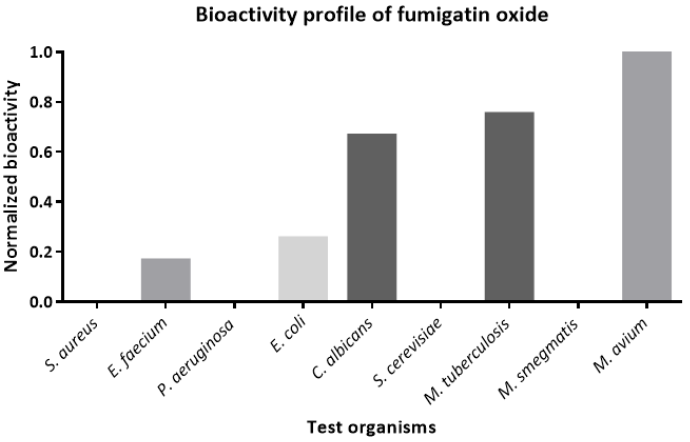
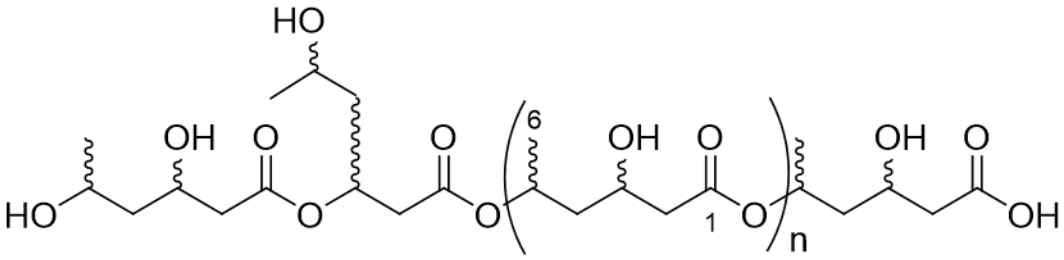


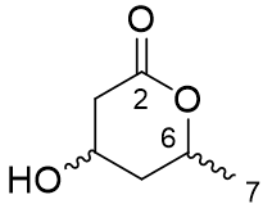
Figure 4.3.2.2. Bioactivity profiles of the extract from KP1-131Q, fumagillin and fumigatin oxide.

4.3.3 Sterile grey filamentous II (KP1-131DD)

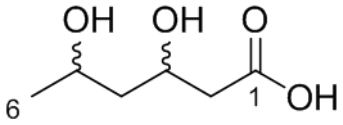
The EtOAc extract of sterile grey filamentous II (KP1-131DD) obtained from a two-week bench-scale (5L) fermentation was fractionated using a modified Kupchan liquid-liquid partition, with the CH_2Cl_2 fraction showing antimicrobial activity. Normal phase flash column chromatography of the CH_2Cl_2 fraction led to the isolation of **5** (Figure 4.3.3.1 & Figure 4.3.3.2).



5



6



7

Figure 4.3.3.1. The structure of poly(3,5-dihydroxyhexanoic acid) (**5**) isolated from an unidentified sterile grey filamentous endophyte, and the proposed structures of 4-hydroxy-6-methyltetrahydropyran-2-one (**6**) and 3,5-dihydroxyhexanoic acid (**7**).

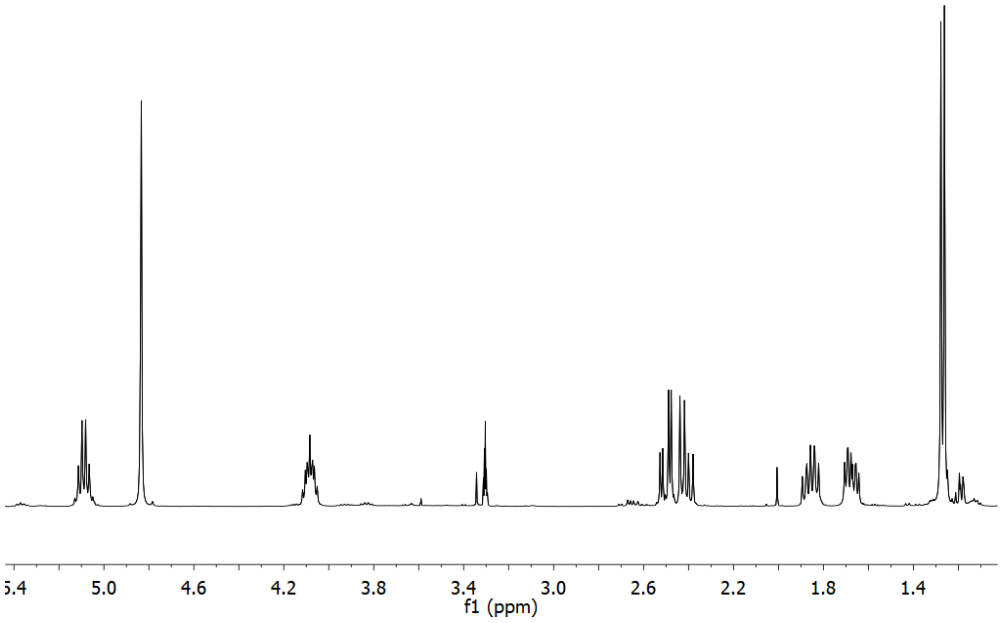


Figure 4.3.3.2. ^{1}H NMR (CD_3OD, 400 MHz) of **5**.

Initially, investigation of the ^{1}H, ^{13}C and 2D NMR data obtained for **5** originally suggested the isolation of 4-hydroxy-6-methyltetrahydropyran-2-one (**6**; Figure 4.3.3.1) due to the presence of a characteristic ester or lactone carbonyl chemical shift of 172 (Pretsch et al. 2009) and the presence of COSY correlations along the entirety of the spin system (Table 4.3.3.1). A peak in the positive mode mass spectrum ($M+H^+$, 131.0704; Figure 4.3.3.3) was indicative of the proposed lactone structure; however, upon comparison of the experimental NMR data obtained for the unknown natural product to the literature NMR data for **6** (Fehr et al. 1999; Gijsen and Wong 1994), discrepancies were observed between the chemical shifts in the ^{13}C NMR spectra (Table 4.3.3.2). As the literature data did not support a cyclized structure for our isolated compound, the linear 3,5-dihydroxyhexanoic acid (**7**; Figure 4.3.3.1) was proposed to be the structure of the isolated natural product.

Table 4.3.3.1. Experimental ^{1}H, ^{13}C and COSY NMR data obtained for **5**[a].

No.	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	COSY coupling to
1	172.8, C	-	-
2	43.9, CH_2	2.41, 1H, dd (*J* = 15.2, 8.1 Hz) 2.51, 1H, dd (*J* = 15.2, 4.7 Hz)	66.7
3	66.6, CH	4.09, 1H, m	43.9, 43.7
4	43.7, CH_2	1.68, 1H, ddd (*J* = 13.8, 6.5, 5.1 Hz) 1.86, 1H, ddd (*J* = 14.0, 7.9, 7.0 Hz)	70.1, 66.7
5	70.1, CH	5.10, 1H, m	43.7, 20.2
6	20.2, CH_3	1.28, 3H, d (*J* = 6.3 Hz)	70.1

[a] Data obtained in CD_3OD.

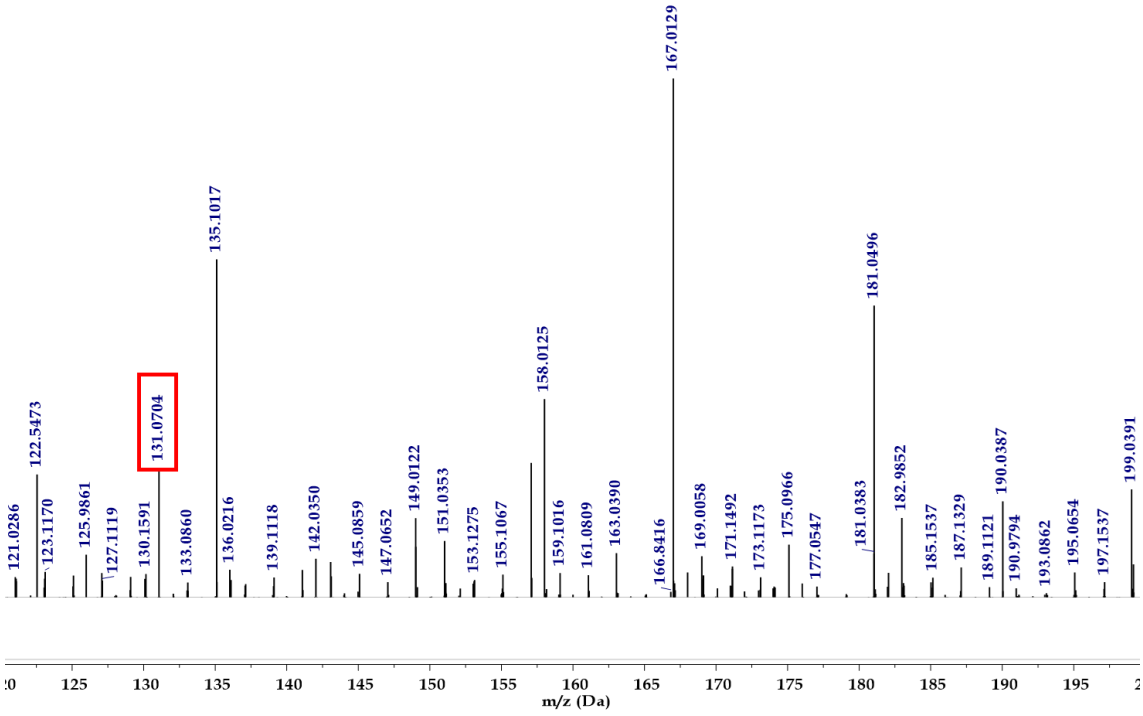


Figure 4.3.3.3. Positive mode mass spectrum (expanded to 120 – 200 m/z) of **5**. A peak in the positive mode mass spectrum ($M+H^+$, 131.0704; red box) was indicative of the proposed lactone structure **6**.

Table 4.3.3.2. A comparison of the experimental ^{13}C NMR data obtained for **5** and the reported ^{13}C NMR data for **6**[a].

δ_C ppm **5**	δ_C ppm **cis-6**[b]	δ_C ppm **trans-6**[b]
20.4	21.4	21.4
42.6	39.2	37.4
42.8	39.4	38.3
66.3	63.5	62.4
69.6	74.0	72.7
172.1	171.7	171.5

[a] Data compared in $CDCl_3$.
[b] Fehr et al. 1999.

Unfortunately, no literature NMR data for **7** could be obtained to confirm the proposed structure for the isolated metabolite. Also, no characteristic $M+H^+$, $M+Na^+$, or $M-H^+$ peaks were observed in the mass spectrum to confirm the isolation of **7** (Figure 4.3.3.3 and 4.3.3.4). In an attempt to confirm the isolation of **7**, the cyclization of the isolated metabolite to **6**, and the acetylation and methylation of the isolated metabolite was attempted to confirm the proposed structure (Figure 4.3.3.5). The cyclization of the isolated metabolite was conducted overnight using DCC, DMAP and CH_2Cl_2; however, no signals in the ^1H NMR spectra obtained for the vacuum liquid chromatography fractions suggested that the isolated metabolite had cyclized or had at all reacted. The acetylation of the isolated metabolite was also attempted overnight using acetic anhydride and pyridine; however, the ^1H NMR data of the reaction product suggested that only one site of the metabolite was acetylated (Figure 4.3.3.6). The methylation of the isolated metabolite was attempted overnight using acetyl chloride and MeOH; however, no signals consistent with the methylation of **7** were observed in the ^1H NMR of the obtained product. In fact, ^1H NMR of the fractions derived from the attempted methylation suggested that a small amount (2 mg) of the isolated metabolite had cyclized to **6** (Figure 4.3.3.7) when compared to literature data (Fehr et al. 1999).

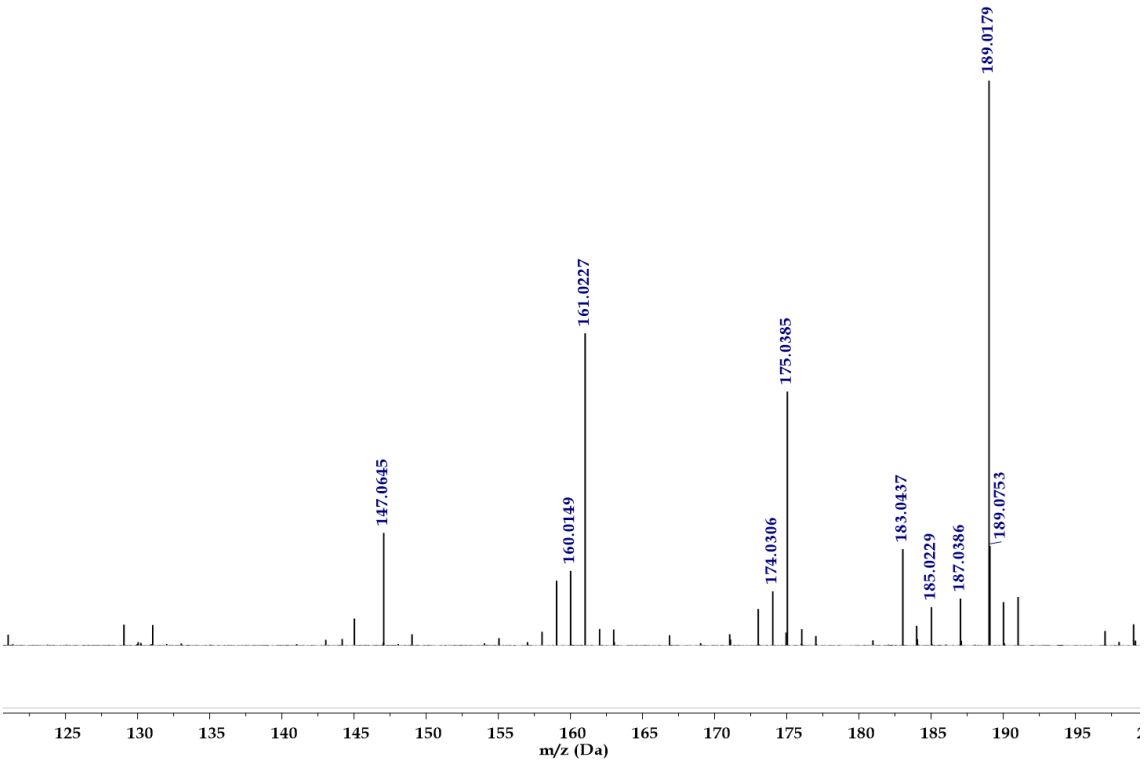


Figure 4.3.3.4. Negative mode mass spectrum (expanded to 120 – 200 m/z) of **5**. No characteristic peak ($M\text{-}H^+$, 129.0557) for the proposed lactone structure **6** was observed.

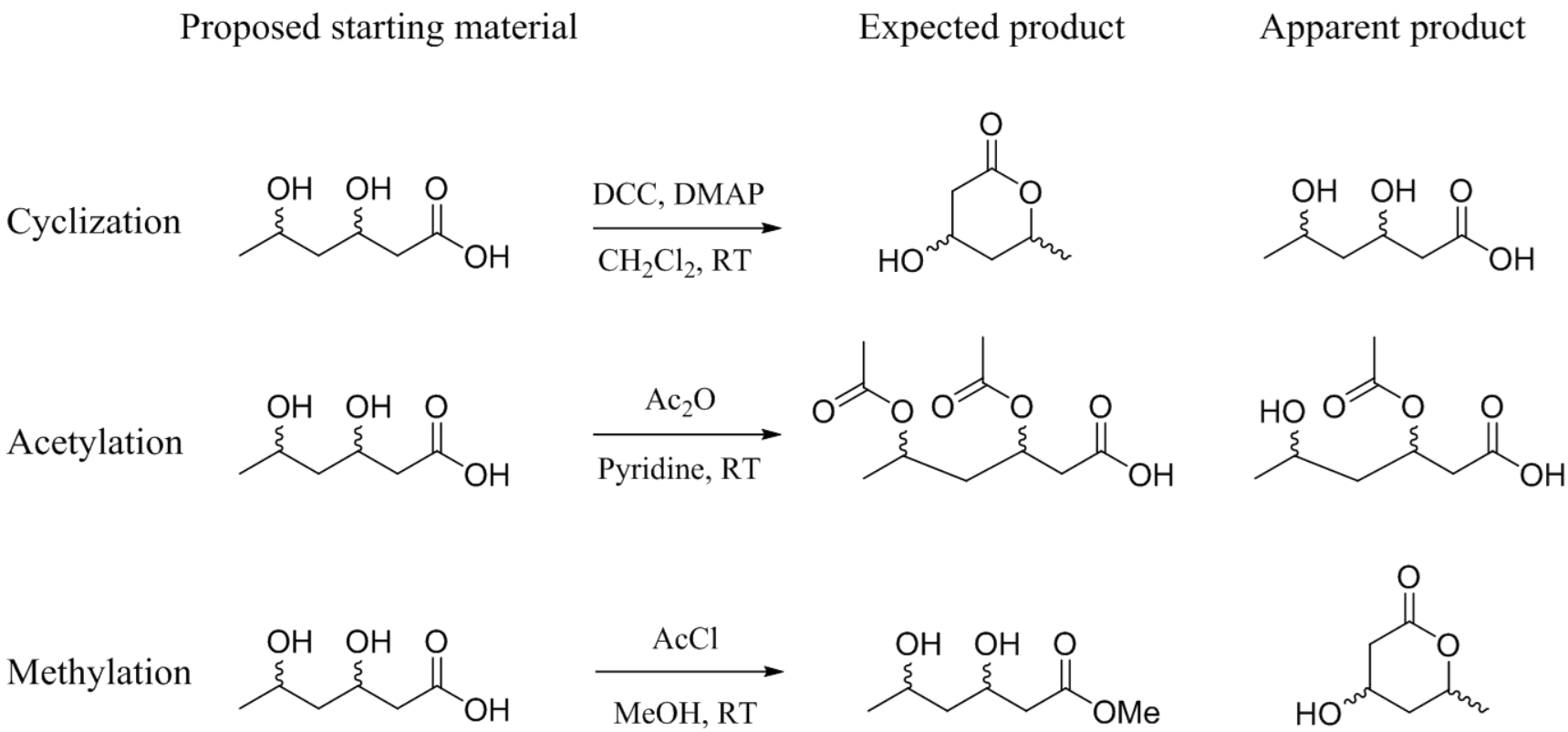


Figure 4.3.3.5. Attempted cyclization, acetylation and methylation of the proposed structure **7**.

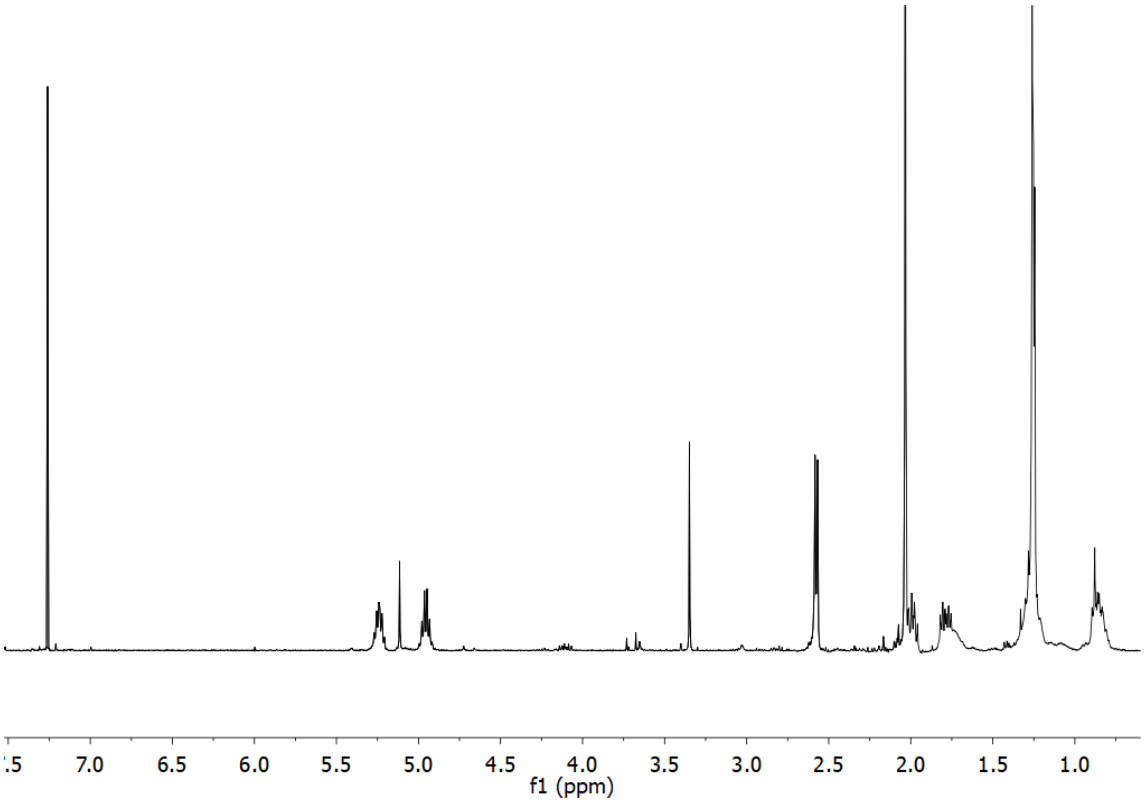


Figure 4.3.3.6. ^{1}H NMR ($CDCl_3$, 400 MHz) of the obtained product from the attempted acetylation of the proposed structure **7**, showing the single acetylated site at 2.03 ppm.

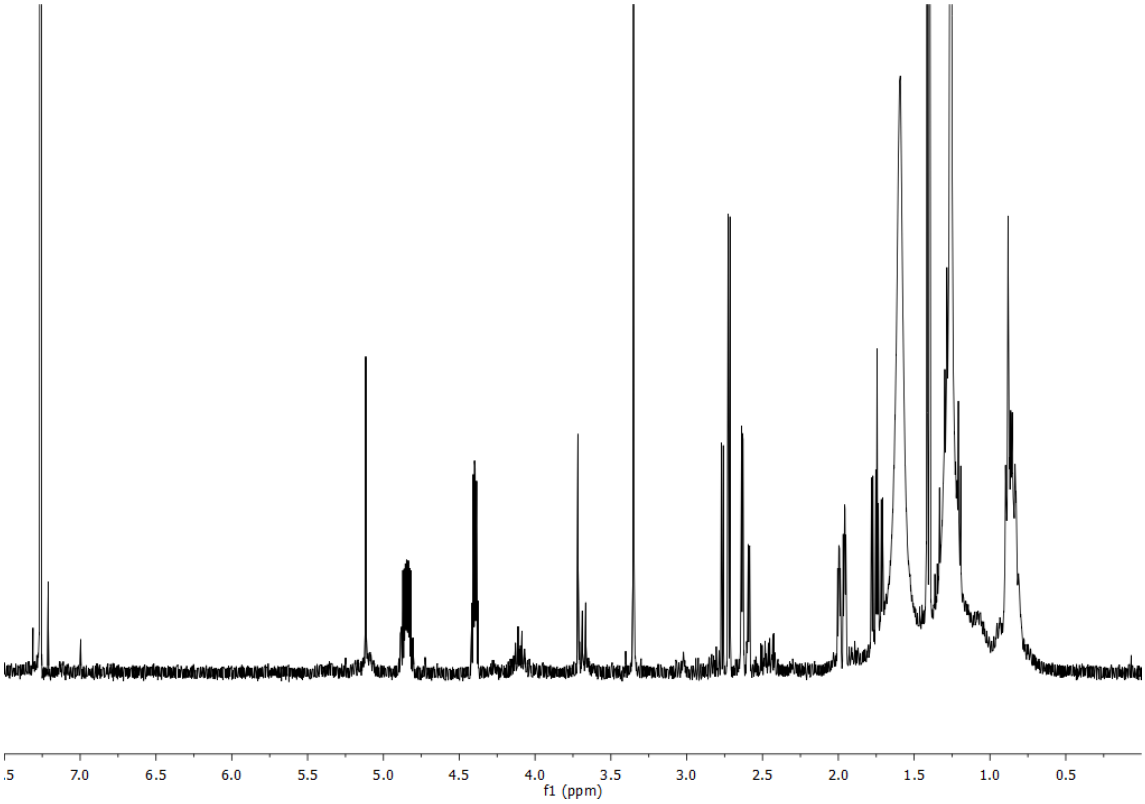
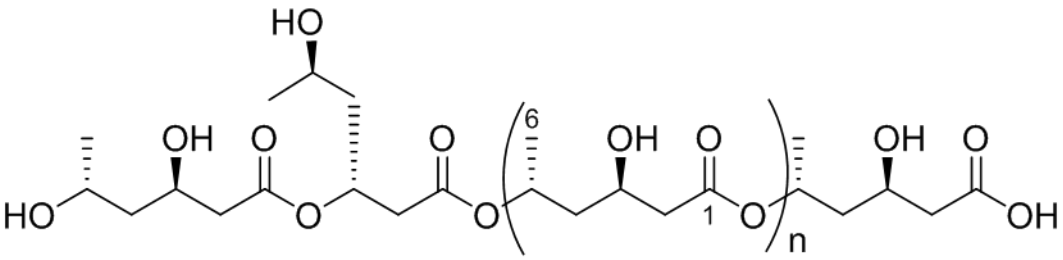


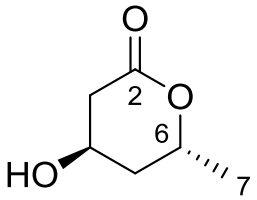
Figure 4.3.3.7. ^{1}H NMR ($CDCl_3$, 400 MHz) of the obtained product from the attempted methylation of the proposed structure **7**, showing the chemical shifts consistent with the cyclized product, **6** (Fehr et al. 1999).

With the inability to cyclize, acetylate or methylate the isolated metabolite as proposed, the acetylation of one site and the lack of representative molecular ions in the HRESIMS data for **7**, it was proposed that the structure of **5** was in fact a polymer of 3,5-dihydroxyhexanoic acid. The isolation of **5** as a polymer of 3,5-dihydroxyhexanoic acid would be consistent with the inability to cyclize and methylate **7** and the presence of only one acetylated methyl signal following an attempted acetylation. Further investigation of the negative mode mass spectrum revealed the presence of $2M\text{-}H^+$ to $19M\text{-}H^+$ peaks representative of the oligomers of 3,5-dihydroxyhexanoic acid (Figures 4.3.3.9 and 4.3.3.10; Table 4.3.3.4). In an attempt to confirm that the 3,5-dihydroxyhexanoic acid oligomers were not a product of the mass spectral analysis and were indeed isolated from

the endophyte, **5** was hydrolyzed under basic conditions using KOH and MeOH for 75 hours and subsequently cyclized under acidic conditions, resulting in the formation of **6**. A comparison of the NMR and optical rotation data of the cyclized product **6** to reported data indicated the isolation of the *R*,*R* product (Figure 4.3.3.8). The hydrolysis product (**6**) and the attempted methylation product (**6**) showed highly similar ^{1}H NMR spectra; however, the hydrolysis product was not pure enough to provide an optical rotation, therefore the cyclized methylation product was used to assess the absolute stereochemistry. This ultimately confirms the isolation of **5** as a collection of 3*R*,5*R*-dihydroxyhexanoic acid oligomers (Figure 4.3.3.8). The ^{1}H NMR and mass spectrometry data of **5** was consistent with literature values (Maloney 2007); however, due to the dark pigmented nature of **5**, optical rotation could not be obtained.



5



6

Figure 4.3.3.8. The structure of poly(3*R*,5*R*-dihydroxyhexanoic acid) (**5**) and 4*R*-hydroxy-6*R*-methyltetrahydropyran-2-one (**6**).

Table 4.3.3.4. Negative mode mass spectrum peaks corresponding to the oligomers of 3*R*,5*R*-dihydroxyhexanoic acid (**5**).

Number of acid units	Theoretical [M-H]	Observed [M-H]	Δppm
1	147.0663	not observed	not applicable
2	277.1293	277.1288	-1.8
3	407.1923	407.1916	-1.7
4	537.2553	537.2544	-1.7
5	667.3183	667.3171	-1.8
6	797.3813	797.3796	-2.1
7	927.4443	927.4424	-2.1
8	1057.5073	1057.5051	-2.1
9	1187.5703	1187.5680	-1.9
10	1317.6333	1317.6309	-1.8
11	1447.6963	1447.6941	-1.5
12	1577.7593	1577.7565	-1.8
13	1707.8223	1707.8189	-2.0
14	1837.8853	1837.8807	-2.5
15	1967.9483	1967.9393	-4.6
16	2098.0113	2098.0056	-2.7
17	2228.0743	2228.0729	-0.6
18	2358.1373	2358.1332	-1.7
19	2488.2003	2488.2065	2.5

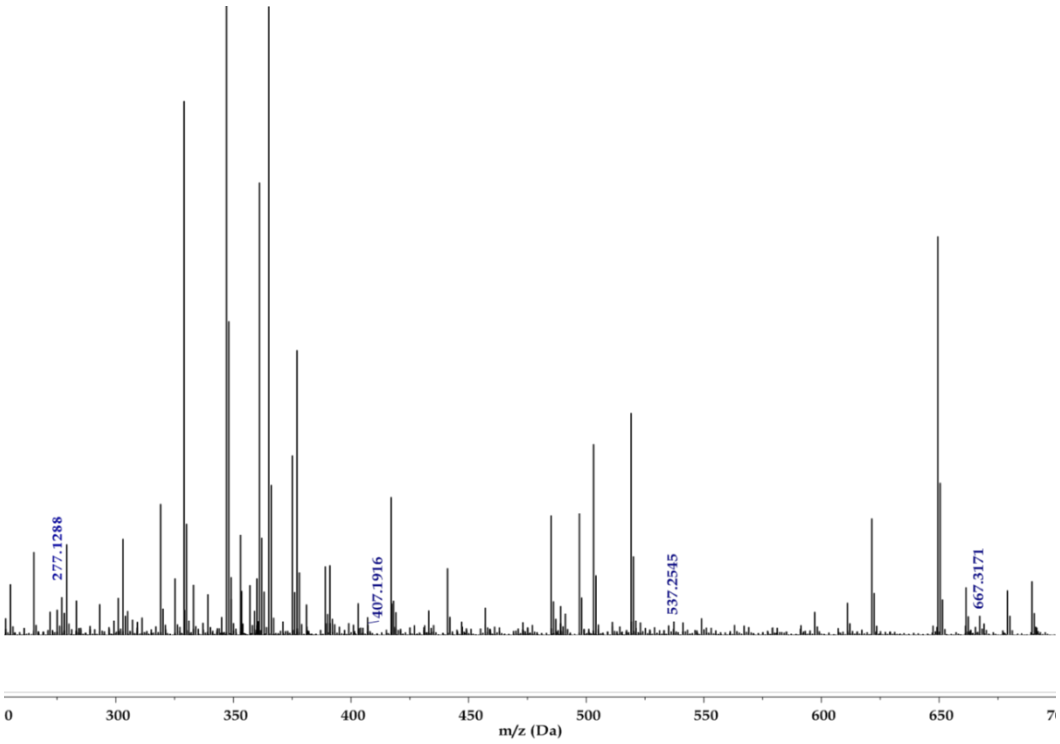


Figure 4.3.3.9. Negative mode mass spectrum (expanded to 250 – 700 m/z) for **5** showing the peaks representing the dimer to the pentamer of 3,5-dihydroxyhexanoic acid.

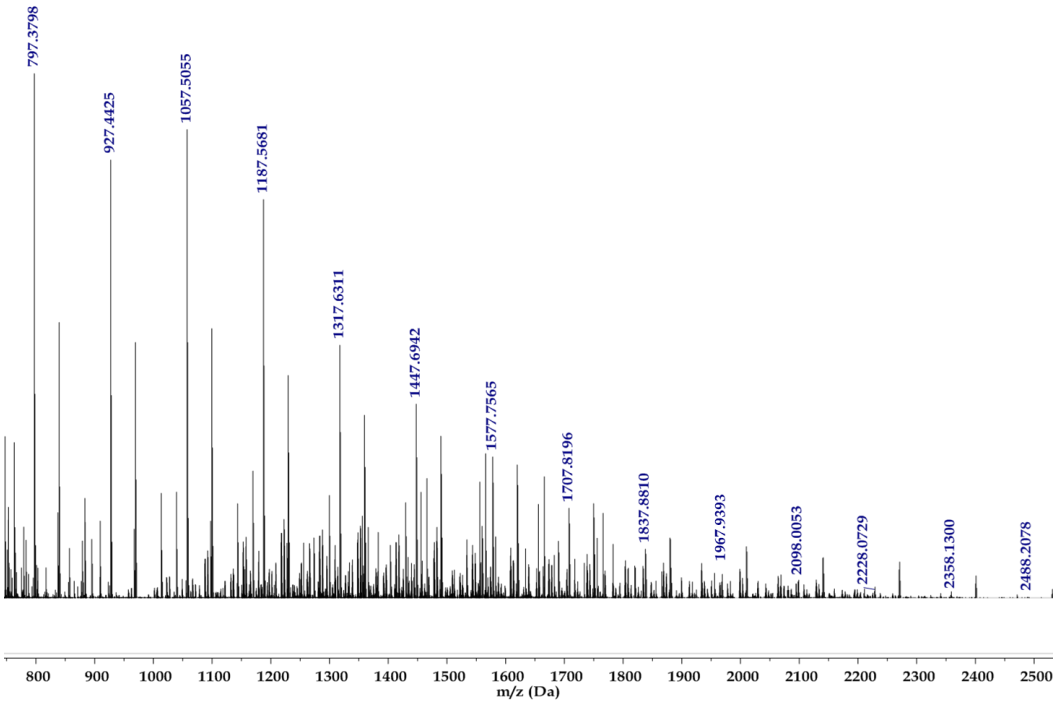


Figure 4.3.3.10. Negative mode mass spectrum (expanded to 750-2500 m/z) for **5** showing the peaks representing the hexamer to the 19-mer of 3,5-dihydroxyhexanoic acid.

3*R*,5*R*-dihydroxyhexanoic acid oligomers have only been previously isolated from the *Manilkara* sp. endophyte *Daldinia concentrica* in a collection of linear oligomers ranging from a pentamer to a 27-mer (Maloney 2007). No biological activity for **5** has been reported; however, **5** showed activity against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Table 4.3.3.5). Poly(3*R*,5*R*-dihydroxyhexanoic acid) showed less than 50% activity towards the human embryonic kidney 293 cell line at 250 µg/mL and therefore a median lethal concentration could not be determined.

The bioactivity profile of poly(3*R*,5*R*-dihydroxyhexanoic acid) showed greater activity towards Gram negative bacteria than seen in the extract from which it was derived (Figure 4.3.3.11; 25 µg/mL test concentration; methodology as reported in Chapter 3). The extract possessed activity towards *Mycobacterium smegmatis* that was not seen from poly(3*R*,5*R*-dihydroxyhexanoic acid) (Figure 4.3.3.11).

Table 4.3.3.5. Biological activity (MIC and IC_{50} in µg/mL) of poly(3*R*,5*R*-dihydroxyhexanoic acid) (**5**) isolated from a sterile grey filamentous endophyte of *Scytosiphon lomentaria*.

Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	> 200	110 (96-126)
Escherichia coli	> 400	> 400
Pseudomonas aeruginosa	> 400	369 (312-436)
Staphylococcus aureus	> 400	101 (89-115)

[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.

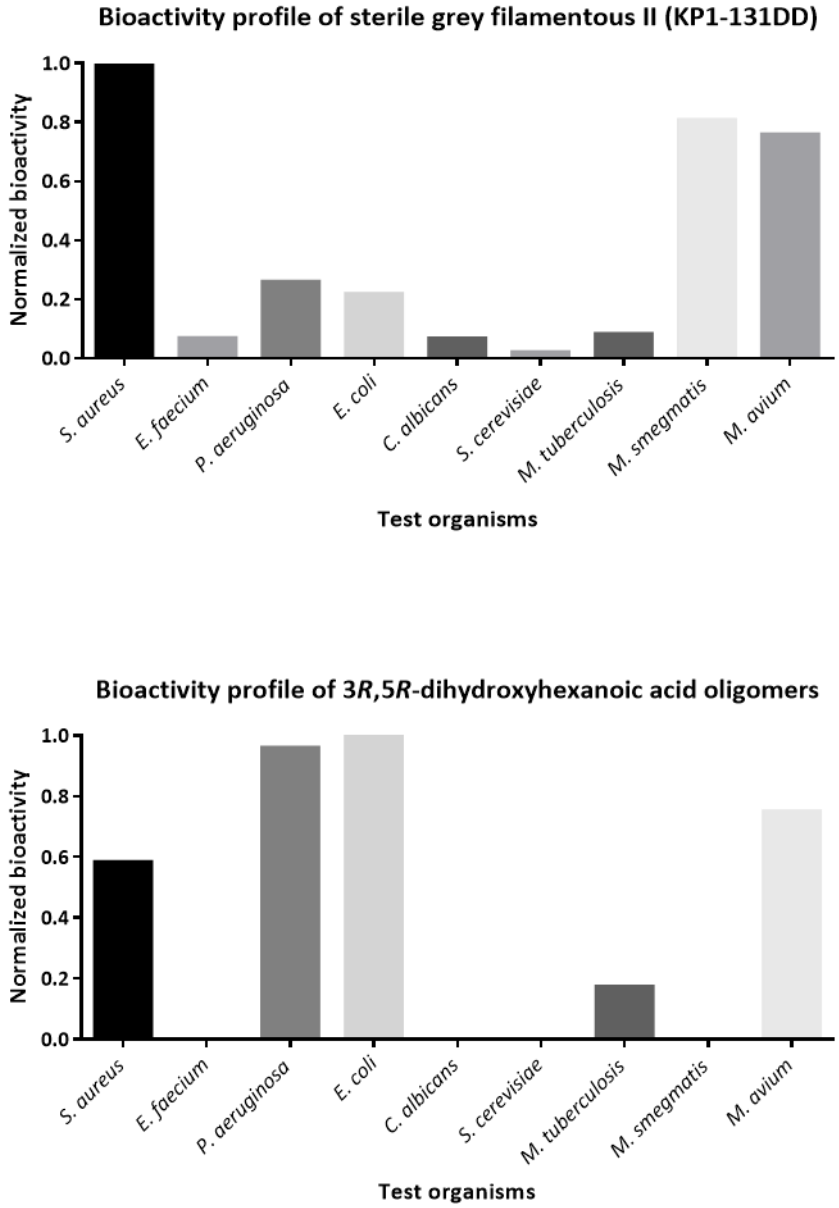


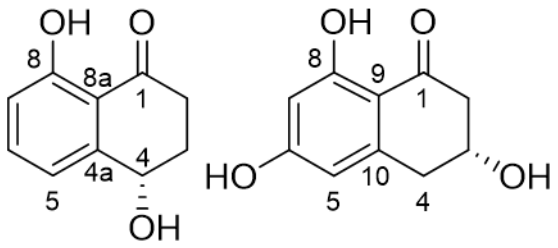
Figure 4.3.3.11. Bioactivity profiles of the extract from KP1-131DD and poly(3*R*,5*R*-dihydroxyhexanoic acid).

HPLC of two normal phase flash column fractions led to the isolation of two tetralones, isosclerone [**8**; (Klaiklay et al. 2012; Morita and Aoki 1974)] and scytalone [**9**; (Findlay and Kwan 1973b; Jordan et al. 2000; Viviani and Gaudry 1990; Xu et al. 2013)] (Figure 4.3.3.12), with the mass spectrometry, NMR spectroscopy (Tables 4.3.3.6 &

4.3.3.7), and infrared spectroscopy obtained for each compound consistent with literature values. While the polarimetry data for **8** confirmed the isolation of (+)-isosclerone, polarimetry of **9** gave an optical rotation of 0. While this may suggest a racemic mixture of enantiomers, (+)-scytalone has been reported to possess a very low specific rotation, and as such, is commonly reported as 0 in the literature (Viviani and Gaudry 1990). As a result of this low specific rotation, it was reported that optical rotation shouldn't be used to determine stereochemistry of **9** from natural sources and methods such as circular dichroism are more reliable for determining the stereochemistry of this compound (Viviani and Gaudry 1990). In the absence of a spectrophotometer for assessing the stereochemistry of **9**, and only one report of a similarly low specific rotation for (-)-scytalone ($[\alpha]^{22}_{D}$ -1.37) for comparison (Wheeler and Stipanovic 1985), the relative stereochemistry of **9** has thus been reported (Figure 4.3.3.12). Unfortunately, prior to the acquisition of a ^{13}C NMR spectrum for **8**, the compound degraded. Mass spectrometry and a comparison of the ^{1}H NMR spectrum with reported values and to a standard obtained by Trevor Clark in the Natural Products Research Group confirmed the structure of **8** as isosclerone.

Isosclerone (Figure 4.3.3.12; Table 4.3.3.6) was first isolated from *Sclerotinia sclerotiorum* (Morita and Aoki 1974) and has subsequently been reported from *Penicillium diversum* (Fujimoto et al. 1986), *Scolecotrichum graminis* (Tabuchi et al. 1994), *Urnula craterium* (Ayer et al. 2000), *Phaeoacremonium aleophilum* (Evidente et al. 2000), *Humicola fuscoatra* (Joshi et al. 2002), *Cytospora eucalypticola* (Kokubun et al. 2003), *Botrytis cinerea* (Evidente et al. 2011), *Pyrenochaeta* sp. (Lin et al. 2011), *Xylaria cubensis* (Klaiklay et al. 2012), *Paraphoma radicina* (El-Elimat et al. 2014),

Aspergillus fumigatus (Li et al. 2014c; Li et al. 2014d), *Neofusicoccum parvum* (Burruano et al. 2016; Evidente et al. 2010), and two unidentified Ascomycetes (Rukachaisirikul et al. 2007; Tian et al. 2015). Isosclerone has been reported for its ability to promote root growth (Morita and Aoki 1974), for its anticancer activity against MCF-7 breast cancer cells (Li et al. 2014c; Li et al. 2014d), and for its antibacterial activity against *Bacillus subtilis* (Kokubun et al. 2003) though unfortunately due to limited material it could not be assessed in this work for its antimicrobial activity.



8 **9**

Figure 4.3.3.12. The structures of isosclerone (**8**) and scytalone (**9**) isolated from an unidentified sterile grey filamentous endophyte.

Scytalone (Figure 4.3.3.12; Table 4.3.3.7) was first isolated from *Scytalidium* sp. (Ayer et al. 1993; Findlay and Kwan 1973a; Findlay and Kwan 1973b) and has also been reported from *Phialophora lagerbergii* (Aldridge et al. 1974), *Verticillium dahlia* (Bell et al. 1976a; Bell et al. 1976b), *Ceratocystis minor* (Hemingway et al. 1977), *Wangiella dermatitidis* (Geis et al. 1984), *Colletotrichum lagenarium* (Kubo et al. 1986), *Valsa ambiens* (Jiao et al. 1994), *Phaeoacremonium aleophilum* (Evidente et al. 2000), *Ceratocystis fimbriata* (Burki et al. 2003), *Alternaria helianthi* (Anitha and Murugesan 2008), *Leptographium qinlingensis* (Li et al. 2012), *X. nigripes* (Chang et al. 2017), *Cytospora rhizophorae* (Liu et al. 2017), *Cladosporium tenuissimum* (Naseer et al. 2017)

and *Trichoderma* sp. (Zhang et al. 2017). Scytalone has previously been reported for its phytotoxic effects (Burki et al. 2003; Evidente et al. 2000; Jiao et al. 1994) as well as possessing weak antimicrobial, anticancer (Naseer et al. 2017) and antineuroinflammatory activities (Chang et al. 2017), though unfortunately due to limited material it could not be assessed in this work for its antimicrobial activity.

Table 4.3.3.6. A comparison of the experimental and reported proton and carbon NMR data obtained for isosclerone (**8**).

No.	Experimental data in $CDCl_3$[a]	Reported data in $CDCl_3$[b]	
	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
1	-	204.3	-
2	2.65, 1H, ddd (*J* = 17.9, 8.4, 4.8 Hz) 3.01, 1H, ddd (*J* = 17.9, 8.4, 4.8 Hz)	34.6	2.65, 1H, ddd (*J* = 18.4, 8.1, 4.6 Hz) 3.01, 1H, ddd (*J* = 18.4, 8.1, 4.6 Hz)
3	2.19, 1H, m 2.35, 1H, m	34.2	2.15-2.22, 1H, m 2.30-2.40, 1H, m
4	4.92, 1H, dd (*J* = 7.4, 3.5 Hz)	67.7	4.92, 1H, dd (*J* = 7.2, 3.6 Hz)
4a	-	145.8	-
5	7.02, 1H, d (*J* = 7.5 Hz)	117.4	7.02, 1H, d (*J* = 8.6 Hz)
6	7.50, 1H, dd (*J* = 8.4, 7.5 Hz)	137.0	7.50, 1H, dd (*J* = 8.6, 7.6 Hz)
7	6.93, 1H, d (*J* = 8.4 Hz)	117.8	6.92, 1H, d (*J* = 8.6 Hz)
8	-	162.7	-
8a	-	115.0	-
8-OH	12.42, 1H, s		12.42, 1H, s

[a] Prior to the acquisition of a ^{13}C NMR spectrum for **8**, the compound degraded.
[b] Klaiklay et al. 2012

Table 4.3.3.7. A comparison of the experimental and reported proton and carbon NMR data obtained for scytalone (**9**).

No.	Experimental data in CD_3OD		Reported data in CD_3OD[a]	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
1	202.3, C	-	202.4	-
2	47.4, CH_2	2.61, 1H, dd (*J* = 17.3, 7.8 Hz) 2.84, 1H, dd (*J* = 16.8, 3.6 Hz)	47.4	2.61, 1H, dd (*J* = 17.2, 7.6 Hz) 2.84, 1H, dd (*J* = 16.8, 3.2 Hz)
3	66.9, CH	4.25, 1H, m	66.9	4.25, 1H, m
4	39.2, CH_2	2.84, 1H, d (*J* = 16.8 Hz) 3.08, 1H, dd (*J* = 16.0, 3.6 Hz)	39.2	2.84, 1H, d (*J* = 16.0 Hz) 3.07, 1H, d (*J* = 16.0 Hz)
5	109.6, CH	6.22, 1H, brs	109.4	6.22, 1H, brs
6	166.6, C	-	166.7	-
7	101.7, CH	6.10, 1H, brd	101.7	6.09, 1H, brs
8	166.6, C	-	166.5	-
9	111.5, C	-	111.7	-
10	145.9, C	-	146.0	-

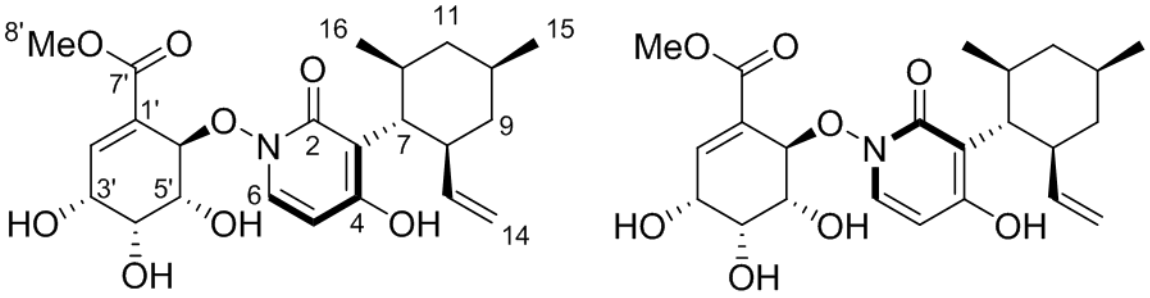
[a] Xu et al. 2013

4.3.4 *Tolypocladium* sp. (KP1-175E)

The EtOAc extract of KP1-175E obtained from a two-week bench-scale (4L) fermentation was fractionated using a modified Kupchan liquid-liquid partition, with the CH_2Cl_2 fraction showing significant antimicrobial activity. Size exclusion chromatography of the CH_2Cl_2 fraction over Sephadex® LH-20 followed by reverse phase high performance liquid chromatography (RP-HPLC) of the subsequent bioactive fraction led to the isolation of an inseparable mixture of (*P*/*M*)-maximiscin (*P*-**10** & *M*-**10**; Figure 4.3.4.1; Tables 4.3.4.1 and 4.3.4.2). (*P*/*M*)-Maximiscin was identified by comparing the experimental mass spectrometry, NMR spectroscopy, and polarimetry data obtained with those of literature values (Du et al. 2014b). Moderate antimicrobial activity was observed for **10** against *Mycobacterium tuberculosis* H37Ra, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and methicillin-resistant *S. aureus* (Table 4.3.4.3); however, **10** was found to be nearly as cytotoxic when tested against the human embryonic kidney HEK-293 cell line (Table 4.3.4.3; therapeutic indices of 1.6 1.1, 2.7, 0.95 and 1.2, respectively). The bioactivity profile of **10** possessed different activity than what was observed for the *Tolypocladium* sp., extract (Figure 4.3.4.2; 25 µg/mL test concentration; methodology as reported in Chapter 3). No activity towards *Candida albicans* or *Mycobacterium smegmatis* was observed for **10**; however, it did possess greater activity towards *S. aureus*, *P. aeruginosa* or *E. coli* (Figure 4.3.4.2).

(*P*/*M*)-Maximiscin is a unique polyketide-shikimate-NRPS hybrid compound that has only been reported once previously from an Alaskan soil-derived *Tolypocladium* sp. and was obtained through chemical epigenetic modification of the fungus, culture medium

variation and co-culturing strategies (Du et al. 2014b). This is the first report of **10** having been produced by a fungal endophyte and while **10** has been shown to be antiproliferative and cytotoxic to several cancer cell lines, including the triple negative breast cancer MDA-MB-468 cell line and the UACC-62 melanoma cell line when tested using *in vitro* and *in vivo* models (Du et al. 2014b; Robles et al. 2016), it has not been reported previously for antibacterial or antimycobacterial activity. (*P/M*)-Maximiscin has shown moderate antifungal activity against *Aspergillus niger*, *Tolypocladium* spp., and *Penicillium* spp. (Du et al. 2016).



***P*-10** ***M*-10**

Figure 4.3.4.1. The structures of (*P*/*M*)-maximiscin (**10**).

Table 4.3.4.1. A comparison of the experimental and reported proton and carbon NMR data obtained for *P*-maximiscin (*P*-**10**).

No.	Experimental data in $(CD_3)_2SO$		Reported data in $(CD_3)_2SO$[a]	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
2	159.3, C	-	159.3	-
3	113.2, C	-	113.1	-
4	162.3, C	-	162.5	-
5	98.3, CH	5.95, 1H, d (*J* = 7.9 Hz)	98.4	5.95, 1H, d (*J* = 7.8 Hz)
6	134.2, CH	7.53, 1H, d (*J* = 7.9 Hz)	134.2	7.53, 1H, d (*J* = 7.8 Hz)
7	45.8, CH	2.35, 1H, t (*J* = 10.3 Hz)	45.8	2.36, 1H, t (*J* = 10.5 Hz)
8	42.0, CH	2.93, 1H, m	42.0	2.93, 1H, m
9	41.7, CH_2	1.67, 1H, m 0.85, 1H, m	41.7	1.68, 1H, m 0.86, 1H, m
10	31.4, CH	1.54, 1H, m	31.4	1.53, 1H, m
11	44.1, CH_2	1.67, 1H, m 0.69, 1H, m	44.1	1.67, 1H, m 0.69, 1H, m
12	31.3, CH	2.33, 1H, m	31.2	2.32, 1H, m
13	143.3, CH	5.46, 1H, m	143.3	5.40, 1H, m
14	112.5, CH_2	4.76, 1H, dd (*J* = 16.9, 1.8 Hz) 4.63, 1H, dd (*J* = 10.3, 2.3 Hz)	112.5	4.73, 1H, dd (*J* = 16.5, 1.8 Hz) 4.62, 1H, dd (*J* = 10.3, 1.8 Hz)
15	22.7, CH_3	0.89, 3H, d (*J* = 6.5 Hz)	22.7	0.89, 3H, d (*J* = 6.2 Hz)
16	20.4, CH_3	0.66, 3H, d (*J* = 6.0 Hz)	20.5	0.65, 3H, d (*J* = 6.2 Hz)
1′	126.9, C	-	127.0	-
2′	145.7, CH	6.72, 1H, m	145.7	6.72, 1H, ddd (*J* = 2.5, 1.0, 1.0 Hz)
3′	66.9, CH	4.26, 1H, brs	67.0	4.26, 1H, brs
4′	71.1, CH	3.92, 1H, brs	71.1	3.92, 1H, brs
5′	71.0, CH	3.97, 1H, dd (*J* = 6.5, 1.9 Hz)	71.0	3.96, 1H, dd (*J* = 6.2, 1.5 Hz)
6′	86.5, CH	4.89, 1H, ddd (*J* = 6.3, 1.1, 0.8 Hz)	86.5	4.89, 1H, ddd, (*J* = 6.2, 1.0, 0.9 Hz)
7′	166.0, C	-	166.0	-
8′	51.2, CH_3	3.69, 3H, s	52.1	3.68, 3H, s
4-OH		10.32, 1H, brs		10.37, 1H, brs
5′-OH		6.08, 1H, brs		6.10, 1H, brs
4′-OH		5.02, 1H, brs		4.96, 1H, brs

[a] Du et al. 2014.

Table 4.3.4.2. A comparison of the experimental and reported proton and carbon NMR data obtained for *M*-maximiscin (*M*-**10**).

No.	Experimental data in $(CD_3)_2SO$		Reported data in $(CD_3)_2SO$[a]	
	δ_C ppm, type	δ_H ppm int., mult., (*J* in Hz)	δ_C ppm	δ_H ppm int., mult., (*J* in Hz)
2	161.5, C	-	161.5	-
3	113.6, C	-	113.6	-
4	161.3, C	-	161.5	-
5	98.9, CH	5.92, 1H, d (*J* = 7.9 Hz)	99.0	5.91, 1H, d (*J* = 7.8 Hz)
6	134.0, CH	7.53, 1H, d (*J* = 7.9 Hz)	134.0	7.52, 1H, d (*J* = 7.8 Hz)
7	45.8, CH	2.59, 1H, t (*J* = 11.0 Hz)	45.9	2.59, 1H, t, (*J* = 10.5 Hz)
8	42.9, CH	2.75, 1H, m	42.9	2.74, 1H, m
9	42.0, CH_2	1.70, 1H, m 0.81, 1H, m	42.0	1.70, 1H, m 0.81, 1H, m
10	31.4, CH	1.54, 1H, m	31.4	1.53, 1H, m
11	44.3, CH_2	1.67, 1H, m 0.69, 1H, m	44.3	1.67, 1H, m 0.69, 1H, m
12	32.3, CH	2.13, 1H, m	32.4	2.15, 1H, m
13	143.1, CH	5.53, 1H, m	143.1	5.53, 1H, m
14	112.6, CH_2	4.77, 1H, dd (*J* = 16.8, 1.8 Hz) 4.67, 1H, dd (*J* = 10.3, 2.3 Hz)	112.6	4.75, 1H, dd (*J* = 16.5, 1.8 Hz) 4.66, 1H, dd (*J* = 10.3, 1.8 Hz)
15	22.6, CH_3	0.89, 3H, d (*J* = 6.5 Hz)	22.6	0.89, 3H, d (*J* = 6.2 Hz)
16	20.4, CH_3	0.63, 3H, d (*J* = 6.6 Hz)	20.4	0.62, 3H, d (*J* = 6.2 Hz)
1′	127.1, C	-	127.1	-
2′	145.5, CH	6.69, 1H, m	145.5	6.69, 1H, ddd (*J* = 2.5, 1.0, 1.0 Hz)
3′	67.0, CH	4.26, 1H, brs	67.0	4.26, 1H, brs
4′	71.2, CH	3.92, 1H, brs	71.2	3.92, 1H, brs
5′	71.3, CH	3.97, 1H, dd (*J* = 6.5, 1.9 Hz)	71.3	3.96, 1H, dd (*J* = 6.2, 1.5 Hz)
6′	86.7, CH	4.86, 1H, m	86.8	4.87, 1H, ddd (*J* = 6.2, 1.0, 0.9 Hz)
7′	166.0, C	-	166.0	-
8′	52.1, CH_3	3.66, 3H, s	52.0	3.66, 3H, s
4-OH		10.36, 1H, brs		10.37, 1H, brs
5′-OH		6.15, 1H, brs		6.16, 1H, brs
4′-OH		4.98, 1H, brs		4.96, 1H, brs

[a] Du et al. 2014.

Table 4.3.4.3. Biological activity (MIC and IC_{50} in μM) of (*P/M*)-maximiscin (**10**) isolated from a *Tolypocladium* sp. endophyte of *Spongomorpha arcta*.

Test organism	MIC[a]	Mean IC_{50}[a] (95% CI)[b]
Mycobacterium tuberculosis H37Ra	250	52 (46-59)
Escherichia coli	250	75 (68-82)
Pseudomonas aeruginosa	250	30 (24-38)
Staphylococcus aureus	125	86 (69-108)
Methicillin resistant *S. aureus*	125	67 (56-80)
Human embryonic kidney 293 cells	> 500	82 (68-98)

[a] IC_{50}: median lethal concentration; MIC: minimum inhibitory concentration.
[b] 95% CI: 95% confidence interval.

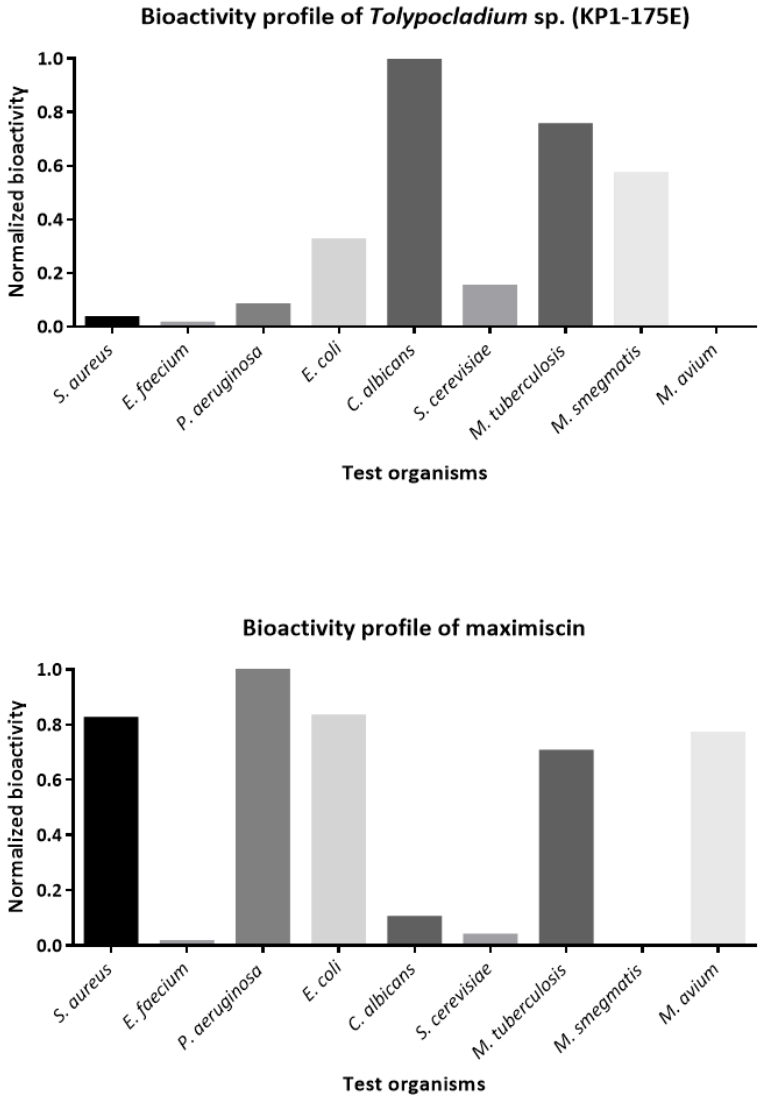


Figure 4.3.4.2. Bioactivity profiles of the extract from KP1-175E and (*P/M*)-maximiscin.

4.4 Conclusions

Bioassay guided fractionation of four fungal extracts led to the isolation of six antimicrobial metabolites: penicillic acid and methylenolactocin from *Penicillium* sp. IX, fumagillin and fumigatin oxide from *Aspergillus fumigatus* III, poly(3*R*,5*R*-dihydroxyhexanoic acid) from sterile grey filamentous II, and (*P/M*)-maximiscin from *Tolypocladium* sp. Two natural products also isolated from sterile grey filamentous II, isosclerone and scytalone, were obtained in a yield insufficient for characterising their bioactivity. While all known chemical entities, the majority of the natural products isolated in this research possessed unique antimicrobial activity. In fact, with the exception of penicillic acid, none of the compounds isolated have been previously reported to possess antimycobacterial activity towards *M. tuberculosis*. In addition, (*P/M*)-maximiscin, reported in the literature as possessing potent anticancer activity, has not been previously reported to possess antibacterial or antimycobacterial activity, highlighting the potential for this natural product in numerous biological applications. Also, poly(3*R*,5*R*-dihydroxyhexanoic acid) has had no reported biological activity. This work has revealed that this collection of oligomers has promising antimicrobial activity and should be investigated to see if it possesses additional bioactivity towards other targets.

None of the natural products isolated in the course of this research have had well defined modes of action. In fact, only penicillic acid has a report regarding its antimicrobial mode of action, whereby it was only broadly described as acting on sulfhydryl groups of bacterial enzymes. As (*P/M*)-maximiscin and poly(3*R*,5*R*-dihydroxyhexanoic acid) have not been reported for their antimicrobial activity, and as

both compounds possessed broad spectrum activity towards Gram positive bacteria, Gram negative bacteria and mycobacteria, future work investigating their antibacterial modes of action is warranted. In order to gather information regarding their potential modes of action, the bioactivity profiles of each natural product were reintroduced into the cluster analysis (Figure 4.4.1). It was observed that both (*P/M*)-maximiscin and poly(3*R*,5*R*-dihydroxyhexanoic acid) clustered closest to the fluoroquinolone ciprofloxacin, an antibiotic that targets the DNA synthesis of bacteria. Future work should investigate this observation to see if these two compounds also target bacteria in the same manner.

In summary, the bioassay-guided fraction of four endophytes extracts led to the isolation of natural products with promising antimicrobial activity. The use of bioactivity profiling highlighted an additional 22 extracts of interest and certainly warrant investigation in order to determine their antimicrobial natural products. With the encouraging results reported in this chapter, we are confident that our bioactivity profiling technique will be a valuable tool for prioritising natural product extracts for bioassay guided fractionation.

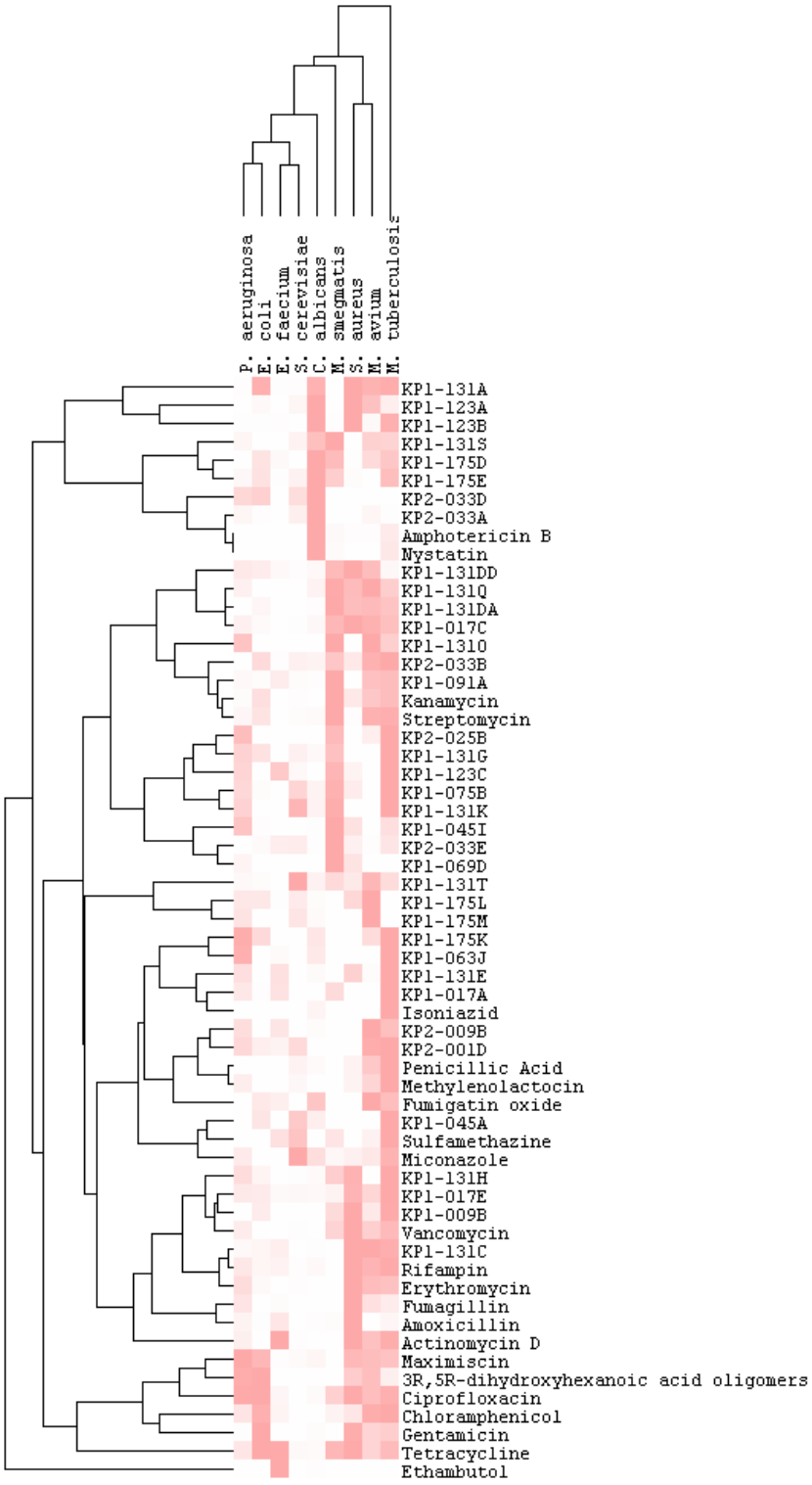


Figure 4.4.1. Hierarchical cluster analysis (Euclidean distance, average linkage) of bioactivity profiles of the isolated natural products and their extracts in relation to the bioactivity profiles of the antimicrobial standards.

Chapter 5: General conclusions and future directions

As we are faced with increased concerns regarding antimicrobial drug resistance, new methods are needed to efficiently identify new antimicrobial drug leads. If we are to succeed in developing new antimicrobial pharmaceuticals, investigations into understudied biological reservoirs, such as fungal endophytes from the marine environment, coupled with improved technologies for the detection and characterization of novel natural products are required. The objective of the work presented in this thesis was to isolate and identify fungal endophytes from 20 species of marine macroalgae of the Bay of Fundy, New Brunswick, Canada and to subsequently prioritise the extracts derived from these endophytes for bioassay-guided fractionation using a bioactivity profiling technique. Furthermore, this work aimed to isolate and identify antimicrobial natural products from four fungal extracts.

Marine macroalgae have been highlighted in the literature as an excellent source of a diverse range of fungal endophytes, despite the fact that such a low proportion of marine macroalgae have been investigated worldwide. One hundred and forty distinct fungal endophytes were isolated from 18 of the 20 marine macroalgae investigated. Seven of the host algae, namely, *Alaria esculenta*, *Desmarestia viridis*, *Dumontia contorta*, *Fucus distichus* subsp. *edentatus*, *F. distichus* subsp. *evanescens*, *Petalonia fascia*, and *Scytosiphon lomentaria*, have not been previously investigated for their endophytic fungi. The study of *S. lomentaria* led to the isolation of 33 distinct fungal endophytes, the highest of any of the hosts investigated in this work. Although a large proportion of sterile isolates and *Penicillium* spp. were isolated from marine macroalgae, eight of the endophytes isolated could not be taxonomically identified due to low sequence homology with entries in GenBank, suggesting the potential isolation of new fungal species as endophytes of

marine macroalgae. Also, eleven endophytes have not been previously isolated from marine macroalgae. Future studies into the assemblages of fungal endophytes from marine macroalgae are certainly warranted, due to not only the array of endophytes obtained from marine macroalgae, but also the great potential for isolating new species of fungi that may possess potent biological activity.

In the current climate of natural products research where the chance for reisolating known compounds is high, strategies for rapidly prioritising and dereplicating natural product libraries are critical to discovering novel chemistry. Metabolomic ventures aim to quickly dereplicate known natural products through NMR or mass spectrometry techniques, whereas antimicrobial bioactivity profiling presents an opportunity to not only prioritise unique extracts within a library, but also glean information about the potential modes of action of the bioactive constituents responsible for the antimicrobial activity by incorporating the profiles of known antimicrobial drugs that act on a range of biological targets. Extracts from each of the 140 fungal endophytes were screened against a suite of nine microorganisms in a microbroth dilution assay. The use of PCA allowed for the comparison of all extracts to each other to identify any outliers within the data set. The analysis successfully identified 37 extracts that were unique to the library as the remaining extracts primarily showed activity only towards *M. smegmatis* and/or *M. tuberculosis*. The bioactivity profiles of these 37 extracts were subsequently compared to the bioactivity profiles of 17 antimicrobial standards covering a range of biological targets. The use of hierarchical cluster analysis revealed the profiles of 26 extracts that did not cluster with the profiles of any of the 17 antimicrobial standards, suggesting that these extracts possess antimicrobial natural products that function differently to the 17 antimicrobials. These

extracts therefore warrant further investigation into their biologically active constituents to identify the natural products responsible for the observed bioactivity. A successful bioactivity profiling platform has been developed, forming the foundation for further expansion using prefractionation of the extracts, incorporation of additional antimicrobial standards and different sample concentrations for identifying extracts with unique bioactivity profiles. This simplified bioactivity profiling technique can also be adapted in other research laboratories to screen natural product extracts from any source (microbial, plant, invertebrate, etc.) and can be modified to prioritise extracts with biological activity towards other biological targets such as various cancerous cell lines.

Four extracts were fractionated using bioassay-guided fractionation. The fractionation of the extract of *Penicillium* sp. IX led to the isolation of two polyketides, penicillic acid and methylenolactocin. Though known for possessing antimicrobial activity, methylenolactocin has not been previously reported for its antimycobacterial activity. The fractionation of the extract of *Aspergillus fumigatus* III led to the isolation of fumagillin and fumigatin oxide. Fumagillin has been the focus of considerable study and has been shown to possess an array of biological activities; however, fumigatin oxide, despite the substantial research into *A. fumigatus*, has rarely been reported from this source. The investigation of the extract of a sterile grey filamentous isolate led to the isolation of poly(3*R*,5*R*-dihydroxyhexanoic acid), isosclerone and scytalone. Originally thought to possess a lactone or free acid structure, poly(3*R*,5*R*-dihydroxyhexanoic acid was found to possess moderate antimicrobial activity for the first time and has only been reported from one other biological source. Isosclerone and scytalone were isolated in low yield, therefore these compounds could not be characterised for their antimicrobial

activity. Lastly, the fractionation of the extract of a *Tolypocladium* sp. led to the isolation of (*P/M*)-maximiscin. This unique inseparable mixture of atropisomers has been reported for its potent anticancer activity; however, this molecule has not been reported to possess the broad spectrum antibacterial activity that was observed in this work. Though (*P/M*)-maximiscin has been previously isolated from a *Tolypocladium* sp., this is only the second report of this metabolite, and the first report of this natural product from an endophyte.

Marine macroalgae are an excellent source of endophytic fungi with a range of antimicrobial activities and will continue to be an important biological reservoir for identifying novel natural products. Bioactivity profiling has been shown to be a valuable tool for prioritising extracts in a library for fractionation by providing a platform that can be adapted and used by any research group interested in identifying novel bioactive natural products. Bioassay-guided fractionation of four extracts led to the isolation of structurally diverse antimicrobial natural products, with an additional 22 promising extracts showing an array of antimicrobial activity remaining prioritised for bioassay-guided fractionation to determine their biologically active constituents. New antimicrobial therapeutics are needed to overcome the drug resistance crisis we are facing and in order to address this issue, we must continue to investigate unique biological sources for their natural products and develop more refined and efficient techniques for their identification. The endophytic fungi isolated from marine macroalgae, the screening technique used and the natural products identified through the course of this research may play important roles in future antimicrobial drug discovery and development.

REFERENCES

Abdel-Lateff, A. 2008. Chaetominedione, a new tyrosine kinase inhibitor isolated from the algicolous marine fungus *Chaetomium* sp. Tetrahedron Letters **49**(45): 6398-6400.

Abdel-Lateff, A., Fisch, K.M., Wright, A.D., and Konig, G.M. 2003a. A new antioxidant isobenzofuranone derivative from the algicolous marine fungus *Epicoccum* sp. Planta Medica **69**(9): 831-834.

Abdel-Lateff, A., Klemke, C., Konig, G.M., and Wright, A.D. 2003b. Two new xanthone derivatives from the algicolous marine fungus *Wardomyces anomalus*. Journal of Natural Products **66**(5): 706-708.

Abdel-Lateff, A., Konig, G.M., Fisch, K.M., Holler, U., Jones, P.G., and Wright, A.D. 2002. New antioxidant hydroquinone derivatives from the algicolous marine fungus *Acremonium* sp. Journal of Natural Products **65**(11): 1605-1611.

Aldridge, D.C., Davies, A.B., Jackson, M.R., and Turner, W.B. 1974. Pentaketide metabolites of the fungus *Phialophora lagerbergii*. Journal of the Chemical Society, Perkin Transactions 1(0): 1540-1541.

Almeida, J.A.S., Barbosa, L.M.S., Pais, A.A.C.C., and Formosinho, S.J. 2007. Improving hierarchical cluster analysis: A new method with outlier detection and automatic clustering. Chemometrics and Intelligent Laboratory Systems **87**(2): 208-217.

Aly, A.H., Debbab, A., and Proksch, P. 2011. Fifty years of drug discovery from fungi. Fungal Diversity **50**(1): 3-19.

Aminov, R. 2017. History of antimicrobial drug discovery: Major classes and health impact. Biochemical Pharmacology **133**: 4-19.

An, C.Y., Li, X.M., Li, C.S., Gao, S.S., Shang, Z., and Wang, B.G. 2013. Triazoles and other N-containing metabolites from the marine-derived endophytic fungus *Penicillium chrysogenum* EN-118. Helvetica Chimica Acta **96**(4): 682-687.

Anderson, H.H. 1952. The use of fumagillin in amoebiasis. Annals of the New York Academy of Sciences **55**(6): 1118-1124.

Andersson, D.I., and Hughes, D. 2010. Antibiotic resistance and its cost: Is it possible to reverse resistance? Nature Reviews Microbiology **8**(4): 260-271.

Andriole, V.T. 2005. The quinolones: Past, present, and future. Clinical Infectious Diseases **41**(Supplement_2): S113-S119.

Anitha, R., and Murugesan, K. 2008. Melanin production in *Alternaria helianthi*. Archives of Phytopathology and Plant Protection **41**(5): 360-364.

Ariffin, S.A., Davis, P., and Ramasamy, K. 2011. Cytotoxic and antimicrobial activities of Malaysian marine endophytic fungi. Botanica Marina **54**(1): 95-100.

Ariza, X., Garcia, J., López, M., and Montserrat, L. 2001. A concise synthesis of (-)-methylenolactocin and (-)-phaseolinic acid from (6S, 9S)-tetradec-7-yne-6, 9-diol. Synlett **2001**(01): 0120-0122.

Ayer, W.A., Lu, P.P., Orszanska, H., and Sigler, L. 1993. Deoxyscytalidin and lignicol: new metabolites from *Scytalidium* species. Journal of Natural Products **56**(10): 1835-1838.

Ayer, W.A., Trifonov, L.S., Hutchison, L.J., and Chakravarty, P. 2000. Metabolites from a wood-inhabiting cup fungus, *Urnula craterium*. Natural Product Letters **14**(6): 405-410.

Barklund, P., and Kowalski, T. 1996. Endophytic fungi in branches of Norway spruce with a particular reference to *Tryblidiopsis pinastri*. Canadian Journal of Botany **74**: 673-678.

Bates, C.R., Saunders, G.W., and Chopin, T. 2009. Historical versus contemporary measures of seaweed biodiversity in the Bay of Fundy. Botany **87**(11): 1066-1076.

Bates, S.E., Fojo, A.T., Weinstein, J.N., Myers, T.G., Alvarez, M., Pauli, K.D., and Chabner, B.A. 1995. Molecular targets in the National Cancer Institute drug screen. Journal of Cancer Research and Clinical Oncology **121**(9): 495-500.

Begg, E.J., and Barclay, M.L. 1995. Aminoglycosides - 50 years on. British Journal of Clinical Pharmacology **39**(6): 597-603.

Bell, A.A., Puhalla, J.E., Tolmsoff, W.J., and Stipanovic, R.D. 1976a. Use of mutants to establish (+)-scytalone as an intermediate in melanin biosynthesis by *Verticillium dahliae*. Canadian Journal of Microbiology **22**(6): 787-799.

Bell, A.A., Stipanovic, R.D., and Puhalla, J.E. 1976b. Pentaketide metabolites of *Verticillium dahliae*. Identification of (+)-scytalone as a natural precursor to melanin. Tetrahedron **32**(12): 1353-1356.

Bernan, V.S., Greenstein, M., and Maiese, W.M. 1997. Marine microorganisms as a source of new natural products. *In* Advances in Applied Microbiology. *Edited by* L.N.a.A. Saul, I. Academic Press. pp. 57-90.

Betina, V., Gasparikova, E., and Nemec, P. 1968. Isolation of penicillic acid from *Penicillium simplicissimum*. Biologia **24**(6): 482-485.

Birkinshaw, J.H., Oxford, A.E., and Raistrick, H. 1936. Studies in the biochemistry of micro-organisms. XLVIII. Penicillic acid, a metabolic product of *Penicillium puberulum* Bainier and *P. cyclopium* Westling. Biochemical Journal **30**((3)): 394-411.

Bladt, T.T., Durr, C., Knudsen, P.B., Kildgaard, S., Frisvad, J.C., Gotfredsen, C.H., Seiffert, M., and Larsen, T.O. 2013. Bio-activity and dereplication-based discovery of ophiobolins and other fungal secondary metabolites targeting leukemia cells. Molecules **18**(12): 14629-14650.

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2007. Marine natural products. Natural Product Reports **24**(1): 31-86.

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2008. Marine natural products. Natural Product Reports **25**(1): 35-94.

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2009. Marine natural products. Natural Product Reports **26**(2): 170-244.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2012. Marine natural products. Natural Product Reports **29**(2): 144-222.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2013. Marine natural products. Natural Product Reports **30**(2): 237-323.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2014. Marine natural products. Natural Product Reports **31**(2): 160-258.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2015. Marine natural products. Natural Product Reports **32**(2): 116-211.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2016. Marine natural products. Natural Product Reports **33**(3): 382-431.

Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H., and Prinsep, M.R. 2017. Marine natural products. Natural Product Reports **34**(3): 235-294.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2003. Marine natural products. Natural Product Reports **20**(1): 1-48.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2004. Marine natural products. Natural Product Reports **21**(1): 1-49.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2005. Marine natural products. Natural Product Reports **22**(1): 15-61.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2006. Marine natural products. Natural Product Reports **23**(1): 26-78.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2010. Marine natural products. Natural Product Reports **27**(2): 165-237.

Blunt, J.W., Copp, B.R., Munro, M.H., Northcote, P.T., and Prinsep, M.R. 2011. Marine natural products. Natural Product Reports **28**(2): 196-268.

Bologa, C.G., Ursu, O., Oprea, T.I., Melancon, C.E., 3rd, and Tegos, G.P. 2013. Emerging trends in the discovery of natural product antibacterials. Current Opinions in Pharmacology **13**(5): 678-687.

Bongiorno, V.A., Rhoden, S.A., Garcia, A., Polonio, J.C., Azevedo, J.L., Pereira, J.O., and Pamphile, J.A. 2016. Genetic diversity of endophytic fungi from *Coffea arabica* cv. IAPAR-59 in organic crops. Annals of Microbiology **66**(2): 855-865.

Bro, R., and Smilde, A.K. 2014. Principal component analysis. Analytical Methods **6**(9): 2812-2831.

Brown, D.G., Lister, T., and May-Dracka, T.L. 2014. New natural products as new leads for antibacterial drug discovery. Bioorganic & Medicinal Chemistry Letters **24**(2): 413-418.

Brown, E.D., and Wright, G.D. 2016. Antibacterial drug discovery in the resistance era. Nature **529**(7586): 336-343.

Bugni, T.S., and Ireland, C.M. 2004. Marine-derived fungi: A chemically and biologically diverse group of microorganisms. Natural Product Reports **21**(1): 143-163.

Burki, N., Michel, A., and Tabacchi, R. 2003. Naphthalenones and isocoumarins of the fungus *Ceratocystis fimbriata* f. sp. *platani*. Phytopathologia Mediterranea **42**(2): 191-198.

Burruano, S., Giambra, S., Mondello, V., Dellagreca, M., Basso, S., Tuzi, A., and Andolfi, A. 2016. Naphthalenone polyketides produced by *Neofusicoccum parvum*, a fungus

associated with grapevine *Botryosphaeria dieback*. Phytopathologia Mediterranea **55**(2): 197-206.

Butinar, L., Frisvad, J.C., and Gunde-Cimerman, N. 2011. Hypersaline waters - a potential source of foodborne toxigenic aspergilli and penicillia. FEMS Microbiology Ecology **77**(1): 186-199.

Caicedo, J.C., Singh, S., and Carpenter, A.E. 2016. Applications in image-based profiling of perturbations. Current Opinions in Biotechnology **39**: 134-142.

Carpenter, C.D., O'Neill, T., Picot, N., Johnson, J.A., Robichaud, G.A., Webster, D., and Gray, C.A. 2012. Anti-mycobacterial natural products from the Canadian medicinal plant *Juniperus communis*. Journal of Ethnopharmacology **143**(2): 695-700.

Carroll, G. 1988. Fungal endophytes in stems and leaves - from latent pathogen to mutualistic symbiont. Ecology **69**(1): 2-9.

Cassell, G.H., and Mekalanos, J. 2001. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. The Journal of the American Medical Association **285**(5): 601-605.

Chain, E., Florey, H.W., Gardner, A.D., Heatley, N.G., Jennings, M.A., Orr-Ewing, J., and Sanders, A.G. 1940. Penicillin as a chemotherapeutic agent. The Lancet **236**(6104): 226-228.

Chakrabarty, K., Defrenza, I., Denora, N., Drioli, S., Forzato, C., Franco, M., Lentini, G., Nitti, P., and Pitacco, G. 2015. Enzymatic resolution of α-methyleneparaconic acids and evaluation of their biological activity. Chirality **27**(3): 239-246.

Chan, Y. 2003. Biostatistics 104: correlational analysis. Singapore Medical Journal **44**(12): 614-619.

Chandrasekharam, M., and Liu, R.-S. 1998. Synthesis of natural α-methylene butyrolactones via tungsten−π-allyl complexes. Total synthesis of (−)-methylenolactocin. The Journal of Organic Chemistry **63**(24): 9122-9124.

Chang, J.-C., Hsiao, G., Lin, R.-K., Kuo, Y.-H., Ju, Y.-M., and Lee, T.-H. 2017. Bioactive constituents from the termite nest-derived medicinal fungus *Xylaria nigripes*. Journal of Natural Products **80**(1): 38-44.

Chen, X., Xie, S., Bhat, S., Kumar, N., Shapiro, T.A., and Liu, J.O. 2009. Fumagillin and fumarranol interact with *P. falciparum* methionine aminopeptidase 2 and inhibit malaria parasite growth *in vitro* and *in vivo*. Chemistry & Biology **16**(2): 193-202.

Choi, E., Park, J., Kim, Y., Jung, J., Lee, J., Kwon, H., and Yang, H. 2011. Apoptosis-inducing effect of diketopiperazine disulfides produced by *Aspergillus* sp. KMD 901 isolated from marine sediment on HCT116 colon cancer cell lines. Journal of Applied Microbiology **110**(1): 304-313.

Chu, M., Mierzwa, R., He, L., Xu, L., Patel, M., Patel, D., and Chan, T.-M. 2001. Structure of Sch 528647: A new antitumor antibiotic related to fumagillin. The Journal of antibiotics **54**(12): 1096-1099.

Church, A.H. 1893. A marine fungus. Annals of Botany **os-7**(3): 399-400.

Clark, A.M. 1996. Natural products as a resource for new drugs. Pharmaceutical Research **13**(8): 1133-1141.

Clay, K., and Schardl, C. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. American Naturalist **160**: S99-S127.

Cole, R.J., and Cox, R.H. 1981. Handbook of toxic fungal metabolites. Academic Press, New York, New York, USA.

Covell, D.G., Huang, R., and Wallqvist, A. 2007. Anticancer medicines in development: assessment of bioactivity profiles within the National Cancer Institute anticancer screening data. Molecular Cancer Therapeutics **6**(8): 2261-2270.

Covington, B.C., McLean, J.A., and Bachmann, B.O. 2017. Comparative mass spectrometry-based metabolomics strategies for the investigation of microbial secondary metabolites. Natural Product Reports **34**(1): 6-24.

Croteau, R., Kutchan, T.M., and Lewis, N.G. 2000. Natural products (secondary metabolites). *In* Biochemistry & Molecular Biology of Plants, B. American Society of Plant Biologists, Rockville, MD. pp. 1250-1318.

Crusemann, M., O'Neill, E.C., Larson, C.B., Melnik, A.V., Floros, D.J., da Silva, R.R., Jensen, P.R., Dorrestein, P.C., and Moore, B.S. 2017. Prioritizing natural product diversity in a collection of 146 bacterial strains based on growth and extraction protocols. Journal of Natural Products **80**(3): 588-597.

Cui, C.M., Li, X.M., Li, C.S., Proksch, P., and Wang, B.G. 2010a. Cytoglobosins A-G, cytochalasans from a marine-derived endophytic fungus, *Chaetomium globosum* QEN-14. Journal of Natural Products **73**(4): 729-733.

Cui, C.M., Li, X.M., Li, C.S., Sun, H.F., Gao, S.S., and Wang, B.G. 2009. Benzodiazepine alkaloids from marine-derived endophytic fungus *Aspergillus ochraceus*. Helvetica Chimica Acta **92**(7): 1366-1370.

Cui, C.M., Li, X.M., Meng, L., Li, C.S., Huang, C.G., and Wang, B.G. 2010b. 7-Nor-ergosterolide, a pentalactone-containing norsteroid and related steroids from the marine-derived endophytic *Aspergillus ochraceus* EN-31. Journal of Natural Products **73**(11): 1780-1784.

Czarnoleski, M., Olejniczak, P., Mikolajczak, P., Lembicz, M., and Kozlowski, J. 2010. Fungal endophytes protect grass seedlings against herbivory and allow economical seed production. Evolutionary Ecology Research **12**(6): 769-777.

D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G.B., Poinar, H.N., and Wright, G.D. 2011. Antibiotic resistance is ancient. Nature **477**(7365): 457-461.

Dai, J., Krohn, K., Flörke, U., Pescitelli, G., Kerti, G., Papp, T., Kövér, K.E., Bényei, A.C., Draeger, S., Schulz, B., and Kurtán, T. 2010. Curvularin-type metabolites from the fungus *Curvularia* sp. isolated from a marine alga. European Journal of Organic Chemistry **2010**(36): 6928-6937.

David, A.S., Haridas, S., LaButti, K., Lim, J., Lipzen, A., Wang, M., Barry, K., Grigoriev, I.V., Spatafora, J.W., and May, G. 2016. Draft genome sequence of *Microdochium bolleyi*, a dark septate fungal endophyte of beach grass. Genome Announcements **4**(2): e00270-00216.

Davies, J., and Davies, D. 2010. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews **74**(3): 417-433.

De Azevedo, M.B.M., Murta, M.M., and Greene, A.E. 1992. Novel enantioselective lactone construction. First synthesis of methylenolactocin, antitumor antibiotic from *Penicillium* sp. The Journal of Organic Chemistry **57**(17): 4567-4569.

de Errasti, A., Carmarán, C.C., and Novas, M.V. 2010. Diversity and significance of fungal endophytes from living stems of naturalized trees from Argentina. Fungal Diversity **41**(1): 29-40.

de Felício, R., Pavão, G.B., de Oliveira, A.L.L., Erbert, C., Conti, R., Pupo, M.T., Furtado, N.A.J.C., Ferreira, E.G., Costa-Lotufo, L.V., Young, M.C.M., Yokoya, N.S., and Debonsi, H.M. 2015. Antibacterial, antifungal and cytotoxic activities exhibited by endophytic fungi from the Brazilian marine red alga *Bostrychia tenella* (Ceramiales). Revista Brasileira de Farmacognosia **25**(6): 641-650.

de Hoon, M.J., Imoto, S., Nolan, J., and Miyano, S. 2004. Open source clustering software. Bioinformatics **20**(9): 1453-1454.

de Silva, E.D., Geiermann, A.-S., Mitova, M.I., Kuegler, P., Blunt, J.W., Cole, A.L.J., and Munro, M.H.G. 2009. Isolation of 2-pyridone alkaloids from a New Zealand marine-derived *Penicillium* species. Journal of Natural Products **72**(3): 477-479.

de Souza Leite, T., Cnossen-Fassoni, A., Pereira, O.L., Mizubuti, E.S., de Araujo, E.F., and de Queiroz, M.V. 2013. Novel and highly diverse fungal endophytes in soybean revealed by the consortium of two different techniques. Journal of Microbiology **51**(1): 56-69.

de Vita-Marques, A.M., Lira, S.P., Berlinck, R.G.S., Seleghim, M.H.R., Sponchiado, S.R.P., Tauk-Tornisielo, S.M., Barata, M., Pessoa, C., de Moraes, M.O., Cavalcanti, B.C., Nascimento, G.G.F., de Souza, A.O., Galetti, F.C.S., Silva, C.L., Silva, M., Pimenta, E.F., Thiemann, O., Passarini, M.R.Z., and Sette, L.D. 2008. A multi-screening approach for marine-derived fungal metabolites and the isolation of cyclodepsipeptides from *Beauveria felina*. Quimica Nova **31**(5): 1099-1103.

Deckert, R., and Garbary, D. 2005a. *Ascophyllum* and its symbionts. VI. Microscopic characterization of the *Ascophyllum nodosum* (Phaeophyceae), *Mycophycias ascophylli* (Ascomycetes) symbiotum. Algae **20**(3): 225-232.

Deckert, R.J., and Garbary, D.J. 2005b. *Ascophyllum* and its symbionts. VIII. Interactions among *Ascophyllum nodosum* (Phaeophyceae), *Mycophycias ascophylli* (Ascomycetes) and *Elachista fucicola* (Phaeophyceae). Algae **20**(4): 363-368.

dela Cruz, T.E., Schulz, B.E., Kubicek, C.P., and Druzhinina, I.S. 2006a. Carbon source utilization by the marine *Dendryphiella* species *D. arenaria* and *D. salina*. FEMS Microbiology Ecology **58**(3): 343-353.

dela Cruz, T.E., Wagner, S., and Schulz, B. 2006b. Physiological responses of marine *Dendryphiella species* from different geographical locations. Mycological Progress **5**(2): 108-119.

Demain, A.L. 2006. From natural products discovery to commercialization: a success story. Journal of Industrial Microbiology & Biotechnology **33**(7): 486-495.

Dethoup, T., Mortagua Gomes, N.G., Chaopongpang, S., and Kijjoa, A. 2016. *Aspergillus similanensis* sp nov from marine sponge in Thailand. Mycotaxon **131**(1): 7-15.

Dickens, F., and Jones, H.E.H. 1961. Carcinogenic activity of a series of reactive lactones and related substances. British Journal of Cancer **15**(1): 85-100.

Didier, E.S. 1997. Effects of albendazole, fumagillin, and TNP-470 on microsporidial replication in vitro. Antimicrobial Agents and Chemotherapy **41**(7): 1541-1546.

Didier, P.J., Phillips, J.N., Kuebler, D.J., Nasr, M., Brindley, P.J., Stovall, M.E., Bowers, L.C., and Didier, E.S. 2006. Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives *in vitro* and *in vivo*. Antimicrobial Agents and Chemotherapy **50**(6): 2146-2155.

Dobranic, J.K., Johnson, J.A., and Alikhan, Q.R. 1995. Isolation of endophytic fungi from eastern larch (*Larix laricina*) leaves from New Brunswick, Canada. Canadian Journal of Microbiology **41**(2): 194-198.

Du, F.-Y., Li, X., Li, X.-M., Zhu, L.-W., and Wang, B.-G. 2017. Indolediketopiperazine alkaloids from *Eurotium cristatum* EN-220, an endophytic fungus isolated from the marine alga *Sargassum thunbergii*. Marine Drugs **15**(2): 24.

Du, F.Y., Li, X.M., Li, C.S., Shang, Z., and Wang, B.G. 2012. Cristatumins A-D, new indole alkaloids from the marine-derived endophytic fungus *Eurotium cristatum* EN-220. Bioorganic & Medicinal Chemistry Letters **22**(14): 4650-4653.

Du, F.Y., Li, X.M., Song, J.Y., Li, C.S., and Wang, B.G. 2014a. Anthraquinone derivatives and an orsellinic acid ester from the marine alga-derived endophytic fungus *Eurotium cristatum* EN-220. Helvetica Chimica Acta **97**(7): 973-978.

Du, L., Robles, A.J., King, J.B., Powell, D.R., Miller, A.N., Mooberry, S.L., and Cichewicz, R.H. 2014b. Crowdsourcing natural products discovery to access uncharted dimensions of fungal metabolite diversity. Angewandte Chemie International Edition **53**(3): 804-809.

Du, L., You, J., Nicholas, K.M., and Cichewicz, R.H. 2016. Chemoreactive natural products that afford resistance against disparate antibiotics and toxins. Angewandte Chemie International Edition **55**: 4220-4225.

Ebel, R. 2010. Terpenes from marine-derived fungi. Marine Drugs **8**(8): 2340-2368.

Eble, T.E., and Hanson, F.R. 1951. Fumaglllin, an antibiotic from *Aspergillus fumigatus* H-3. Antibiotics & Chemotherapy **1**(1): 54-58.

El-Elimat, T., Raja, H.A., Figueroa, M., Falkinham, J.O., III, and Oberlies, N.H. 2014. Isochromenones, isobenzofuranone, and tetrahydronaphthalenes produced by *Paraphoma radicina*, a fungus isolated from a freshwater habitat. Phytochemistry **104**: 114-120.

Elsebai, M.F., El Maddah, F., Kehraus, S., and Koenig, G.M. 2015. New bicyclo-spartinols from the marine-derived fungus *Phaeosphaeria spartinae*. Natural Product Communications **10**(4): 637-639.

Elsebai, M.F., Kehraus, S., Gutschow, M., and Konig, G.M. 2009. New polyketides from the marine-derived fungus *Phaeosphaeria spartinae*. Natural Product Communications **4**(11): 1463-1468.

Elsebai, M.F., Kehraus, S., Gutschow, M., and Konig, G.M. 2010. Spartinoxide, a new enantiomer of A82775C with inhibitory activity toward HLE from the marine-derived fungus *Phaeosphaeria spartinae*. Natural Product Communications **5**(7): 1071-1076.

Elsebai, M.F., Kehraus, S., and Konig, G.M. 2013. Caught between triterpene- and steroid-metabolism: 4alpha-carboxylic pregnane-derivative from the marine alga-derived fungus *Phaeosphaeria spartinae*. Steroids **78**(9): 880-883.

Elsebai, M.F., Kehraus, S., Lindequist, U., Sasse, F., Shaaban, S., Gutschow, M., Josten, M., Sahl, H.G., and Konig, G.M. 2011a. Antimicrobial phenalenone derivatives from the marine-derived fungus *Coniothyrium cereale*. Organic & Biomolecular Chemistry **9**(3): 802-808.

Elsebai, M.F., Natesan, L., Kehraus, S., Mohamed, I.E., Schnakenburg, G., Sasse, F., Shaaban, S., Gutschow, M., and Konig, G.M. 2011b. HLE-inhibitory alkaloids with a polyketide skeleton from the marine-derived fungus *Coniothyrium cereale*. Journal of Natural Products **74**(10): 2282-2285.

Elsebai, M.F., Nazir, M., Kehraus, S., Egereva, E., Ioset, K.N., Marcourt, L., Jeannerat, D., Gütschow, M., Wolfender, J.L., and König, G.M. 2012. Polyketide skeletons from the marine alga-derived fungus *Coniothyrium cereale*. European Journal of Organic Chemistry **2012**(31): 6197-6203.

Erbert, C., Lopes, A.A., Yokoya, N.S., Furtado, N.A.J.C., Conti, R., Pupo, M.T., Lopes, J.L.C., and Debonsi, H.M. 2012. Antibacterial compound from the endophytic fungus *Phomopsis longicolla* isolated from the tropical red seaweed *Bostrychia radicans*. Botanica Marina **55**(4).

Evidente, A., Punzo, B., Andolfi, A., Cimmino, A., Melck, D., and Luque, J. 2010. Lipophilic phytotoxins produced by *Neofusicoccum parvum*, a grapevine canker agent. Phytopathologia Mediterranea **49**(1): 74-79.

Evidente, A., Sparapano, L., Andolfi, A., and Bruno, G. 2000. Two naphthalenone pentaketides from liquid cultures of *Phaeoacremonium aleophilum*, a fungus associated with esca of grapevine. Phytopathologia Mediterranea **39**(1): 162-168.

Evidente, A., Superchi, S., Cimmino, A., Mazzeo, G., Mugnai, L., Rubiales, D., Andolfi, A., and Villegas-Fernández, A.M. 2011. Regiolone and isosclerone, two enantiomeric phytotoxic naphthalenone pentaketides: Computational assignment of absolute configuration and its relationship with phytotoxic activity. European Journal of Organic Chemistry **2011**(28): 5564-5570.

Faeth, S.H., and Hammon, K.E. 1997. Fungal endophytes in oak trees: Long-term patterns of abundance and associations with leafminers. Ecology **78**(3): 810-819.

Fang, W., Lin, X., Wang, J., Liu, Y., Tao, H., and Zhou, X. 2016. Asperpyrone-type bis-naphtho-gamma-pyrones with COX-2-inhibitory activities from marine-derived fungus *Aspergillus niger*. Molecules **21**(7).

Faulkner, D.J. 1977. Interesting aspects of marine natural products chemistry. Tetrahedron **33**(12): 1421-1443.

Faulkner, D.J. 1984a. Marine natural products: metabolites of marine algae and herbivorous marine molluscs. Natural Product Reports **1**(3): 251.

Faulkner, D.J. 1984b. Marine natural products: metabolites of marine invertebrates. Natural Product Reports **1**(6): 551.

Faulkner, D.J. 1986. Marine natural products. Natural Product Reports **3**(1): 1-33.

Faulkner, D.J. 1987. Marine natural products. Natural Product Reports **4**(5): 539-576.

Faulkner, D.J. 1988. Marine natural products. Natural Product Reports **5**(6): 613-663.

Faulkner, D.J. 1990. Marine natural products. Natural Product Reports **7**(4): 269-309.

Faulkner, D.J. 1991. Marine natural products. Natural Product Reports **8**(2): 97-147.

Faulkner, D.J. 1992. Marine natural products. Natural Product Reports **9**(4): 323-364.

Faulkner, D.J. 1993. Marine natural products. Natural Product Reports **10**(5): 497-539.

Faulkner, D.J. 1994. Marine natural products. Natural Product Reports **11**(4): 355-395.

Faulkner, D.J. 1995. Marine natural products. Natural Product Reports **12**(3): 223-269.

Faulkner, D.J. 1996. Marine natural products. Natural Product Reports **13**(2): 75-125.

Faulkner, D.J. 1997. Marine natural products. Natural Product Reports **14**(3): 259-302.

Faulkner, D.J. 1998. Marine natural products. Natural Product Reports **15**(2): 113-158.

Faulkner, D.J. 1999. Marine natural products. Natural Product Reports **16**(2): 155-198.

Faulkner, D.J. 2000. Marine natural products. Natural Product Reports **17**(1): 7-55.

Faulkner, D.J. 2001. Marine natural products. Natural Product Reports **18**(1): 1-49.

Faulkner, D.J. 2002. Marine natural products. Natural Product Reports **19**(1): 1-48.

Fehr, M.J., Consiglio, G., Scalone, M., and Schmid, R. 1999. Asymmetric hydrogenation of substituted 2-pyrones. The Journal of Organic Chemistry **64**(16): 5768-5776.

Fenical, W. 1993. Chemical studies of marine bacteria - developing a new resource. Chemical Reviews **93**(5): 1673-1683.

Fernandes, R.A., and Chowdhury, A.K. 2011. A concise synthesis of paraconic acids: (−)-methylenolactocin and (−)-phaseolinic acid. Tetrahedron: Asymmetry **22**(10): 1114-1119.

Findlay, J.A., and Kwan, D. 1973a. Metabolites from a *Scytalidium* species. Canadian Journal of Chemistry **51**(19): 3299-3301.

Findlay, J.A., and Kwan, D. 1973b. Scytalone (3,6,8-trihydroxytetralone), a metabolite from a *Scytalidium* species. Canadian Journal of Chemistry **51**(10): 1617-1619.

Fischbach, M.A., and Walsh, C.T. 2009. Antibiotics for emerging pathogens. Science **325**(5944): 1089-1093.

Fleming, A. 1929. On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. Influenzae*. British Journal of Experimental Pathology **10**(3): 226-236.

Flewelling, A., Ellsworth, K., Sanford, J., Forward, E., Johnson, J., and Gray, C. 2013a. Macroalgal endophytes from the Atlantic coast of Canada: A potential source of antibiotic natural products? Microorganisms **1**(1): 175-187.

Flewelling, A.J. 2012. Bioactive natural products from marine macroalgal endophytes of the Shetland Islands, UK. Thesis. Department of Biology. University of New Brunswick, Saint John Campus, 198.

Flewelling, A.J., Currie, J., Gray, C.A., and Johnson, J.A. 2015. Endophytes from marine macroalgae: promising sources of novel natural products. Current Science **109**(1): 88-111.

Flewelling, A.J., Johnson, J.A., and Gray, C.A. 2013b. Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Botanica Marina **56**(3): 287-297.

Florey, H.W., and Jennings, M.A. 1942. Some biological properties of highly purified penicillin. British Journal of Experimental Pathology **23**(3): 120-123.

Forbes, J.E., Saicic, R.N., and Zard, S.Z. 1999. New radical reactions of S-alkoxycarbonyl xanthates. Total synthesis of (+-)-cinnamolide and (+-)-methylenolactocin. Tetrahedron **55**(12): 3791-3802.

Fredricks, D.N., Smith, C., and Meier, A. 2005. Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR. Journal of Clinical Microbiology **43**(10): 5122-5128.

Fries, L. 1988. *Ascophyllum nodosum* (Phaeophyta) in axenic culture and its response to the endophytic fungus *Mycosphaerella ascophylli* and epiphytic bacteria. Journal of Phycology **24**(3): 333-337.

Fries, N. 1979. Physiological characteristics of *Mycosphaerella ascophylli*, a fungal endophyte of the marine brown alga *Ascophyllum nodosum*. Physiologia Plantarum **45**(1): 117-121.

Fries, N., and Thorentolling, K. 1978. Identity of fungal endophyte of *Ascophyllum* with *Mycosphaerella ascophylli* established by means of fluorescent-antibody technique. Botanica Marina **21**(7): 409-411.

Frisvad, J., and Thrane, U. 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indexes and UV-VIS spectra (diode array detection). Journal of Chromatography **404**(1): 195-214.

Fujimoto, Y., Yokoyama, E., Takahashi, T., Uzawa, J., Morooka, N., Tsunoda, H., and Tatsuno, T. 1986. Studies on the metabolites of *Penicillium diversum* var. *aureum* I. Chemical & Pharmaceutical Bulletin **34**(4): 1497-1500.

Gamal-Eldeen, A.M., Abdel-Lateff, A., and Okino, T. 2009. Modulation of carcinogen metabolizing enzymes by chromanone A; a new chromone derivative from algicolous marine fungus *Penicillium* sp. Environmental Toxicology and Pharmacology **28**(3): 317-322.

Gao, S.-S., Li, X.-M., and Wang, B.-G. 2009. Perylene derivatives produced by *Alternaria alternata*, an endophytic fungus isolated from *Laurencia* species. Natural Product Communications **4**(11): 1477-1480.

Gao, S.S., Li, X.M., Du, F.Y., Li, C.S., Proksch, P., and Wang, B.G. 2011a. Secondary metabolites from a marine-derived endophytic fungus *Penicillium chrysogenum* QEN-24S. Marine Drugs **9**(1): 59-70.

Gao, S.S., Li, X.M., Li, C.S., Proksch, P., and Wang, B.G. 2011b. Penicisteroids A and B, antifungal and cytotoxic polyoxygenated steroids from the marine alga-derived endophytic fungus *Penicillium chrysogenum* QEN-24S. Bioorganic & Medicinal Chemistry Letters **21**(10): 2894-2897.

Gao, S.S., Li, X.M., Zhang, Y., Li, C.S., and Wang, B.G. 2011c. Conidiogenones H and I, two new diterpenes of cyclopiane class from a marine-derived endophytic fungus *Penicillium chrysogenum* QEN-24S. Chemistry & Biodiversity **8**(9): 1748-1753.

Garbary, D.J., Brackenbury, A., McLean, A.M., and Morrison, D. 2006. Structure and development of air bladders in *Fucus* and *Ascophyllum* (Fucales, Phaeophyceae). Phycologia **45**(5): 557-566.

Garbary, D.J., Burke, J., and Tian, L.N. 1991. The *Ascophyllum Polysiphonia Mycosphaerella* symbiosis. 2. Aspects of the ecology and distribution of *Polysiphonia lanosa* in Nova Scotia. Botanica Marina **34**(5): 391-401.

Garbary, D.J., Deckert, R.J., and Hubbard, C.B. 2005a. *Ascophyllum* and its symbionts. VII. Three-way interactions among *Ascophyllum nodosum* (Phaeophyceae), *Mycophycias ascophylli* (Ascomycetes) and *Vertebrata lanosa* (Rhodophyta). Algae **20**(4): 353-361.

Garbary, D.J., and Gautam, A. 1989. The *Ascophyllum*, *Polysiphonia*, *Mycosphaerella* symbiosis. I. Population ecology of *Mycosphaerella* from Nova Scotia. Botanica Marina **32**(2): 181-186.

Garbary, D.J., Jamieson, M.M., Fraser, S.J., Ferguson, C.A., and Cranston, P.S. 2005b. *Ascophyllum* (Phaeophyceae) and its symbionts. IX. A novel symbiosis between *Halocladius variabilis* (Chironomidae, Insecta) and *Elachista fucicola* (Elachistaceae, Phaeophyceae) from marine rocky shores of Nova Scotia. Symbiosis **40**(2): 61-68.

Garbary, D.J., and London, J.F. 1995. The *Ascophyllum Polysiphonial Mycosphaerella* symbiosis. V. Fungal infection protects *A. nosodum* from desiccation. Botanica Marina **38**(6): 529-533.

Garbary, D.J., and Macdonald, K.A. 1995. The *Ascophyllum Polysiphonia Mycosphaerella* symbiosis. IV. Mutualism in the *Ascophyllum Mycosphaerella* Interaction. Botanica Marina **38**(3): 221-225.

Gaudencio, S.P., and Pereira, F. 2015. Dereplication: racing to speed up the natural products discovery process. Natural Product Reports **32**(6): 779-810.

Geiger, W.B., and Conn, J.E. 1945. The Mechanism of the antibiotic action of clavacin and penicillic acid. Journal of the American Chemical Society **67**(1): 112-116.

Geis, P.A., Wheeler, M.H., and Szaniszlo, P.J. 1984. Pentaketide metabolites of melanin synthesis in the dematiaceous fungus *Wangiella dermatitidis*. Archives of Microbiology **137**(4): 324-328.

Genilloud, O. 2014. The re-emerging role of microbial natural products in antibiotic discovery. Antonie Van Leeuwenhoek **106**(1): 173-188.

Georgopapadakou, N.H., and Walsh, T.J. 1996. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrobial Agents and Chemotherapy **40**(2): 279-291.

Ghannoum, M.A., and Rice, L.B. 1999. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clinical Microbiology Reviews **12**(4): 501-517.

Ghatak, A., Sarkar, S., and Ghosh, S. 1997. Strategic use of retro diels-alder reaction in the construction of beta-carboxy-alpha-methylene-gamma-lactones. Total synthesis of methylenolactocin and protolichesterinic acid. Tetrahedron **53**(51): 17335-17342.

Ghosh, M., Bose, S., Maity, S., and Ghosh, S. 2009. Enantiodivergent synthesis of (−)-methylenolactocin and (+)-methylenolactocin from d-mannitol. Tetrahedron Letters **50**(50): 7102-7104.

Gijsen, H.J.M., and Wong, C.-H. 1994. Unprecedented asymmetric aldol reactions with three aldehyde substrates catalyzed by 2-deoxyribose-5-phosphate aldolase. Journal of the American Chemical Society **116**(18): 8422-8423.

Golinska, P., Wypij, M., Agarkar, G., Rathod, D., Dahm, H., and Rai, M. 2015. Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek **108**(2): 267-289.

Gotelli, N.J., and Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters **4**(4): 379-391.

Gould, I.M., and Bal, A.M. 2013. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence **4**(2): 185-191.

Greve, H., Schupp, P.J., Eguereva, E., Kehraus, S., Kelter, G., Maier, A., Fiebig, H.H., and Konig, G.M. 2008a. Apralactone A and a new stereochemical class of curvularins from the marine-derived fungus *Curvularia* sp. European Journal of Organic Chemistry **2008**(30).

Greve, H., Schupp, P.J., Eguereva, E., Kehraus, S., and König, G.M. 2008b. Ten-membered lactones from the marine-derived fungus *Curvularia* sp. Journal of Natural Products **71**(9): 1651-1653.

Griffith, E.C., Su, Z., Niwayama, S., Ramsay, C.A., Chang, Y.-H., and Liu, J.O. 1998. Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. Proceedings of the National Academy of Sciences of the United States of America **95**(26): 15183-15188.

Guiry, M.D. 2012. How many species of algae are there? Journal of Phycology **48**(5): 1057-1063.

Gunde-Cimerman, N., Ramos, J., and Plemenitas, A. 2009. Halotolerant and halophilic fungi. Mycological Research **113**(11): 1231-1241.

Guo, L.D., Huang, G.R., and Wang, Y. 2008. Seasonal and tissue age influences on endophytic fungi of *Pinus tabulaeformis* (Pinaceae) in the Dongling Mountains, Beijing. Journal of Integrative Plant Biology **50**(8): 997-1003.

Guo, L.D., Hyde, K.D., and Liew, E.C.Y. 2000. Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytologist **147**(3): 617-630.

Guo, Z., Ren, F., Che, Y., Liu, G., and Liu, L. 2015. New bergamotane sesquiterpenoids from the plant endophytic fungus *Paraconiothyrium brasiliense*. Molecules **20**(8): 14611-14620.

Haefner, B. 2003. Drugs from the deep: marine natural products as drug candidates. Drug Discovery Today **8**(12): 536-544.

Hajra, S., Karmakar, A., Giri, A.K., and Hazra, S. 2008. Concise syntheses of (+)- and (-)-methylenolactocins and phaseolinic acids. Tetrahedron Letters **49**(22): 3625-3627.

Hamayun, M., Khan, S.A., Iqbal, I., Na, C.I., Khan, A.L., Hwang, Y.H., Lee, B.H., and Lee, I.J. 2009. *Chrysosporium pseudomerdarium* produces gibberellins and promotes plant growth. Journal of Microbiology **47**(4): 425-430.

Hammond, S., Lambert, P., and Kliger, B. 1974. The mode of action of polyene antibiotics; induced potassium leakage in *Candida albicans*. Microbiology **81**(2): 325-330.

Hanson, F.R., and Eble, T.E. 1949. An antiphage agent isolated from *Aspergillus* sp. Journal of Bacteriology **58**(4): 527-529.

Haroon, M.H., Premaratne, S.R., Choudhry, M.I., and Dharmaratne, H.R. 2013. A new beta-glucuronidase inhibiting butyrolactone from the marine endophytic fungus *Aspergillus terreus*. Natural Product Research **27**(12): 1060-1066.

Harvey, J.B., and Goff, L.J. 2010. Genetic covariation of the marine fungal symbiont *Haloguignardia irritans* (Ascomycota, Pezizomycotina) with its algal hosts *Cystoseira* and *Halidrys* (Phaeophyceae, Fucales) along the west coast of North America. Fungal Biology **114**(1): 82-95.

Haslam, E. 1986. Secondary metabolism - fact and fiction. Natural Product Reports **3**: 217.

Hawas, U.W., and Al-Farawati, R. 2017. Chemical constituents and antiviral activity from marine endophytic fungi from red sea alga *Padina pavonica*. Journal of the Chemical Society of Pakistan **39**(3): 479-483.

Hawas, U.W., Al-Farawati, R., Abou El-Kassem, L.T., and Turki, A.J. 2016. Different culture metabolites of the Red sea fungus *Fusarium equiseti* optimize the inhibition of hepatitis C virus NS3/4A protease (HCV PR). Marine Drugs **14**(10).

Hawas, U.W., El-Beih, A.A., and El-Halawany, A.M. 2012. Bioactive anthraquinones from endophytic fungus *Aspergillus versicolor* isolated from red sea algae. Archives of Pharmacal Research **35**(10): 1749-1756.

Hawas, U.W., El-Halawany, A.M., and Ahmede, E.F. 2013. Hepatitis C virus NS3-NS4A protease inhibitors from the endophytic *Penicillium chrysogenum* isolated from the red alga *Liagora viscida*. Zeitschrift für Naturforschung C **68**(9-10): 355-366.

Hemingway, R.W., McGraw, G.W., and Barras, S.J. 1977. Polyphenols in *Ceratocystis minor* infected *Pinus taeda*: fungal metabolites, phloem and xylem phenols. Journal of Agricultural and Food Chemistry **25**(4): 717-722.

Higginbotham, S., Wong, W.R., Linington, R.G., Spadafora, C., Iturrado, L., and Arnold, A.E. 2014. Sloth Hair as a novel source of fungi with potent anti-parasitic, anti-cancer and anti-bacterial bioactivity. PLOS One **9**(1): e84549-Article No.: e84549.

Higgins, K.L., Coley, P.D., Kursar, T.A., and Arnold, A.E. 2011. Culturing and direct PCR suggest prevalent host generalism among diverse fungal endophytes of tropical forest grasses. Mycologia **103**(2): 247-260.

Hilarino, M.P.A., de Oliveira e Silveira, F.A., Oki, Y., Rodrigues, L., Santos, J.C., Correa Junior, A., Fernandes, G.W., and Rosa, C.A. 2011. Distribution of the endophytic fungi community in leaves of *Bauhinia brevipes* (Fabaceae). Acta Botanica Brasilica **25**(4): 815-821.

Hollants, J., Leliaert, F., Clerck, O., and Willems, A. 2010. How endo- is endo-? Surface sterilization of delicate samples: a *Bryopsis* (Bryopsidales, Chlorophyta) case study. Symbiosis **51**(1): 131-138.

Hon, Y.-S., Hsieh, C.-H., and Liu, Y.-W. 2005. Dibromomethane as one-carbon source in organic synthesis: total synthesis of (±)- and (−)-methylenolactocin. Tetrahedron **61**(10): 2713-2723.

Hou, Y., Braun, D.R., Michel, C.R., Klassen, J.L., Adnani, N., Wyche, T.P., and Bugni, T.S. 2012. Microbial strain prioritization using metabolomics tools for the discovery of natural products. Analytical Chemistry **84**(10): 4277-4283.

Hrenoff, A.K., and Nakamuraj, M. 1951. *In vitro* and *in vivo* studies of a new antibiotic, fumagillin, with *Endamoeba histolytica*. Proceedings of the Society for Experimental Biology and Medicine **77**(1): 162-164.

Hsiao, G., Chi, W.C., Pang, K.L., Chen, J.J., Kuo, Y.H., Wang, Y.K., Cha, H.J., Chou, S.C., and Lee, T.H. 2017. Hirsutane-type sesquiterpenes with inhibitory activity of microglial nitric oxide production from the red alga-derived fungus *Chondrostereum* sp. NTOU4196. Journal of Natural Products **80**(5): 1615-1622.

Huang, H., Feng, X., Xiao, Z., Liu, L., Li, H., Ma, L., Lu, Y., Ju, J., She, Z., and Lin, Y. 2011. Azaphilones and *p*-terphenyls from the mangrove endophytic fungus *Penicillium chermesinum* (ZH4-E2) isolated from the South China Sea. Journal of Natural Products **74**(5): 997-1002.

Huang, H.B., Feng, X.J., Liu, L., Chen, B., Lu, Y.J., Ma, L., She, Z.G., and Lin, Y.C. 2010. Three dimeric naphtho-gamma-pyrones from the mangrove endophytic fungus *Aspergillus tubingensis* isolated from *Pongamia pinnata*. Planta Medica **76**(16): 1888-1891.

Huang, Q., An, H., Song, H., Mao, H., Shen, W., and Dong, J. 2015. Diversity and biotransformative potential of endophytic fungi associated with the medicinal plant *Kadsura angustifolia*. Research in Microbiology **166**(1): 45-55.

Hughes, C.C., and Fenical, W. 2010. Antibacterials from the Sea. Chemistry - A European Journal **16**(42): 12512-12525.

Hurlbert, S.H. 1971. Nonconcept of species diversity - Critique and alternative parameters. Ecology **52**(4): 577-586.

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.

Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., and Folkman, J. 1990. Synthetic analogues of fumagillin that inhibit angiogenesis and supress tumor growth. Nature **348**(6301): 555-556.

Jayachandran, S., Lleras-Muney, A., and Smith, K.V. 2010. Modern medicine and the twentieth century decline in mortality: Evidence on the impact of sulfa drugs. American Economic Journal: Applied Economics **2**(2): 118-146.

Jesus, H.C.R.d., Jeller, A.H., Debonsi, H.M., Alves, P.B., and Porto, A.L.M. 2017. Multiple monohydroxylation products from *rac*-camphor by marine fungus *Botryosphaeria* sp. isolated from marine alga *Bostrychia radicans*. Journal of the Brazilian Chemical Society **28**(3): 498-504.

Ji, N.-Y., Liu, X.-H., Miao, F.-P., and Qiao, M.-F. 2013. Aspeverin, a new alkaloid from an algicolous strain of *Aspergillus versicolor*. Organic Letters **15**(10): 2327-2329.

Ji, N.-Y., and Wang, B.-G. 2016. Mycochemistry of marine algicolous fungi. Fungal Diversity **80**(1): 301-342.

Jiao, Y., Yoshihara, T., Akimoto, M., and Ichihara, A. 1994. Two phenolic compounds from *Valsa ambiens*. Bioscience, Biotechnology, and Biochemistry **58**(4): 784-785.

Johnson, J.A., and Whitney, N.J. 1989a. An investigation of needle endophyte colonization patterns with respect to height and compass direction in a single crown of balsam fir (*Abies balsamea*). Canadian Journal of Botany **67**(3): 723-725.

Johnson, J.A., and Whitney, N.J. 1989b. A study of fungal endophytes of needles of balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*) in New Brunswick, Canada, using culture and electron-microscope techniques. Canadian Journal of Botany **67**(12): 3513-3516.

Johnson, J.A., and Whitney, N.J. 1992. Isolation of fungal endophytes from black spruce (*Picea mariana*) dormant buds and needles from New Brunswick, Canada. Canadian Journal of Botany **70**(9): 1754-1757.

Johnsson, K., King, D.S., and Schultz, P.G. 1995. Studies on the mechanism of action of isoniazid and ethionamide in the chemotherapy of Tuberculosis. Journal of the American Chemical Society **117**(17): 5009-5010.

Jones, E.B.G. 2011a. Are there more marine fungi to be described? Botanica Marina **54**(4): 343-354.

Jones, E.B.G. 2011b. Fifty years of marine mycology. Fungal Diversity **50**(1): 73-112.

Jones, E.B.G., Stanley, S.J., and Pinruan, U. 2008. Marine endophyte sources of new chemical natural products: a review. Botanica Marina **51**(3): 163-170.

Jones, E.B.G., Suetrong, S., Sakayaroj, J., Bahkali, A.H., Abdel-Wahab, M.A., Boekhout, T., and Pang, K.-L. 2015. Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Diversity **73**(1): 1-72.

Jongkol, R., Choommongkol, R., Tarnchompoo, B., Nimmanpipug, P., and Meepowpan, P. 2009. Syntheses of methylenolactocin and nephrosterinic acid via diastereoselective acylation and chemoselective reduction-lactonization. Tetrahedron **65**(32): 6382-6389.

Jordan, D.B., Zheng, Y.-J., Lockett, B.A., and Basarab, G.S. 2000. Stereochemistry of the enolization of scytalone by scytalone dehydratase. Biochemistry **39**(9): 2276-2282.

Joshi, B.K., Gloer, J.B., and Wicklow, D.T. 2002. Bioactive natural products from a sclerotium-colonizing isolate of *Humicola fuscoatra*. Journal of Natural Products **65**(11): 1734-1737.

Kanafani, Z.A., and Perfect, J.R. 2008. Resistance to antifungal agents: mechanisms and clinical impact. Clinical Infectious Diseases **46**(1): 120-128.

Kanetsuna, F. 1985. Bactericidal effect of fatty acids on mycobacteria, with particular reference to the suggested mechanism of intracellular killing. Microbiology and Immunology **29**(2): 127-141.

Kang, S., and Kim, S. 2004. New antifungal activity of penicillic acid against *Phytophthora* species. Biotechnology Letters **26**(9): 695-698.

Kathiravan, M.K., Salake, A.B., Chothe, A.S., Dudhe, P.B., Watode, R.P., Mukta, M.S., and Gadhwe, S. 2012. The biology and chemistry of antifungal agents: A review. Bioorganic & Medicinal Chemistry **20**(19): 5678-5698.

Kathiresan, K., Manivannan, S., Nabeel, M., and Dhivya, B. 2009. Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. Colloids and Surfaces B-Biointerfaces **71**(1): 133-137.

Kaushik, N.K., Murali, T.S., Sahal, D., and Suryanarayanan, T.S. 2014. A search for antiplasmodial metabolites among fungal endophytes of terrestrial and marine plants of southern India. Acta Parasitologica **59**(4): 745-757.

Kavanagh, F. 1947. Activities of twenty-two antibacterial substances against nine species of bacteria. Journal of Bacteriology **54**(6): 761.

Kawahara, T., Takagi, M., and Shin-ya, K. 2012. Three new depsipeptides, JBIR-113, JBIR-114 and JBIR-115, isolated from a marine sponge-derived *Penicillium* sp fS36. The Journal of Antibiotics **65**(3): 147-150.

Kealey, C., Creaven, C.A., Murphy, C.D., and Brady, C.B. 2017. New approaches to antibiotic discovery. Biotechnology Letters.

Khalid, E.B., Ayman, E.-M.E.-K., Rahman, H., Abdelkarim, G., and Najda, A. 2016. Natural products against cancer angiogenesis. Tumor Biology **37**(11): 14513-14536.

Killough, J.H., Magill, G.B., and Smith, R.C. 1952. The treatment of amebiasis with fumagillin. Science: 71-72.

Kimura, Y., Nakahara, S., and Fujioka, S. 2014. Aspyrone, a nematicidal compound isolated from the fungus, *Aspergillus melleus*. Bioscience, Biotechnology, and Biochemistry **60**(8): 1375-1376.

Kis-Papo, T., Oren, A., Wasser, S.P., and Nevo, E. 2003. Survival of filamentous fungi in hypersaline dead sea water. Microbial Ecology **45**(2): 183-190.

Kjer, J., Debbab, A., Aly, A.H., and Proksch, P. 2010. Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nature Protocols **5**(3): 479-490.

Klaiklay, S., Rukachaisirikul, V., Sukpondma, Y., Phongpaichit, S., Buatong, J., and Bussaban, B. 2012. Metabolites from the mangrove-derived fungus *Xylaria cubensis* PSU-MA34. Archives of Pharmacal Research **35**(7): 1127-1131.

Klemke, C., Kehraus, S., Wright, A.D., and König, G.M. 2004. New secondary metabolites from the marine endophytic fungus *Apiospora montagnei*. Journal of Natural Products **67**(6): 1058-1063.

Kobayashi, J., and Ishibashi, M. 1993. Bioactive metabolites of symbiotic marine microorganisms. Chemical Reviews **93**(5): 1753-1769.

Koeman, R.P.T., and van den Hoek, C. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. British Phycological Journal **16**(1): 9-53.

Kohanski, M.A., Dwyer, D.J., and Collins, J.J. 2010. How antibiotics kill bacteria: from targets to networks. Nature Reviews Microbiology **8**(6): 423-435.

Kohlmeyer, J. 1972. Is *Ascophyllum nodosum* lichenized? Botanica Marina **15**(2): 109-112.

Kokubun, T., Veitch, N.C., Bridge, P.D., and Simmonds, M.S.J. 2003. Dihydroisocoumarins and a tetralone from *Cytospora eucalypticola*. Phytochemistry **62**(5): 779-782.

Kongsaeree, P., Meepowpan, P., and Thebtaranonth, Y. 2001. Synthesis of both enantiomers of methylenolactocin, nephrosterinic acid and protolichesterinic acid via tandem aldol–lactonization reactions. Tetrahedron: Asymmetry **12**(13): 1913-1922.

Konig, G.M., Kehraus, S., Seibert, S.F., Abdel-Lateff, A., and Muller, D. 2006. Natural products from marine organisms and their associated microbes. ChemBioChem **7**(2): 229-238.

Kontoyiannis, D.P., and Lewis, R.E. 2002. Antifungal drug resistance of pathogenic fungi. The Lancet **359**(9312): 1135-1144.

Kozak, M. 2009. What is strong correlation? Teaching Statistics **31**(3): 85-86.

Kralj, A., Kehraus, S., Krick, A., Eguereva, E., Kelter, G., Maurer, M., Wortmann, A., Fiebig, H.H., and Konig, G.M. 2006. Arugosins G and H: prenylated polyketides from the marine-derived fungus *Emericella nidulans* var. *acristata*. Journal of Natural Products **69**(7): 995-1000.

Krohn, K., Dai, J., Florke, U., Aust, H.J., Drager, S., and Schulz, B. 2005. Botryane metabolites from the fungus *Geniculosporium* sp. isolated from the marine red alga *Polysiphonia*. Journal of Natural Products **68**(3): 400-405.

Kubo, Y., Kato, M., Furusawa, I., and Shishiyama, J. 1986. Inhibition of melanin biosynthesis by cerulenin in appressoria of *Colletotrichum lagenarium*. Experimental Mycology **10**(4): 301-306.

Kumar, R., Subramani, R., Feussner, K.D., and Aalbersberg, W. 2012. Aurantoside K, a new antifungal tetramic acid glycoside from a Fijian marine sponge of the genus *Melophlus*. Marine Drugs **10**(1): 200-208.

Kurita, K.L., Glassey, E., and Linington, R.G. 2015. Integration of high-content screening and untargeted metabolomics for comprehensive functional annotation of natural product libraries. Proceedings of the National Academy of Sciences **112**(39): 11999-12004.

Lamrani, K., Lakhtar, H., Ismaili Alaoui, M., Ettalibi, M., Boiron, P., Augur, C., Gaime Perraud, I., and Roussos, S. 2008. Production of fumagillin by *Aspergillus fumigatus* isolated from traditional trituration units, Maasra, in Morocco. Micología Aplicada International **20**(1).

Lavine, B.K. 2006. Clustering and classification of analytical data. *In* Encyclopedia of Analytical Chemistry. John Wiley & Sons, Ltd.

Lee, M.-S., Wang, S.-W., Wang, G.-J., Pang, K.-L., Lee, C.-K., Kuo, Y.-H., Cha, H.-J., Lin, R.-K., and Lee, T.-H. 2016. Angiogenesis inhibitors and anti-inflammatory agents from *Phoma* sp NTOU4195. Journal of Natural Products **79**(12): 2983-2990.

Levy, S.B., and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine **10**(12s): S122.

Lewis, K. 2013. Platforms for antibiotic discovery. Nature Reviews Drug Discovery **12**(5): 371-387.

Lewis, K. 2015. Challenges of antibiotic discovery. *In* Microbe Magazine. pp. 363-369.

Lewis, K. 2017. New approaches to antimicrobial discovery. Biochemical Pharmacology **134**: 87-98.

Lewis, L.A., and McCourt, R.M. 2004. Green algae and the origin of land plants. American Journal of Botany **91**(10): 1535-1556.

Li, F., Li, K., Li, X., and Wang, B. 2011. Chemical constituents of marine algal-derived endophytic fungus *Exophiala oligosperma* EN-21. Chinese Journal of Oceanology and Limnology **29**(1): 63-67.

Li, H.-L., Li, X.-M., Li, X., Wang, C.-Y., Liu, H., Kassack, M.U., Meng, L.-H., and Wang, B.-G. 2017. Antioxidant hydroanthraquinones from the marine algal-derived endophytic fungus *Talaromyces islandicus* EN-501. Journal of Natural Products **80**(1): 162-168.

Li, H.-L., Li, X.-M., Liu, H., Meng, L.-H., and Wang, B.-G. 2016a. Two new diphenylketones and a new xanthone from *Talaromyces islandicus* EN-501, an endophytic fungus derived from the marine red alga *Laurencia okamurai*. Marine Drugs **14**(12): 223.

Li, X.-D., Li, X.-M., Li, X., Xu, G.-M., Liu, Y., and Wang, B.-G. 2016b. Aspewentins D-H, 20-nor-isopimarane derivatives from the deep sea sediment-derived fungus *Aspergillus wentii* SD-310. Journal of Natural Products **79**(5): 1347-1353.

Li, X.-J., Gao, J.-M., Chen, H., Zhang, A.-L., and Tang, M. 2012. Toxins from a symbiotic fungus, *Leptographium qinlingensis* associated with *Dendroctonus armandi* and their in vitro toxicities to *Pinus armandi* seedlings. European Journal of Plant Pathology **134**(2): 239-247.

Li, X., Li, X.-M., Xu, G.-M., Li, C.-S., and Wang, B.-G. 2014a. Antioxidant metabolites from marine alga-derived fungus *Aspergillus wentii* EN-48. Phytochemistry Letters **7**: 120-123.

Li, X., Li, X.M., Zhang, P., and Wang, B.G. 2015. A new phenolic enamide and a new meroterpenoid from marine alga-derived endophytic fungus *Penicillium oxalicum* EN-290. Journal of Asian Natural Products Research **17**(12): 1204-1212.

Li, X.D., Miao, F.P., Liang, X.R., and Ji, N.Y. 2014b. Meroterpenes from an algicolous strain of *Penicillium echinulatum*. Magnetic Resonance in Chemistry **52(**5): 247-250.

Li, Y.-X., Himaya, S.W.A., Dewapriya, P., Kim, H.J., and Kim, S.-K. 2014c. Anti-proliferative effects of isosclerone isolated from marine fungus *Aspergillus fumigatus* in MCF-7 human breast cancer cells. Process Biochemistry **49**(12): 2292-2298.

Li, Y.-X., Kang, K.-H., Kim, H.J., and Kim, S.-K. 2014d. In vitro induction of apoptosis by isosclerone from marine-derived fungus *Aspergillus fumigatus*. Bioorganic & Medicinal Chemistry Letters **24**(16): 3923-3927.

Liang, X.R., Miao, F.P., Song, Y.P., Guo, Z.Y., and Ji, N.Y. 2016a. Trichocitrin, a new fusicoccane diterpene from the marine brown alga-endophytic fungus *Trichoderma citrinoviride* cf-27. Natural Product Research **30**(14): 1605-1610.

Liang, X.R., Miao, F.P., Song, Y.P., Liu, X.H., and Ji, N.Y. 2016b. Citrinovirin with a new norditerpene skeleton from the marine algicolous fungus *Trichoderma citrinoviride*. Bioorganic & Medicinal Chemistry Letters **26**(20): 5029-5031.

Liao, L., You, M., Chung, B.K., Oh, D.C., Oh, K.B., and Shin, J. 2015. Alkaloidal metabolites from a marine-derived *Aspergillus* sp. fungus. Journal of Natural Products **78**(3): 349-354.

Lin, T., Lin, X., Lu, C., and Shen, Y. 2011. Secondary metabolites of *Pyrenochaeta* sp. B36, an endophytic fungus from *Annona squamosa* L. Natural Product Research **25**(10): 1008-1013.

Lindenfelser, L., and Ciegler, A. 1977. Penicillic acid production in submerged culture. Applied and Environmental Microbiology **34**(5): 553-556.

Lining, T., and Garbary, D. 1992. The *Ascophyllum/Polysiphonia/Mycosphaerella* symbiosis. III. Experimental studies on the interactions between *P. lanosa* and *A. nodosum*. Botanica Marina **35**(4): 341-350.

Liu, H.-X., Tan, H.-B., Liu, Y., Chen, Y.-C., Li, S.-N., Sun, Z.-H., Li, H.-H., Qiu, S.-X., and Zhang, W.-M. 2017. Three new highly-oxygenated metabolites from the endophytic fungus *Cytospora rhizophorae* A761. Fitoterapia **117**: 1-5.

Liu, H., Li, X.M., Liu, Y., Zhang, P., Wang, J.N., and Wang, B.G. 2016a. Chermesins A-D: Meroterpenoids with a drimane-type spirosesquiterpene skeleton from the marine algal-derived endophytic fungus *Penicillium chermesinum* EN-480. Journal of Natural Products **79**(4): 806-811.

Liu, L., Chen, X., Li, D., Zhang, Y., Li, L., Guo, L., Cao, Y., and Che, Y. 2015. Bisabolane sesquiterpenoids from the plant endophytic fungus *Paraconiothyrium brasiliense*. Journal of Natural Products **78**(4): 746-753.

Liu, S., Widom, J., Kemp, C.W., Crews, C.M., and Clardy, J. 1998. Structure of human methionine aminopeptidase-2 complexed with fumagillin. Science **282**(5392): 1324-1327.

Liu, X.-H., Miao, F.-P., Li, X.-D., Yin, X.-L., and Ji, N.-Y. 2012. A new sesquiterpene from an endophytic *Aspergillus versicolor* strain. Natural Product Communications **7**(7): 819-820.

Liu, X.-H., Miao, F.-P., Qiao, M.-F., Cichewicz, R.H., and Ji, N.-Y. 2013. Terretonin, ophiobolin, and drimane terpenes with absolute configurations from an algicolous *Aspergillus ustus*. RSC Advances **3**(2): 588-595.

Liu, X.-H., Tang, X.-Z., Miao, F.-P., and Ji, N.-Y. 2011. A new pyrrolidine derivative and steroids from an algicolous *Gibberella zeae* strain. Natural Product Communications.

Liu, X., Ashforth, E., Ren, B., Song, F., Dai, H., Liu, M., Wang, J., Xie, Q., and Zhang, L. 2010. Bioprospecting microbial natural product libraries from the marine environment for drug discovery. The Journal of Antibiotics **63**(8): 415-422.

Liu, Z., Chen, Y., Chen, S., Liu, Y., Lu, Y., Chen, D., Lin, Y., Huang, X., and She, Z. 2016b. Aspterpenacids A and B, two sesterterpenoids from a mangrove endophytic fungus *Aspergillus terreus* H010. Organic Letters **18**(6): 1406-1409.

Löffler, J., Hebart, H., Schumacher, U., Reitze, H., and Einsele, H. 1997. Comparison of different methods for extraction of DNA of fungal pathogens from cultures and blood. Journal of Clinical Microbiology **35**(12): 3311-3312.

Loh, T.-P., and Lye, P.-L. 2001. A concise synthesis of (+-)-methylenolactocin and the formal synthesis of (+-)-phaseolinic acid. Tetrahedron Letters **42**(20): 3511-3514.

Lu, K., Bhat, M., and Basu, S. 2016. Plants and their active compounds: natural molecules to target angiogenesis. Angiogenesis **19**(3): 287-295.

Lupetti, A., Danesi, R., Campa, M., Tacca, M.D., and Kelly, S. 2002. Molecular basis of resistance to azole antifungals. Trends in Molecular Medicine **8**(2): 76-81.

Maaroufi, Y., Ahariz, N., Husson, M., and Crokaert, F. 2004. Comparison of different methods of isolation of DNA of commonly encountered Candida species and its quantitation by using a real-time PCR-based assay. Journal of Clinical Microbiology **42**(7): 3159-3163.

Maloney, K. 2007. Biologically active natural products from plants and their endophytes. Cornell University, 126.

Mann, J. 1987. Secondary metabolism. 2nd ed. Oxford University Press, New York, New York. pp. 390.

Markager, S., and Sand-Jensen, K. 1996. Implications of thallus thickness for growth-irradiance relationships of marine macroalgae. European Journal of Phycology **31**(1): 79-87.

Mathan, S., Smith, A., Kumaran, J., and Prakash, S. 2011. Anticancer and antimicrobial activity of *Aspergillus protuberus* SP1 isolated from marine sediments of South Indian coast. Chinese Journal of Natural Medicines **9**(4): 286-292.

Mawson, S.D., and Weavers, R.T. 1995. Application of radical cyclisation/iodine atom transfer to the chiral synthesis of (-)-methylenolactocin. Tetrahedron **51**(41): 11257-11270.

McAleece, N., Lambshead, P.J.D., and Paterson, G.L.J. 1997. BioDiversity professional statistics analysis software. Jointly developed by the Scottish Association for Marine Science and the Natural History Museum London.

McCullough, J.L., and Maren, T.H. 1973. Inhibition of dihydropteroate synthetase from *Escherichia coli* by sulfones and sulfonamides. Antimicrobial Agents and Chemotherapy **3**(6): 665-669.

Meinwald, J. 2011. Natural products as molecular messengers. Journal of Natural Products **74**(3): 305-309.

Miao, F.-P., Liang, X.-R., Yin, X.-L., Wang, G., and Ji, N.-Y. 2012a. Absolute configurations of unique harziane diterpenes from *Trichoderma* species. Organic Letters **14**(15): 3815-3817.

Miao, F.P., Li, X.D., Liu, X.H., Cichewicz, R.H., and Ji, N.Y. 2012b. Secondary metabolites from an algicolous *Aspergillus versicolor* strain. Marine Drugs **10**(1): 131-139.

Miao, F.P., Liang, X.R., Liu, X.H., and Ji, N.Y. 2014. Aspewentins A-C, norditerpenes from a cryptic pathway in an algicolous strain of *Aspergillus wentii*. Journal of Natural Products **77**(2): 429-432.

Mikusova, K., Slayden, R.A., Besra, G.S., and Brennan, P.J. 1995. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrobial Agents and Chemotherapy **39**(11): 2484-2489.

Miller, J.R., and Waldrop, G.L. 2010. Discovery of novel antibacterials. Expert opinion on drug discovery **5**(2): 145-154.

Mills, R. 1955. The action of fumagillin on a bacteriophage of *Staphylococcus aureus*. Microbiology **13**(1): 39-44.

Molina, J.-M., Goguel, J., Sarfati, C., Michiels, J.-F., Desportes-Livage, I., Balkan, S., Chastang, C., Cotte, L., Maslo, C., and Struxiano, A. 2000. Trial of oral fumagillin for the treatment of intestinal microsporidiosis in patients with HIV infection. Aids **14**(10): 1341-1348.

Molina, J.-M., Tourneur, M., Sarfati, C., Chevret, S., de Gouvello, A., Gobert, J.-G., Balkan, S., and Derouin, F. 2002. Fumagillin treatment of intestinal microsporidiosis. New England Journal of Medicine **346**(25): 1963-1969.

Morita, T., and Aoki, H. 1974. Isosclerone, a new metabolite of *Sclerotinia sclerotiorum*. Agricultural and Biological Chemistry **38**(8): 1501-1505.

Mukaka, M.M. 2012. A guide to appropriate use of correlation coefficient in medical research. Malawi Medical Journal : The Journal of Medical Association of Malawi **24**(3): 69-71.

Muller, C.B., and Krauss, J. 2005. Symbiosis between grasses and asexual fungal endophytes. Current Opinion in Plant Biology **8**(4): 450-456.

Müller, M.M., and Hallaksela, A.-M. 1998a. A chemotaxonomical method based on FAST-profiles for the determination of phenotypic diversity of spruce needle endophytic fungi. Mycological Research **102**(10): 1190-1197.

Müller, M.M., and Hallaksela, A.-M. 1998b. Diversity of Norway spruce needle endophytes in various mixed and pure Norway spruce stands. Mycological Research **102**(10): 1183-1189.

Murnaghan, M.F. 1946. The pharmacology of penicillic acid. Journal of Pharmacology and Experimental Therapeutics **88**(2): 119-132.

Nagano, Y., Konishi, M., Nagahama, T., Kubota, T., Abe, F., and Hatada, Y. 2016. Retrieval of deeply buried culturable fungi in marine subsurface sediments, Suruga-Bay, Japan. Fungal Ecology **20**: 256-259.

Nalini, M.S., and Prakash, H.S. 2017. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Letters in Applied Microbiology **64**(4): 261-270.

Nallasivam, J.L., and Fernandes, R.A. 2017. A protecting-group-free synthesis of (+)-nephrosteranic, (+)-protolichesterinic, (+)-nephrosterinic, (+)-phaseolinic, (+)-rocellaric acids and (+)-methylenolactocin. Organic & Biomolecular Chemistry **15**(3): 708-716.

Namikoshi, M., Negishi, R., Nagai, H., Dmitrenok, A., and Kobayashi, H. 2003. Three new chlorine containing antibiotics from a marine-derived fungus *Aspergillus ostianus* collected in Pohnpei. The Journal of Antibiotics **56**(9): 755-761.

Naseer, S., Bhat, K.A., Qadri, M., Riyaz-Ul-Hassan, S., Malik, F.A., and Khuroo, M.A. 2017. Bioactivity-guided isolation, antimicrobial and cytotoxic evaluation of secondary

metabolites from *Cladosporium tenuissimum* associated with *Pinus wallichiana*. ChemistrySelect **2**(3): 1311-1314.

Nelson, M.L., and Levy, S.B. 2011. The history of the tetracyclines. Annals of the New York Academy of Sciences **1241**(1): 17-32.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science **257**(5073): 1064-1073.

Newell, S.Y. 1981. Fungi and bacteria in or on leaves of eelgrass (*Zostera marina* L) from Chesapeake Bay. Applied and Environmental Microbiology **41**(5): 1219-1224.

Newman, D.J., and Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. Journal of Natural Products **70**(3): 461-477.

Newman, D.J., and Cragg, G.M. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products **75**(3): 311-335.

Newman, D.J., and Cragg, G.M. 2016. Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products **79**(3): 629-661.

Newman, D.J., Cragg, G.M., and Snader, K.M. 2003. Natural products as sources of new drugs over the period 1981− 2002. Journal of Natural Products **66**(7): 1022-1037.

Nguyen, H.T., Yu, N.H., Jeon, S.J., Lee, H.W., Bae, C.H., Yeo, J.H., Lee, H.B., Kim, I.S., Park, H.W., and Kim, J.C. 2016. Antibacterial activities of penicillic acid isolated from *Aspergillus persii* against various plant pathogenic bacteria. Letters in Applied Microbiology **62**(6): 488-493.

Nichols, D., Cahoon, N., Trakhtenberg, E., Pham, L., Mehta, A., Belanger, A., Kanigan, T., Lewis, K., and Epstein, S. 2010. Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. Applied and Environmental Microbiology **76**(8): 2445-2450.

Nielsen, J., Yu, S., Bojko, M., and Marcussen, J. 2000. α-1,4-Glucan lyase-producing endophyte of *Gracilariopsis* sp. (Rhodophyta) from China. European Journal of Phycology **35**(3): 207-212.

Norrby, S.R., Nord, C.E., and Finch, R. 2005. Lack of development of new antimicrobial drugs: a potential serious threat to public health. The Lancet Infectious Diseases **5**(2): 115-119.

Nozawa, K., Nakajima, S., Udagawa, S.-i., and Kawai, K.-i. 1991. Isolation and structure of a new 1-decalone derivative, rapiculine, from *Ramichloridium apiculatum*. Journal of the Chemical Society, Perkin Transactions 1(3): 537-539.

O'Neill, T.E., Li, H., Colquhoun, C.D., Johnson, J.A., Webster, D., and Gray, C.A. 2014. Optimisation of the microplate resazurin assay for screening and bioassay-guided fractionation of phytochemical extracts against *Mycobacterium tuberculosis*. Phytochemical Analysis **25**(5): 461-467.

Ochi, K., and Hosaka, T. 2013. New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. Applied Microbiology and Biotechnology **97**(1): 87-98.

Olivigni, F., and Bullerman, L. 1978a. A microbiological assay for penicillic acid. Journal of Food Protection **41**(6): 432-434.

Olivigni, F., and Bullerman, L. 1978b. Production of penicillic acid and patulin by an atypical *Penicillium roqueforti* isolate. Applied and Environmental Microbiology **35**(2): 435-438.

Onaka, H. 2017. Novel antibiotic screening methods to awaken silent or cryptic secondary metabolic pathways in actinomycetes. The Journal of Antibiotics **70**(8): 865-870.

Osterhage, C., Kaminsky, R., König, G.M., and Wright, A.D. 2000. Ascosalipyrrolidinone A, an antimicrobial alkaloid, from the obligate marine fungus *Ascochyta salicorniae*. The Journal of Organic Chemistry **65**(20): 6412-6417.

Osterhage, C., Konig, G.M., Holler, U., and Wright, A.D. 2002a. Rare sesquiterpenes from the algicolous fungus *Drechslera dematioidea*. Journal of Natural Products **65**(3): 306-313.

Osterhage, C., König, G.M., Jones, P.G., and Wright, A.D. 2002b. 5-hydroxyramulosin, a new natural product produced by *Phoma tropica*, a marine-derived fungus isolated from the alga *Fucus spiralis*. Planta medica **68**(11): 1052-1054.

Page, M.G.P. 2012. Beta-lactam antibiotics. *In* Antibiotic Discovery and Development. *Edited by* T.J. Dougherty and M. Pucci, J. Springer, New York, New York, USA. pp. 79-117.

Pang, K.-L., Overy, D.P., Jones, E.B.G., Calado, M.d.L., Burgaud, G., Walker, A.K., Johnson, J.A., Kerr, R.G., Cha, H.-J., and Bills, G.F. 2016. 'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: Toward a new consensual definition. Fungal Biology Reviews **30**(4): 163-175.

Park, B.K., Nakagawa, M., Hirota, A., and Nakayama, M. 1987. Methylenolactocin, a novel antitumoral antibiotic from *Penicillium* sp. Agricultural and Biological Chemistry **51**(12): 3443-3444.

Park, B.K., Nakagawa, M., Hirota, A., and Nakayama, M. 1988. Methylenolactocin, a novel antitumoral antibiotic from *Penicillium* sp. The Journal of Antibiotics **41**(6): 751-758.

Park, E.S., Rabinovsky, R., Carey, M., Hennessy, B.T., Agarwal, R., Liu, W., Ju, Z., Deng, W., Lu, Y., and Woo, H.G. 2010. Integrative analysis of proteomic signatures, mutations, and drug responsiveness in the NCI 60 cancer cell line set. Molecular Cancer Therapeutics **9**(2): 257-267.

Patterson, A.E., Flewelling, A.J., Clark, T.N., Geier, S.J., Vogels, C.M., Masuda, J.D., Gray, C.A., and Westcott, S.A. 2015. Antimicrobial and antimycobacterial activities of aliphatic amines derived from vanillin. Canadian Journal of Chemistry **93**(11): 1305-1311.

Pavao, G.B., Venancio, V.P., de Oliveira, A.L., Hernandes, L.C., Almeida, M.R., Antunes, L.M., and Debonsi, H.M. 2016. Differential genotoxicity and cytotoxicity of phomoxanthone A isolated from the fungus *Phomopsis longicolla* in HL60 cells and peripheral blood lymphocytes. Toxicology in Vitro **37**: 211-217.

Paz, Z., Komon-Zelazowska, M., Druzhinina, I.S., Aveskamp, M.M., Shnaiderman, A., Aluma, Y., Carmeli, S., Ilan, M., and Yarden, O. 2010. Diversity and potential antifungal properties of fungi associated with a Mediterranean sponge. Fungal Diversity **42**(1): 17-26.

Peng, J., Zhang, X., Wang, W., Zhu, T., Gu, Q., and Li, D. 2016. Austalides S-U, new meroterpenoids from the sponge-derived fungus *Aspergillus aureolatus* HDN14-107. Marine drugs **14**(7).

Perfect, J.R. 2017. The antifungal pipeline: a reality check. Nature Reviews Drug Discovery.

Petrini, O., Fisher, P., and Petrini, L. 1992. Fungal endophytes of bracken (*Pteridium aquilinum*), with some reflections on their use in biological control. Sydowia **44**(2): 282-293.

Phainuphong, P., Rukachaisirikul, V., Tadpetch, K., Sukpondma, Y., Saithong, S., Phongpaichit, S., Preedanon, S., and Sakayaroj, J. 2017. gamma-Butenolide and furanone derivatives from the soil-derived fungus *Aspergillus sclerotiorum* PSU-RSPG178. Phytochemistry **137**: 165-173.

Pinheiro, A., Dethoup, T., Bessa, J., Silva, A.M., and Kijjoa, A. 2012. A new bicyclic sesquiterpene from the marine sponge associated fungus *Emericellopsis minima*. Phytochemistry Letters **5**(1): 68-70.

Pontius, A., Krick, A., Kehraus, S., Brun, R., and KoÝˆnig, G.M. 2008a. Antiprotozoal activities of heterocyclic-substituted xanthones from the marine-derived fungus *Chaetomium* sp. Journal of Natural Products **71**(9): 1579-1584.

Pontius, A., Krick, A., Kehraus, S., Foegen, S.E., Muller, M., Klimo, K., Gerhauser, C., and Konig, G.M. 2008b. Noduliprevenone: a novel heterodimeric chromanone with cancer chemopreventive potential. Chemistry **14**(32): 9860-9863.

Pontius, A., Krick, A., Mesry, R., Kehraus, S., Foegen, S.E., Müller, M., Klimo, K., Gerhäuser, C., and König, G.M. 2008c. Monodictyochromes A and B, dimeric xanthone derivatives from the marine algicolous fungus *Monodictys putredinis*. Journal of Natural Products **71**(11): 1793-1799.

Pontius, A., Mohamed, I., Krick, A., Kehraus, S., and Konig, G.M. 2008d. Aromatic polyketides from marine algicolous fungi. Journal of Natural Products **71**(2): 272-274.

Pounder, J.I., Simmon, K.E., Barton, C.A., Hohmann, S.L., Brandt, M.E., and Petti, C.A. 2007. Discovering potential pathogens among fungi identified as nonsporulating molds. Journal of Clinical Microbiology **45**(2): 568-571.

Prado, S., Buisson, D., Ndoye, I., Vallet, M., and Nay, B. 2013. One-step enantioselective synthesis of (4*S*)-isosclerone through biotransformation of juglone by an endophytic fungus. Tetrahedron Letters **54**: 1189-1191.

Pretsch, E., Buhlmann, P., and Badertscher, M. 2009. Structure determination of organic compounds. 4th ed. Springer.

Proksch, P., Ebel, R., Edrada, R., Riebe, F., Liu, H., Diesel, A., Bayer, M., Li, X., Lin, W.H., Grebenyuk, V., Mueller, W.E., Draeger, S., Zuccaro, A., and Schulz, B. 2008. Sponge-associated fungi and their bioactive compounds: the *Suberites* case. Botanica Marina **51**(3): 209-218.

Public Health Agency of Canada. 2015. Canadian antimicrobial resistance surveillance system - Report 2015. Government of Canada, Ottawa, Ontario, Canada. p. 62.

Public Health Agency of Canada. 2016. Canadian antimicrobial resistance surveillance system - Report 2016. Government of Canada, Ottawa, Ontario, Canada. p. 118.

Qi, F., Jing, T., and Zhan, Y. 2012. Characterization of endophytic fungi from *Acer ginnala* maxim. in an artificial plantation: media effect and tissue-dependent variation. PLOS One **7**(10): e46785.

Qiao, M.F., Ji, N.Y., Liu, X.H., Li, F., and Xue, Q.Z. 2010a. Asporyergosterol, a new steroid from an algicolous isolate of *Aspergillus oryzae*. Natural Product Communications **5**(10): 1575-1578.

Qiao, M.F., Ji, N.Y., Liu, X.H., Li, K., Zhu, Q.M., and Xue, Q.Z. 2010b. Indoloditerpenes from an algicolous isolate of *Aspergillus oryzae*. Bioorganic & Medicinal Chemistry Letters **20**(19): 5677-5680.

Qiao, M.F., Ji, N.Y., Miao, F.P., and Yin, X.L. 2011. Steroids and an oxylipin from an algicolous isolate of *Aspergillus flavus*. Magnetic Resonance in Chemistry **49**(6): 366-369.

Raghukumar, C. 2008. Marine fungal biotechnology: an ecological perspective. Fungal Diversity **31**: 19-35.

Raja, H.A., Miller, A.N., Pearce, C.J., and Oberlies, N.H. 2017. Fungal identification using molecular tools: A primer for the natural products research community. Journal of Natural Products **80**(3): 756-770.

Rakshith, D., Santosh, P., Pradeep, T.P., Gurudatt, D.M., Baker, S., Yashavantha Rao, H.C., Pasha, A., and Satish, S. 2016. Application of bioassay-guided fractionation coupled with a molecular approach for the dereplication of antimicrobial metabolites. Chromatographia **79**(23-24): 1625-1642.

Rateb, M.E., and Ebel, R. 2011. Secondary metabolites of fungi from marine habitats. Natural Product Reports **28**(2): 290-344.

Ratner, B. 2009. The correlation coefficient: Its values range between +1/−1, or do they? Journal of Targeting, Measurement and Analysis for Marketing **17**(2): 139-142.

Reddy, M.S., Murali, T.S., Suryanarayanan, T.S., Govinda Rajulu, M.B., and Thirunavukkarasu, N. 2016. *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. Fungal Ecology **24, Part A**: 70-75.

Redman, R.S., Dunigan, D.D., and Rodriguez, R.J. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytologist **151**(3): 705-716.

Redman, R.S., Kim, Y.O., Woodward, C.J., Greer, C., Espino, L., Doty, S.L., and Rodriguez, R.J. 2011. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. PLOS One **6**(7).

Ringnér, M. 2008. What is principal component analysis? Nature Biotechnology **26**(3): 303-304.

Robles, A.J., Du, L., Cichewicz, R.H., and Mooberry, S.L. 2016. Maximiscin induces DNA damage, activates DNA damage response pathways, and has selective cytotoxic activity against a subtype of triple-negative breast cancer. Journal of Natural Products **79**(7): 1822-1827.

Robles, C.A., Lopez, S.E., and Carmarán, C.C. 2015. Basidiomicetes endofíticos de madera en *Platanus acerifolia* (Platanaceae) de Argentina: notas y estudios de cultivo. Boletín de la Sociedad Argentina de Botánica **50**(4): 437-445.

Romanelli, A.M., Sutton, D.A., Thompson, E.H., Rinaldi, M.G., and Wickes, B.L. 2010. Sequence-based identification of filamentous basidiomycetous fungi from clinical specimens: A cautionary note. Journal of Clinical Microbiology **48**(3): 741-752.

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

Rukachaisirikul, V., Sommart, U., Phongpaichit, S., Hutadilok-Towatana, N., Rungjindamai, N., and Sakayaroj, J. 2007. Metabolites from the Xylariaceous fungus PSU-A80. Chemical & Pharmaceutical Bulletin **55**(9): 1316-1318.

Saari, S., Helander, M., Faeth, S.H., and Saikkonen, K. 2010. The effects of endophytes on seed production and seed predation of tall fescue and meadow fescue. Microbial Ecology **60**(4): 928-934.

Saha, S., and Roy, S.C. 2010. Titanocene (III) chloride mediated radical induced synthesis of (−)-methylenolactocin and (−)-protolichesterinic acid. Tetrahedron **66**(24): 4278-4283.

Saldanha, A.J. 2004. Java Treeview - Extensible visualization of microarray data. Bioinformatics **20**(17): 3246-3248.

Sarasan, M., Puthumana, J., Job, N., Han, J., Lee, J.-S., and Philip, R. 2017. Marine algicolous endophytic fungi- A promising drug resource of the era. Journal of Microbiology and Biotechnology **27**(6): 1039-1052.

Sarkar, S., and Ghosh, S. 1996. A short synthesis of (racemic)-methylenolactocin. Tetrahedron Letters **37**(27): 4809-4810.

Sausville, E., and Johnson, J. 2000. Molecules for the millennium: how will they look? New drug discovery year 2000. British Journal of Cancer **83**(11): 1401.

Schinke, C., Martins, T., Queiroz, S.C.N., Melo, I.S., and Reyes, F.G.R. 2017. Antibacterial compounds from marine bacteria, 2010-2015. Journal of Natural Products **80**(4): 1215-1228.

Schulz, B., and Boyle, C. 2005. The endophytic continuum. Mycological Research **109**(6): 661-686.

Schulz, B., Boyle, C., Draeger, S., Römmert, A.-K., and Krohn, K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research **106**(9): 996-1004.

Schulz, B., Draeger, S., dela Cruz, T.E., Rheinheimer, J., Siems, K., Loesgen, S., Bitzer, J., Schloerke, O., Zeeck, A., Kock, I., Hussain, H., Dai, J., and Krohn, K. 2008. Screening strategies for obtaining novel, biologically active, fungal secondary metabolites from marine habitats. Botanica Marina **51**(3): 219-234.

Schulz, B., Guske, S., Dammann, U., and Boyle, C. 1998. Endophyte-host interactions. II. Defining symbiosis of the endophyte-host interaction. Symbiosis **25**(1-3): 213-227.

Seibert, S.F., Eguereva, E., Krick, A., Kehraus, S., Voloshina, E., Raabe, G., Fleischhauer, J., Leistner, E., Wiese, M., Prinz, H., Alexandrov, K., Janning, P., Waldmann, H., and Konig, G.M. 2006. Polyketides from the marine-derived fungus *Ascochyta salicorniae* and their potential to inhibit protein phosphatases. Organic & Biomolecular Chemistry **4**(11): 2233-2240.

Shadduck, J.A. 1980. Effect of fumagillin on *in vitro* multiplication of *Encephalitozoon Cuniculi*. The Journal of Protozoology **27**(2): 202-208.

Sharma, D.K., Jaglan, S., and Mukherjee, D. 2016. Isolation and conversion of penicillic acid to 6-iodo-α-pyran-2,5-dione: A key intermediate for the synthesis of pectinolide F. Synthetic Communications **46**(10): 902-908.

Sibi, M.P., Deshpande, P.K., and La Loggia, A.J. 1996. Regio-and stereoselective functionalization of linear dicarboxylic acid derivatives. A sequential aldol-lactonization strategy for the synthesis of (-)-roccellaric acid,(-)-protolichesterinic acid, and (-)-methylenolactocin. Synlett **1996**(04): 343-345.

Sieber-Canavesi, F., and Sieber, T.N. 1993. Successional patterns of fungal communities in needles of European silver fir (*Abies alba* Mill.). New Phytologist **125**: 149-161.

Sin, N., Meng, L., Margaret, Q.W.W., Wen, J.J., Bornmann, W.G., and Crews, C.M. 1997. The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. Proceedings of the National Academy of Sciences of the United States of America **94**(12): 6099-6103.

Sobell, H.M. 1985. Actinomycin and DNA transcription. Proceedings of the National Academy of Sciences **82**(16): 5328-5331.

Somjaipeng, S., Medina, A., Kwasna, H., Ordaz Ortiz, J., and Magan, N. 2015. Isolation, identification, and ecology of growth and taxol production by an endophytic strain of *Paraconiothyrium variabile* from English yew trees (*Taxus baccata*). Fungal Biology **119**(11): 1022-1031.

Somjaipeng, S., Medina, A., and Magan, N. 2016. Environmental stress and elicitors enhance taxol production by endophytic strains of *Paraconiothyrium variabile* and *Epicoccum nigrum*. Enzyme and Microbial Technology **90**: 69-75.

Song, Y.X., Qiao, L.T., Wang, J.J., Zeng, H.M., She, Z.G., Miao, C.D., Hong, K., Gu, Y.C., Liu, L., and Lin, Y.C. 2011. Two new meroterpenes from the mangrove endophytic fungus *Aspergillus* sp 085241B. Helvetica Chimica Acta **94**(10): 1875-1880.

South, G.R. 1984. A checklist of marine algae of eastern Canada, second revision. Canadian Journal of Botany **62**(4): 680-704.

Stanley, S.J. 1992. Observations on the seasonal occurrence of marine endophytic and parasitic fungi. Canadian Journal of Botany **70**(10): 2089-2096.

Stetina, R. 1986. Induction of DNA single-strand breaks and DNA synthesis inhibition in CHO and AWRF cells after exposure to sterigmaticyctic and penicillic acid. Folia Biologica **32**(6): 406-413.

Strobel, G., and Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Reviews **67**(4): 491-502.

Strobel, G., Daisy, B., Castillo, U., and Harper, J. 2004. Natural products from endophytic microorganisms. Journal of Natural Products **67**(2): 257-268.

Su, G., Burant, C.F., Beecher, C.W., Athey, B.D., and Meng, F. 2011. Integrated metabolome and transcriptome analysis of the NCI60 dataset. BMC Bioinformatics **12**(1): S36.

Sultan, S., Noor, M.Z., Anouar el, H., Shah, S.A., Salim, F., Rahim, R., Al Trabolsy, Z.B., and Weber, J.F. 2014. Structure and absolute configuration of 20beta-

Hydroxyprednisolone, a biotransformed product of predinisolone by the marine endophytic fungus *Penicillium lapidosum*. Molecules **19**(9): 13775-13787.

Sun, H.F., Li, X.M., Meng, L., Cui, C.M., Gao, S.S., Li, C.S., Huang, C.G., and Wang, B.G. 2012a. Asperolides A-C, tetranorlabdane diterpenoids from the marine alga-derived endophytic fungus *Aspergillus wentii* EN-48. Journal of Natural Products **75**(2): 148-152.

Sun, H.F., Li, X.M., Meng, L.H., Cui, C.M., Gao, S.S., Li, C.S., and Wang, B.G. 2013a. Two new secoanthraquinone derivatives from the marine-derived endophytic fungus *Aspergillus wentii* EN-48. Helvetica Chimica Acta **96**(3): 458-462.

Sun, L.L., Shao, C.L., Chen, J.F., Guo, Z.Y., Fu, X.M., Chen, M., Chen, Y.Y., Li, R., de Voogd, N.J., She, Z.G., Lin, Y.C., and Wang, C.Y. 2012b. New bisabolane sesquiterpenoids from a marine-derived fungus *Aspergillus* sp. isolated from the sponge *Xestospongia testudinaria*. Bioorganic & Medicinal Chemistry Letters **22**(3): 1326-1329.

Sun, R.R., Miao, F.P., Zhang, J., Wang, G., Yin, X.L., and Ji, N.Y. 2013b. Three new xanthone derivatives from an algicolous isolate of *Aspergillus wentii*. Magnetic Resonance in Chemistry **51**(1): 65-68.

Suryanarayanan, T.S. 2012. Fungal endosymbionts of seaweeds. Progress in Molecular and Subcellular Biology **53**: 53-69.

Suryanarayanan, T.S., and Johnson, J.A. 2014. Fungal endosymbionts of macroalgae: Need for enquiries into diversity and technological potential. Oceanography: Open Access **2**(1): 1-3.

Suryanarayanan, T.S., Kumaresan, V., and Johnson, J.A. 1998. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Canadian Journal of Microbiology **44**(10): 1003-1006.

Suryanarayanan, T.S., and Thennarasan, S. 2004. Temporal variation in endophyte assemblages of *Plumeria rubra* leaves. Fungal Diversity **15**: 197-204.

Suryanarayanan, T.S., Venkatachalam, A., Thirunavukkarasu, N., Ravishankar, J.P., Doble, M., and Geetha, V. 2010. Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and biotechnological potential. Botanica Marina **53**(5): 457-468.

Suryanarayanan, T.S., Venkatachalam, G., and Murali, T.S. 2003. Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns. Current Science **85**(4): 489-493.

Tabuchi, H., Tajimi, A., and Ichihara, A. 1994. Phytotoxic metabolites isolated from *Scolecotrichum graminis* Fuckel. Bioscience, Biotechnology and Biochemistry **58**(11): 1956-1959.

Takahata, H., Uchida, Y., and Momose, T. 1995. Concise syntheses of natural gamma-butyrolactones, (+)-trans-whisky lactone, (+)-trans-cognac lactone, (-)-methylenolactocin, (+)-nephrosteranic acid, and (+)-roccellaric acid using novel chiral butenolide synthons. The Journal of Organic Chemistry **60**(17): 5628-5633.

Tan, R.X., and Zou, W.X. 2001. Endophytes: a rich source of functional metabolites. Natural Product Reports **18**(4): 448-459.

Tang, Q., Guo, K., Li, X.Y., Zheng, X.Y., Kong, X.J., Zheng, Z.H., Xu, Q.Y., and Deng, X. 2014. Three new asperentin derivatives from the algicolous fungus *Aspergillus* sp. F00785. Marine Drugs **12**(12): 5993-6002.

Tarman, K., Palm, G.J., Porzel, A., Merzweiler, K., Arnold, N., Wessjohann, L.A., Unterseher, M., and Lindequist, U. 2012. Helicascolide C, a new lactone from an Indonesian marine algicolous strain of *Daldinia eschscholzii* (Xylariaceae, Ascomycota). Phytochemistry Letters **5**(1): 83-86.

Tejesvi, M.V., Ruotsalainen, A.L., Markkola, A.M., and Pirttilä, A.M. 2010. Root endophytes along a primary succession gradient in northern Finland. Fungal Diversity **41**(1): 125-134.

Teuscher, F., Lin, W., Wray, V., Edrada, R., Padmakumar, K., Proksch, P., and Ebel, R. 2006. Two new cyclopentanoids from the endophytic fungus *Aspergillus sydowii* associated with the marine alga *Acanthophora spicifera*. Natural Product Communications **1**(11): 927-933.

Thirunavukkarasu, N., Jahnes, B., Broadstock, A., Govinda Rajulu, M.B., Murali, T.S., Gopalan, V., and T.S., S. 2015. Screening marine-derived endophytic fungi for xylan-degrading enzymes. Current Science **109**(1): 112-120.

Tian, Y.-q., Lin, X.-p., Liu, J., Kaliyaperumal, K., Ai, W., Ju, Z.-r., Yang, B., Wang, J., Yang, X.-w., and Liu, Y. 2015. Ascomycotin A, a new citromycetin analogue produced by Ascomycota sp. Ind19F07 isolated from deep sea sediment. Natural Product Research **29**(9): 820-826.

Tian, Y., Amand, S., Buisson, D., Kunz, C., Hachette, F., Dupont, J., Nay, B., and Prado, S. 2014. The fungal leaf endophyte *Paraconiothyrium variabile* specifically metabolizes the host-plant metabolome for its own benefit. Phytochemistry **108**: 95-101.

Toxopeus, J., Kozera, C.J., O'Leary, S.J.B., and Garbary, D.J. 2011. A reclassification of *Mycophycias ascophylli* (Ascomycota) based on nuclear large ribosomal subunit DNA sequences. Botanica Marina **54**(3).

Ukoima, N., Amakiri, M., Adeniji, M., and Daniel-Kalio, L. 2010. Survey of fungi on some red and white mangrove forest trees in Rivers State, Nigeria. African Journal of Ecology **48**(3): 844-846.

Upson, R., Read, D.J., and Newsham, K.K. 2009. Nitrogen form influences the response of *Deschampsia antarctica* to dark septate root endophytes. Mycorrhiza **20**(1): 1-11.

Van Eijk, G. 1969. Isolation and identification of orsellinic acid and penicillic acid produced by *Penicillium fennelliae* Stolk. Antonie Van Leeuwenhoek **35**(1): 497-504.

Vansteelandt, M., Blanchet, E., Egorov, M., Petit, F., Toupet, L., Bondon, A., Monteau, F., Le Bizec, B., Thomas, O.P., Pouchus, Y.F., Le Bot, R., and Grovel, O. 2013. Ligerin, an antiproliferative chlorinated sesquiterpenoid from a marine-derived *Penicillium* strain. Journal of Natural Products **76**(2): 297-301.

Vasudev, N.S., and Reynolds, A.R. 2014. Anti-angiogenic therapy for cancer: Current progress, unresolved questions and future directions. Angiogenesis **17**(3): 471-494.

Vaupel, A., and Knochel, P. 1995. A short formal synthesis (-)-methylenolactocin via a nickel catalyzed intramolecular carbozincation. Tetrahedron Letters **36**(2): 231-232.

Venkatachalam, A., Govinda Rajulu, M.B., Thirunavukkarasu, N., and T.S., S. 2015. Endophytic fungi of marine algae and seagrasses: a novel source of chitin modifying enzymes. Mycosphere **6**(3): 345-355.

Ventola, C.L. 2015. The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy and Therapeutics **40**(4): 277.

Viviani, F., and Gaudry, M. 1990. Enantiomeric purity of scytalone from different fungal sources. Tetrahedron **46**(8): 2827-2834.

Wang, F.-W. 2012. Bioactive metabolites from *Guignardia* sp., an endophytic fungus residing in *Undaria pinnatifida*. Chinese Journal of Natural Medicines **10**(1): 72-76.

Wang, S., Li, X.M., Teuscher, F., Li, D.L., Diesel, A., Ebel, R., Proksch, P., and Wang, B.G. 2006. Chaetopyranin, a benzaldehyde derivative, and other related metabolites from *Chaetomium globosum*, an endophytic fungus derived from the marine red alga *Polysiphonia urceolata*. Journal of Natural Products **69**(11): 1622-1625.

Webber, F.C. 1967. Observations on the structure, life history and biology of *Mycosphaerella ascophylli*. Transactions of the British Mycological Society **50**(4): 583-601.

Wheeler, M.H., and Stipanovic, R.D. 1985. Melanin biosynthesis and the metabolism of flaviolin and 2-hydroxyjuglone in *Wangiella dermatitidis*. Archives of Microbiology **142**(3): 234-241.

White, R.J. 2012. The early history of antibiotic discovery: Empiricism ruled. *In* Antibiotic Discovery and Development. *Edited by* T.J. Dougherty and M. Pucci, J. Springer, New York, New York, USA. pp. 3-31.

White, T.J., Bruns, T., Lee, S., and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: A guide to methods and applications. Academic Press, Inc., New York. pp. 315-322.

Wiemann, P., Guo, C.J., Palmer, J.M., Sekonyela, R., Wang, C.C., and Keller, N.P. 2013. Prototype of an intertwined secondary-metabolite supercluster. Proceedings of the National Academy of Sciences **110**(42): 17065-17070.

Williams, D.H., Stone, M.J., Hauck, P.R., and Rahman, S.K. 1989. Why are secondary metabolites (natural products) biosynthesized. Journal of Natural Products **52**(6): 1189-1208.

Wilson, D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. Oikos: 274-276.

Wilson, D., Barr, M.E., and Faeth, S.H. 1997. Ecology and description of a new species of *Ophiognomonia* endophytic in the leaves of *Quercus emoryi*. Mycologia **89**(4): 537-546.

Wirth, J.C., Gilmore, T.E., and Noval, J.J. 1956. Penicillic acid, the antibiotic responsible for the activity of a culture filtrate of a strain of *Penicillium martensii* Biourge. Archives of Biochemistry and Biophysics **63**(2): 452-453.

Wold, S., Esbensen, K., and Geladi, P. 1987. Principal component analysis. Chemometrics and Intelligent Laboratory Systems **2**(1): 37-52.

Wong, Weng R., Oliver, Allen G., and Linington, Roger G. 2012. Development of antibiotic activity profile screening for the classification and discovery of natural product antibiotics. Chemistry & Biology **19**(11): 1483-1495.

World Health Organization. 2007. WHO report 2007 - Global tuberculosis control - Surveillance, planning, financing. World Health Organization, Geneva, Switzerland. p. 277.

World Health Organization. 2008. Global tuberculosis control 2008 - Surveillance, planning, financing. World Health Organization, Geneva, Switzerland. p. 304.

World Health Organization. 2009a. Global tuberculosis control 2009 - Epidemiology, strategy, financing. World Health Organization, Geneva, Switzerland. p. 314.

World Health Organization. 2009b. Global tuberculosis control - A short update to the 2009 report. World Health Organisation, Geneva, Switzerland. p. 48.

World Health Organization. 2010. Global tuberculosis control 2010. World Health Organization, Geneva, Switzerland. p. 218.

World Health Organization. 2011. Global tuberculosis control 2011. World Health Organization, Geneva, Switzerland. p. 258.

World Health Organization. 2012. Global tuberculosis report 2012. World Health Organization, Geneva, Switzerland. p. 100.

World Health Organization. 2013. Global tuberculosis report 2013. World Health Organization, Geneva, Switzerland. p. 306.

World Health Organization. 2014a. Antimicrobial resistance: Global report on surveillance. World Health Organization, Geneva, Switzerland. p. 256.

World Health Organization. 2014b. Global tuberculosis report 2014. Wolrd Health Organization, Geneva, Switzerland. p. 171.

World Health Organization. 2015. Global tuberculosis report 2015. World Health Organization, Geneva, Switzerland. p. 204.

World Health Organization. 2016. Global tuberculosis report 2016. World Health Organization, Geneva, Switzerland. p. 214.

Wright, G.D. 2014. Something old, something new: revisiting natural products in antibiotic drug discovery. Canadian Journal of Microbiology **60**(3): 147-154.

Wright, G.D. 2017. Opportunities for natural products in 21st century antibiotic discovery. Natural Product Reports **34**(7): 694-701.

Wu, B., Wu, X., Sun, M., and Li, M. 2013. Two novel tyrosinase inhibitory sesquiterpenes induced by $CuCl_2$ from a marine-derived fungus *Pestalotiopsis* sp. Z233. Marine Drugs **11**(8): 2713-2721.

Wu, G., Yu, G., Kurtan, T., Mandi, A., Peng, J., Mo, X., Liu, M., Li, H., Sun, X., Li, J., Zhu, T., Gu, Q., and Li, D. 2015. Versixanthones A-F, cytotoxic xanthone-chromanone dimers from the marine-derived fungus *Aspergillus versicolor* HDN1009. Journal of Natural Products **78**(11): 2691-2698.

Wu, Q., X, Jin, X., Draskovic, M., Crews, M., Tenney, K., Valeriote, F., Yao, X., and Crews, P. 2012. Unraveling the numerous biosynthetic products of the marine sediment-derived fungus, *Aspergillus insulicola*. Phytochemistry Letters **5**(1): 114-117.

Xiang, L., Gong, S., Yang, L., Hao, J., Xue, M., Zeng, F., Zhang, X., Shi, W., Wang, H., and Yu, D. 2016. Biocontrol potential of endophytic fungi in medicinal plants from Wuhan Botanical Garden in China. Biological Control **94**: 47-55.

Xu, H., Deckert, R.J., and Garbary, D.J. 2008. *Ascophyllum* and its symbionts. X. ultrastructure of the interaction between *A. nodosum* (Phaeophyceae) and *Mycophycias ascophylli* (Ascomycetes). Botany **86**(2): 185-193.

Xu, R., Li, X., Xu, G., and Wang, B. 2017. Optimizing production of asperolide A, a potential anti-tumor tetranorditerpenoid originally produced by the algal-derived endophytic fungus *Aspergillus wentii* EN-48. Chinese Journal of Oceanology and Limnology **35**(3): 658-663.

Xu, S., Shang, M.-Y., Liu, G.-X., Xu, F., Wang, X., Shou, C.-C., and Cai, S.-Q. 2013. Chemical constituents from the rhizomes of *Smilax glabra* and their antimicrobial activity. Molecules **18**: 5265-5287.

Yamamoto, Y., Nitta, K., and Jinbo, A. 1967. Studies on the metabolic products of a strain of *Aspergillus fumigatus* (DH 413). III. Biosynthesis of toluquinones. Chemical & Pharmaceutical Bulletin **15**(4): 427-431.

Yamamoto, Y., Nitta, K., Tango, K., Saito, T., and Tsuchimuro, M. 1965. Studies on the metaboic products of a strain of *Aspergillus fumigatus* (DH 413). I. Isolation and chemical structures of metabolites. Chemical & Pharmaceutical Bulletin **13**(8): 935-941.

Yang, J.Y., Sanchez, L.M., Rath, C.M., Liu, X., Boudreau, P.D., Bruns, N., Glukhov, E., Wodtke, A., de Felicio, R., Fenner, A., Wong, W.R., Linington, R.G., Zhang, L., Debonsi, H.M., Gerwick, W.H., and Dorrestein, P.C. 2013. Molecular networking as a dereplication strategy. Journal of Natural Products **76**(9): 1686-1699.

Yang, R.Y., Li, C.Y., Lin, Y.C., Peng, G.T., She, Z.G., and Zhou, S.N. 2006. Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities. Bioorganic & Medicinal Chemistry Letters **16**(16): 4205-4208.

Yin, Y., Ding, Y., Feng, G., Li, J., Xiao, L., Karuppiah, V., Sun, W., Zhang, F., and Li, Z. 2015. Modification of artificial sea water for the mass production of (+)-terrein by *Aspergillus terreus* strain PF26 derived from marine sponge *Phakellia fusca*. Letters in Applied Microbiology **61**(6): 580-587.

Yoon, H.S., Muller, K.M., Sheath, R.G., Ott, F.D., and Bhattacharya, D. 2006. Defining the major lineages of red algae (Rhodophyta). Journal of Phycology **42**(2): 482-492.

Zaffiri, L., Gardner, J., and Toledo-Pereyra, L.H. 2012. History of antibiotics. From salvarsan to cephalosporins. Journal of Investigative Surgery **25**(2): 67-77.

Zaffiri, L., Gardner, J., and Toledo-Pereyra, L.H. 2013. History of antibiotics: From fluoroquinolones to daptomycin (Part 2). Journal of Investigative Surgery **26**(4): 167-179.

Zeller, M.A., Riener, M., and Nicewicz, D.A. 2014. Butyrolactone synthesis via polar radical crossover cycloaddition reactions: Diastereoselective syntheses of methylenolactocin and protolichesterinic acid. Organic Letters **16**(18): 4810-4813.

Zhang, G., Li, J., Zhu, T., Gu, Q., and Li, D. 2016a. Advanced tools in marine natural drug discovery. Current Opinions in Biotechnology **42**: 13-23.

Zhang, J.-c., Chen, G.-Y., Li, X.-Z., Hu, M., Wang, B.-Y., Ruan, B.-H., Zhou, H., Zhao, L.-X., Zhou, J., Ding, Z.-T., and Yang, Y.-B. 2017. Phytotoxic, antibacterial, and antioxidant activities of mycotoxins and other metabolites from *Trichoderma* sp. Natural Product Research: Ahead of Print.

Zhang, P., Li, X.-M., Li, X., and Wang, B.-G. 2015a. New indole-diterpenoids from the algal-associated fungus *Aspergillus nidulans*. Phytochemistry Letters **12**: 182-185.

Zhang, P., Li, X.-M., Mao, X.-X., Mándi, A., Kurtán, T., and Wang, B.-G. 2016b. Varioloid A, a new indolyl-6,10b-dihydro-5aH-[1]benzofuro[2,3-b]indole derivative from the marine alga-derived endophytic fungus *Paecilomyces variotii* EN-291. Beilstein Journal of Organic Chemistry **12**: 2012-2018.

Zhang, P., Li, X.-M., Wang, J.-N., Li, X., and Wang, B.-G. 2015b. New butenolide derivatives from the marine-derived fungus *Paecilomyces variotii* with DPPH radical scavenging activity. Phytochemistry Letters **11**: 85-88.

Zhang, P., Li, X.-M., Wang, J.-N., Li, X., and Wang, B.-G. 2015c. Prenylated indole alkaloids from the marine-derived fungus *Paecilomyces variotii*. Chinese Chemical Letters **26**(3): 313-316.

Zhang, P., Li, X., and Wang, B.G. 2016c. Secondary metabolites from the marine algal-derived endophytic fungi: Chemical diversity and biological activity. Planta Medica **82**(9-10): 832-842.

Zhang, P., Li, X.M., Wang, J.N., and Wang, B.G. 2015d. Oxepine-containing diketopiperazine alkaloids from the algal-derived endophytic fungus *Paecilomyces variotii* EN-291. Helvetica Chimica Acta **98**(6): 800-804.

Zhang, P., Mandi, A., Li, X.M., Du, F.Y., Wang, J.N., Li, X., Kurtan, T., and Wang, B.G. 2014. Varioxepine A, a 3H-oxepine-containing alkaloid with a new oxa-cage from the marine algal-derived endophytic fungus *Paecilomyces variotii*. Organic Letters **16**(18): 4834-4837.

Zhang, W., Krohn, K., Draeger, S., and Schulz, B. 2008. Bioactive isocoumarins isolated from the endophytic fungus *Microdochium bolleyi*. Journal of Natural Products **71**(6): 1078-1081.

Zhang, Y., Li, X.-M., and Wang, B.-G. 2007a. Nigerasperones A~C, new monomeric and dimeric naphtho-[gamma]-pyrones from a marine alga-derived endophytic fungus *Aspergillus niger* EN-13. The Journal of Antibiotics **60**(3): 204-210.

Zhang, Y., LI, X.-M., and Wang, B.-G. 2012. Anthraquinone derivatives produced by marine-derived fungus *Aspergillus versicolor* EN-7. Bioscience, Biotechnology, and Biochemistry **76**(9): 1774-1776.

Zhang, Y., Li, X.M., Feng, Y., and Wang, B.G. 2010. Phenethyl-alpha-pyrone derivatives and cyclodipeptides from a marine algous endophytic fungus *Aspergillus niger* EN-13. Natural Product Research **24**(11): 1036-1043.

Zhang, Y., Li, X.M., Proksch, P., and Wang, B.G. 2007b. Ergosterimide, a new natural Diels-Alder adduct of a steroid and maleimide in the fungus *Aspergillus niger*. Steroids **72**(9-10): 723-727.

Zhang, Y., Li, X.M., Wang, C.Y., and Wang, B.G. 2007c. A new naphthoquinoneimine derivative from the marine algal-derived endophytic fungus *Aspergillus niger* EN-13. Chinese Chemical Letters **18**(8): 951-953.

Zhang, Y., Mu, J., Feng, Y., Kang, Y., Zhang, J., Gu, P.J., Wang, Y., Ma, L.F., and Zhu, Y.H. 2009. Broad-spectrum antimicrobial epiphytic and endophytic fungi from marine organisms: isolation, bioassay and taxonomy. Marine Drugs **7**(2): 97-112.

Zhang, Y., Wang, S., Li, X.M., Cui, C.M., Feng, C., and Wang, B.G. 2007d. New sphingolipids with a previously unreported 9-methyl-C20-sphingosine moiety from a marine algous endophytic fungus *Aspergillus niger* EN-13. Lipids **42**(8): 759-764.

Zhang, Y., Yeh, J.R., Mara, A., Ju, R., Hines, J.F., Cirone, P., Griesbach, H.L., Schneider, I., Slusarski, D.C., Holley, S.A., and Crews, C.M. 2006. A chemical and genetic approach to the mode of action of fumagillin. Chemistry & Biology **13**(9): 1001-1009.

Zhang, Y., Zhang, Z., Wang, B., Liu, L., and Che, Y. 2016d. Design and synthesis of natural product derivatives with selective and improved cytotoxicity based on a sesquiterpene scaffold. Bioorganic & Medicinal Chemistry Letters **26**(8): 1885-1888.

Zhang, Z., Miao, L., Lv, C., Sun, H., Wei, S., Wang, B., Huang, C., and Jiao, B. 2013. Wentilactone B induces G2/M phase arrest and apoptosis via the Ras/Raf/MAPK signaling pathway in human hepatoma SMMC-7721 cells. Cell Death and Disease **4**: e657.

Zhou, H., Zhu, T., Cai, S., Gu, Q., and Li, D. 2011. Drimane sesquiterpenoids from the mangrove-derived fungus *Aspergillus ustus*. Chemical & Pharmaceutical Bulletin **59**(6): 762-766.

Zhu, G., and Lu, X. 1995a. A palladium-catalyzed construction of enantiomerically pure α-methylene-γ-butyrolactones. Enantiospecific synthesis of both enantiomers of methylenolactocin. Tetrahedron: Asymmetry **6**(4): 885-892.

Zhu, G., and Lu, X. 1995b. A palladium(II)-catalyzed construction of alpha-methylene-gamma-butyrolactones in optically active form. Total synthesis of (-)-methylenolactocin. The Journal of Organic Chemistry **60**(4): 1087-1089.

Ziemert, N., Alanjary, M., and Weber, T. 2016. The evolution of genome mining in microbes - a review. Natural Product Reports **33**(8): 988-1005.

Zuccaro, A., Schoch, C.L., Spatafora, J.W., Kohlmeyer, J., Draeger, S., and Mitchell, J.I. 2008. Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. Applied and Environmental Microbiology **74**(4): 931-941.

Zuccaro, A., Schulz, B., and Mitchell, J.I. 2003. Molecular detection of ascomycetes associated with *Fucus serratus*. Mycological Research **107**: 1451-1466.

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Appendix 1: Algal collection data

Table A1.1. Collection data for the marine macroalgae collected from Green's Point, L'Etete, New Brunswick, Canada.

Algae species	Collection date	GIS N[b] & GIS W[b]
Alaria esculenta	June 23 2013	N 45 02.316; W 066 53.549
Ascophyllum nodosum	May 27 2013	N 45 02.370; W 066 53.471
Chondrus crispus	June 2 2013	N 45 02.323; W 066 53.550
Desmarestia viridis	July 25 2013	N 45 02.309; W 066 53.542
Devaleraea ramentacea	June 2 2013	N 45 02.376; W 066 53.428
Dumontia contorta	June 23 2013	N 45 02.346; W 066 53.532
Fucus distichus subsp. *edentatus*	June 10 2013	N 45 02.351; W 066 53.505
Fucus distichus subsp. *evanescens*	May 27 2013	N 45 02.355; W 066 53.503
Fucus spiralis	July 25 2013	N 45 02.367; W 066 53.500
Fucus vesiculosus	June 23 2013	N 45 02.344; W 066 53.522
Mastocarpus stellatus	May 27 2013	N 45 02.354; W 066 53.500
Palmaria palmata	June 2 2013	N 45 02.328; W 066 53.545
Petalonia fascia	July 11 2013	N 45 02.369; W 066 53.477
Polysiphonia lanosa	June 23 2013	N 45 02.288; W 066 53.523
Porphyra sp.	May 27 2013	N 45 02.355; W 066 53.508
Saccharina latissima	June 23 2013	N 45 02.328; W 066 53.548
Scytosiphon lomentaria	June 10 2013	N 45 02.330; W 066 53.543
Spongomorpha arcta	June 23 2013	N 45 18.348; W 066 05.132
Ulva intestinalis	July 11 2013	N 45 02.368; W 066 53.472
Ulva lactuca	June 10 2013	N 45 02.333; W 066 53.539

[a] GIS N – Geographic Information System North
[b] GIS W – Geographic Information System West

Appendix 2: Raw isolation data of endophytic fungi

Table A2.1. Raw isolation data of endophytic fungi isolated from marine algae of collected from the Bay of Fundy, New Brunswick, Canada. Data was used for the calculation of rarefaction curves.

Isolate	Number observed	Isolate	Number observed	Isolate	Number observed	Isolate	Number observed	Isolate	Number observed
KP1-045A	1	KP1-131B	1	KP1-131Z	1	KP2-033H	1	KP1-123B	1
KP1-045B	1	KP1-131C	1	KP1-131AA	1	KP1-171A	1	KP1-123C	1
KP1-045C	1	KP1-131DA	1	KP1-131BB	1	KP1-135B	1	KP1-175A	2
KP1-045D	5	KP1-131DB	1	KP1-131CC	1	KP1-135C	1	KP1-175C	1
KP1-045G	1	KP1-131E	1	KP1-131DD	4	KP1-135D	1	KP1-175D	1
KP1-045I	2	KP1-131F	1	KP1-017A	1	KP1-135E	1	KP1-175E	1
KP1-045J	1	KP1-131G	1	KP1-017C	2	KP1-135E2	4	KP1-175F	2
KP1-045K	2	KP1-131H	1	KP1-017D	2	KP1-135F	1	KP1-175G	3
KP1-013B	1	KP1-131I	1	KP1-017E	1	KP2-013A	13	KP1-175H	1
KP2-029B	2	KP1-131J	1	KP1-099A	1	KP2-013E	14	KP1-175J	2
KP2-029C	5	KP1-131K	2	KP1-143A	4	KP2-013F	11	KP1-175K	2
KP2-029D	2	KP1-131L	3	KP1-143B	1	KP2-013G	7	KP1-175L	1
KP2-029E	1	KP1-131M	1	KP1-143C	1	KP2-009A	4	KP1-175M	2
KP2-029F	1	KP1-131N	1	KP1-143D	1	KP2-009B	1	KP2-001A	1
KP1-091A	1	KP1-131O	1	KP2-017A	14	KP2-025A	1	KP2-001B	3
KP2-005A	4	KP1-131Q	1	KP1-089A	1	KP2-025B	8	KP2-001C	1
KP1-021A	1	KP1-131R	1	KP2-033A	1	KP2-025C	1	KP2-001D	1
KP1-069A	1	KP1-131S	1	KP2-033B	1	KP2-025D	1	KP2-001E	1
KP1-069B	1	KP1-131T	1	KP2-033C	3	KP2-025E	1	KP2-001F	1
KP1-069C	1	KP1-131U	1	KP2-033D	1	KP1-095A	1	KP2-001G	1
KP1-069D	1	KP1-131V	1	KP2-033E	3	KP1-139A	1	KP1-009A	1
KP1-069E	1	KP1-131W	2	KP2-033F	1	KP1-139B	1	KP1-009B	1
KP1-131A	2	KP1-131Y	1	KP2-033G	1	KP1-123A	1	KP1-009C	1

Table A2.1. Raw isolation data of endophytic fungi isolated from marine algae of collected from the Bay of Fundy, New Brunswick, Canada. Data was used for the calculation of rarefaction curves, continued.

Isolate	Number observed	Isolate	Number observed	Isolate	Number observed	Isolate	Number observed	Isolate	Number observed
KP1-075A	5	KP1-063B	1	KP1-063L	1	KP1-115A	1	KP1-119A	1
KP1-075B	1	KP1-063C	1	KP1-063M	1	KP1-179A	1	KP1-119B	5
KP1-025B	1	KP1-063E	1	KP1-063N	1	KP1-179B	1	KP1-119C	1
KP1-025C	1	KP1-063F	9	KP1-063O	1	KP1-179C	1	KP1-119D	1
KP1-063A	10	KP1-063J	2	KP1-063P	1	KP1-081A	1	KP1-119E	1

Appendix 3: DNA sequences of endophytic fungi

KP1-013B

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 121  aaaactttca acaacggatc tcttggttcc ggcatcgatg aagaacgcag cgaaatgcga
 181  tacgtaatgt gaattgcaga attcagtgaa tcatcgagtc tttgaacgca cattgcgccc
 241  cttggtattc cgggggggcat gcctgtccga gcgtcattgc tgccctcaag cacggcttgt
 301  gtgttgggct ccgtcctcct tcccgggggga cgggcccgaa aggcagcggc ggcaccgcgt
 361  ccggtcctcg agcgtatggg gcttcgtctt ccgctcttgt aggcccggcc ggcgcttgcc
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 481  aagcatat
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ITS 4

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 241  atgcgatacg taatgtgaat tgcagaattc agtgaatcat cgagtctttg aacgcacatt
 301  gcgccccttg gtattccggg gggcatgcct gtccgagcgt cattgctgcc ctcaagcacg
 361  gcttgtgtgt tgggctccgt cctccttccc gggggacggg cccgaaaggc agcggcggca
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KP1-017A

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 121  tagttaaaac tttcaacaac ggatctcttg gttctggcat cgatgaagaa cgcagcgaaa
 181  tgcgataagt aatgtgaatt gcagaattca gtgaatcatc gaatctttga acgcacattg
 241  cgccccttgg tattccgggg ggcatgcctg ttcgagcgtc atttcaaccc tcaagctctg
 301  cttggtattg agccccgcca gcgatggcgg gccctaaaat cagtggcggc gccgctgggt
 361  cctgagcgta gtaattctct cgctacaggg tccccgcgtg cttctgccaa caaccccaaa
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 241  aatgcgataa gtaatgtgaa ttgcagaatt cagtgaatca tcgaatcttt gaacgcacat
 301  tgcgcccctt ggtattccgg ggggcatgcc tgttcgagcg tcatttcaac cctcaagctc
 361  tgcttggtat tgagccccgc cagcgatggc gggccctaaa atcagtggcg gcgccgctgg
 421  gtcctgagcg tagtaattct ctcgctacag ggtccccgcg t
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KP1-017C

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 121  ggcagcctgc acaaaccccc acatccaagc ctcggagaac cgttcggaaa acgggtgagg
 181  ttgagaattt aatgacactc aaacaggcat gctcctcgga ataccaagga gcgcaaggtg
 241  cgttcaaaga ttcgatgatt cactgaattc tgcaattcac attacttatc gcatttcgct
 301  gcgttcttca tcgatgcgag agccaagaga tccgttgctg aaagttgtat agtgttttat
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 421  agagcctgga aattcgagga gagacacctc aagagggcaa tcctcgcatc cgcactcaga
 481  gagcacgaga gtcatccaga cctacagtcg gtgcacaggt ggatagataa aaatggcggg
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 601  ccttccgc
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ITS 4

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 181  ggcagcctgc acaaaccccc acatccaagc ctcggagaac cgttcggaaa acgggtgagg
 241  ttgagaattt aatgacactc aaacaggcat gctcctcgga ataccaagga gcgcaaggtg
 301  cgttcaaaga ttcgatgatt cactgaattc tgcaattcac attacttatc gcatttcgct
 361  gcgttcttca tcgatgcgag agccaagaga tccgttgctg aaagttgtat agtgttttat
 421  aggcgatcaa gcccattgac tacattctat atcatacttg tggggtgtgt aaaaagacgt
 481  agagcctgga aattcgagga gagacacctc aagagggcaa tcctcgcatc cgcactcaga
 541  gagcacgaga gtcatccaga cctacagtcg gtgcacaggt ggatagataa aaatggcggg
 601  cgtgcacaat
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KP1-017E

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 421  gcccgccggc gaccccaatc aatctttcca ggttgacctc ggatcaggta gggatacccg
 481  ctgaacttaa gcatat
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ITS 4

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 181  aatcagttaa aactttcaac aacggatctc ttggttccgg catcgatgaa gaacgcagcg
 241  aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgagtctt tgaacgcaca
 301  ttgcgccccc tggtattccg gggggcatgc ctgtccgagc gtcattgctg ccctcaagca
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KP1-045A

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 181  gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 241  attgcgccct ctggtattcc ggagggcatg cctgtccgag cgtcattgct gccctccagc
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KP1-045C

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KP1-045K

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 181  aaagattcga tgattcactg aattctgcaa ttcacactac gtatcgcatt tcgctgcgtt
 241  cttcatcgat gccagaacca agagatccgt tgttgaaagt tgtaataatt acattgttta
 301  ctgacgctga ttgcaattac aaaaaaaggt ttatggttgg gtcctggtgg cgggcgaacc
 361  cgcccaggaa acaagaagtg cgcaaaagac atgggtgaat aattcagaca agctggagcc
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ITS 4

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 181  ccaacaccaa gcaaagcttg agggtacaaa tgacgctcga acaggcatgc cctttggaat
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 301  tacgtatcgc atttcgctgc gttcttcatc gatgccagaa ccaagagatc cgttgttgaa
 361  agttgtaata attacattgt ttactgacgc tgattgcaat tacaaaaaaa ggtttatggt
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KP1-063J

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 121  gcatcgatga agaacgcagc gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat
 181  catcgagtct ttgaacgcac attgcgccct ctggtattcc ggagggcatg cctgtccgag
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ITS 4

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KP1-063N

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ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt accgagtgag ggccctctgg gtccaacctc
  61  ccacccgtgt ctatcgtacc ttgttgcttc ggcgggcccg ccgtttcgac ggccgccggg
 121  gaggccttgc gccccccgggc ccgcgcccgc cgaagacccc aacatgaacg ctgttctgaa
 181  agtatgcagt ctgagttgat tatcgtaatc agttaaaact ttcaacaacg gatctcttgg
 241  ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag
 301  tgaatcatcg agtctttgaa cgcacattgc gccccctggt attccgggggg gcatgcctgt
 361  ccgagcgtca ttgctgccct caagcacggc ttgtgtgttg ggcccccgtc cccctctccc
 421  gggggacggg cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg tatggggctt
 481  tgtcacctg
```

KP1-075B

ITS 1

```
   1  cgccgggggg catctgcccc cgggcccgcg cccgccgaag ccacctgtga actctgtctg
  61  aagtatgcag tctgagacaa ttattaaatt aattaaaact ttcaacaacg gatctcttgg
 121  ttccggcatc gatgaagaac gcagcgaaat gcgataacta atgtgaattg cagaattcag
 181  tgaatcatcg agtctttgaa cgcacattgc gccctctggt attccggagg gcatgcctgt
 241  ccgagcgtca ttgctgccct ccagcccggc tggtgtgttg ggcccccgccc cccttcccgg
 301  gggggcgggc ccgaaaggca gcggcggcac cgcgtccggt cctcgagcgt atggggcttt
 361  gtcacccgct cttgtaggcc cggccggcgc cagccgaccc cctcaatcta ttttttcagg
 421  ttgacctcgg atcaggtagg gatacccgct gaacttaagc atatc
```

ITS 4

```
   1  acctgcggaa ggatcattac tgagtgaggg cccctcgggg tccaacctcc cacccgtgtt
  61  taacgaacct tgttgctttg gcgggcccgc ctcacggccg ccgggggggca tctgcccccg
 121  ggcccgcgcc cgccgaagcc acctgtgaac tctgtctgaa gtatgcagtc tgagacaatt
 181  attaaattaa ttaaaacttt caacaacgga tctcttggtt ccggcatcga tgaagaacgc
 241  agcgaaatgc gataactaat gtgaattgca gaattcagtg aatcatcgag tctttgaacg
 301  cacattgcgc cctctggtat tccggagggc atgcctgtcc gagcgtcatt gctgccctcc
 361  agcccggctg gtgtgttggg ccccgccccc cttcccgggg gggcgggccc gaaaggcagc
 421  ggcggcaccg cgtccggtcc tcgagcgtat ggggctttgt cacccgctc
```

KP1-089A

ITS 1

```
   1  tgtattatta ccttgttgct ttggcggtgc cgcgtggctt cggccgcgcc ttgggctctc
  61  gagcccgagt gtgcccgcca gaggaaaccc aaactctgaa tatttttgtc gtctgagtac
 121  tatataatag ttaaaacttt caacaacgga tctcttggtt ctggcatcga tgaagaacgc
 181  agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgaa tctttgaacg
 241  cacattgcgc cccttggtat tccgggggggc atgcctgttc gagcgtcatt tcaaccctca
 301  agctctgctt ggtattgagc cccgccagcg atggcgggct ctaaaatcag tggcggcgcc
 361  gctgggtcct gagcgtagta attctctcgc tacagggtcc ccgcgtgctt ctgccaacaa
 421  ccccaaattt tctatggttg acctcggatc aggtagggat acccgctgaa cttaagcata
 481  t
```

KP1-091A

ITS 1

```
   1  gccgggggggc acctgccccc gggcccgcgc ccgccgaaga caccattgaa ctctgtctga
  61  agattgcagt ctgagcgatt aactaaatca gttaaaactt tcaacaacgg atctcttggt
 121  tccggcatcg atgaagaacg cagcgaaatg cgataagtaa tgtgaattgc agaattcagt
 181  gaatcatcga gtctttgaac gcacattgcg ccccctggta ttccgggggg catgcctgtc
 241  cgagcgtcat tgctgccctc aagcacggct tgtgtgttgg gctccgcccc cctcccgggg
 301  ggcgggcccg aaaggcagcg gcggcaccgc gtccggtcct cgagcgtatg gggctttgtc
 361  acccgctctg taggcccggc cggcgcccgc cggcgacccc aatcaatctt tccaggttga
 421  cctcggatca ggtagggata cccgctgaac ttaagcatat caataagcgg ag
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt accgagtgag ggccctctgg gtccaacctc
  61  ccacccgtgt ttatcgtacc ttgttgcttc ggcgggcccg cctcacggcc gccgggggggc
 121  acctgccccc gggcccgcgc ccgccgaaga caccattgaa ctctgtctga agattgcagt
 181  ctgagcgatt aactaaatca gttaaaactt tcaacaacgg atctcttggt tccggcatcg
 241  atgaagaacg cagcgaaatg cgataagtaa tgtgaattgc agaattcagt gaatcatcga
 301  gtctttgaac gcacattgcg ccccctggta ttccgggggg catgcctgtc cgagcgtcat
 361  tgctgccctc aagcacggct tgtgtgttgg gctccgcccc cctcccgggg ggcgggcccg
 421  aaaggcagcg gcggcaccgc gtccggtcct cgagcgtatg gggctttgtc accc
```

KP1-119C

ITS 1

```
   1  ggggcttgct cccgggtggt aggggtaaca ccctcacgcg ccgcctgcct gtaccctctt
  61  tttacgagca cctttcgttc tccttcggcg gggcaacctg ccgctggaac caaaataaaa
 121  cctttttttgc atctagcatt acctgttctg atacaaacaa tcgttacaac tttcaacaat
 181  ggatctcttg gctctggcat cgatgaagaa cgcagcgaaa tgcgataagt agtgtgaatt
 241  gcagaattca gtgaatcatc gaatctttga acgcacattg cgccccttgg tattccatgg
 301  ggcatgcctg ttcgagcgtc atctacaccc tcaagctctg cttggtgttg ggcgtctgtc
 361  ccgcctctgc gcgcggactc gccccaaatt cattggcagc ggtctttgcc tcctctcgcg
 421  cagcacaatt gcgtctgcgg gggggcgcgg cccgcgtcca cgaagcaaca ttaccgtctt
 481  tgacctcgga tcaggtaggg atacccgctg aacttaagc
```

ITS 4

```
   1  gcggaaggat cattatccat ctcaaaccag gtgcggtcgc ggcccccggg ggcttgctcc
  61  cgggtggtag gggtaacacc ctcacgcgcc gcctgcctgt accctctttt tacgagcacc
 121  tttcgttctc cttcggcggg gcaacctgcc gctggaacca aaataaaacc ttttttgcat
 181  ctagcattac ctgttctgat acaaacaatc gttacaactt tcaacaatgg atctcttggc
 241  tctggcatcg atgaagaacg cagcgaaatg cgataagtag tgtgaattgc agaattcagt
 301  gaatcatcga atctttgaac gcacattgcg ccccttggta ttccatgggg catgcctgtt
 361  cgagcgtcat ctacaccctc aagctctgct tggtgttggg cgtctgtccc gcctctgcgc
 421  gcggactcgc cccaaattca ttggcagcgg tctttgcctc ctctcgcgca gcacaattgc
 481  gtctgcgggg gggcgcggcc cgcgtccacg aagcaaca
```

KP1-119E

ITS 1

```
   1  gttgcttcgg cggcgcggcc tccctcacgg gggcgccgca gccccgcctc tccggaggtg
  61  tggggcgccc gccggaggta cgaaactctg tattatagtg gcatctctga gtaaaaaaca
 121  aataagttaa aactttcaac aacggatctc ttggttctgg catcgatgaa gaacgcagcg
 181  aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgaatctt tgaacgcaca
 241  ttgcgcccgc tagtactcta gcgggcatgc ctgttcgagc gtcatttcaa ccctcaagcc
 301  ctgcttggtg ttggggccct acggctgccg taggccctga aaggaagtgg cgggctcgct
 361  acaactccga gcgtagtaat tcattatctc gctagggacg ttgcggcgcg ctcctgccgt
 421  taaagaccat ctttaactca aggttgacct cggatcaggt aggaataccc gctgaactta
 481  agcatatca
```

ITS 4

```
   1  cggagggatc attattagaa gccgaaaggc tacttaaaac catcgcgaac tcgtccaagt
  61  tgcttcggcg gcgcggcctc cctcacgggg gcgccgcagc cccgcctctc cggaggtgtg
 121  gggcgcccgc cggaggtacg aaactctgta ttatagtggc atctctgagt aaaaaacaaa
 181  taagttaaaa ctttcaacaa cggatctctt ggttctggca tcgatgaaga acgcagcgaa
 241  atgcgataag taatgtgaat tgcagaattc agtgaatcat cgaatctttg aacgcacatt
 301  gcgcccgcta gtactctagc gggcatgcct gttcgagcgt catttcaacc ctcaagccct
 361  gcttggtgtt ggggccctac ggctgccgta ggccctgaaa ggaagtggcg ggctcgctac
 421  aactccgagc gtagtaattc attatctcgc tagggacgtt gcggcgcgct cctgccgtta
 481  aagaccatc
```

KP1-123A

ITS 4

```
   1  acctgcggaa ggatcattac cgagtgaggg ccctctgggt ccaacctccc acccgtgttt
  61  atcgtacctt gttgcttcgg cgggcccgcc tcacggccgc cgggggcac ctgcccccgg
 121  gcccgcgccc gccgaagaca ccattgaact ctgtctgaag attgcagtct gagcgattaa
 181  ctaaatcagt taaaactttc aacaacggat ctcttggttc cggcatcgat gaagaacgca
 241  gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgagt ctttgaacgc
 301  acattgcgcc ccctggtatt ccgggggggca tgcctgtccg agcgtcattg ctgccctcaa
 361  gcacggcttg tgtgttgggc tccgccccc tcccgggggg cgggcccgaa aggcagcggc
 421  gg
```

KP1-123B

ITS 1

```
   1  gattggggtc gccggcgggc gccggccggg cctacagagc gggtgacaaa gccccatacg
  61  ctcgaggacc ggacgcggtg ccgccgctgc ctttcgggcc cgccccccgg gaggggggcg
 121  gagcccaaca cacaagccgt gcttgagggc agcaatgacg ctcggacagg catgcccccc
 181  ggaataccag ggggcgcaat gtgcgttcaa agactcgatg attcactgaa ttctgcaatt
 241  cacattactt atcgcatttc gctgcgttct tcatcgatgc cggaaccaag agatccgttg
 301  ttgaaagttt taactgattt agttaatcgc tcagactgca atcttcagac agagttcaat
 361  ggtgtcttcg gcgggcgcgg gcccgggggc aggtgccccc cggcggccgt gaggcgggcc
 421  cgccgaagca acaaggtacg ataaacacgg gtgggaggtt ggacccagag ggccctcact
 481  cggtaatgat ccttccgca
```

ITS 4

```
   1  attgatatgc ttaagttcag cgggtatccc tacctgatcc gaggtcaacc tggaaagatt
  61  gattggggtc gccggcgggc gccggccggg cctacagagc gggtgacaaa gccccatacg
 121  ctcgaggacc ggacgcggtg ccgccgctgc ctttcgggcc cgccccccgg gaggggggcg
 181  gagcccaaca cacaagccgt gcttgagggc agcaatgacg ctcggacagg catgcccccc
 241  ggaataccag ggggcgcaat gtgcgttcaa agactcgatg attcactgaa ttctgcaatt
 301  cacattactt atcgcatttc gctgcgttct tcatcgatgc cggaaccaag agatccgttg
 361  ttgaaagttt taactgattt agttaatcgc tcagactgca atcttcagac agagttcaat
 421  ggtgtcttcg gcgggcgcgg gcccgggggc aggtgccccc cggcggccgt gaggcgggcc
 481  cgccgaagca acaaggta
```

KP1-123C

ITS 1

```
   1  gcgaaagcgt gtacgcgccg tcactcttac ccttttttta cgagtacctt cgttctcctt
  61  cggtggggca acctgccgct ggaatcaaca aaacctttt tgcatctagc attacctgtt
 121  ctgatacaaa taatcgttac aactttcaac aatggatctc ttggctctgg catcgatgaa
 181  gaacgcagcg aaatgcgata agtagtgtga attgcagaat tcagtgaatc atcgaatctt
 241  tgaacgcaca ttgcgccccc tggtattcca tggggcatgc ctgttcgagc gtcatctaca
 301  ccctcaagct ctgcttggtg ttgggcgtct gtcccgcctc tgcgcgtgga ctcgccccaa
 361  attcattggc agcggtcttt gcctcctctc gcgcagcaca attgcgtttc ttgggggggt
 421  gggtcgcatc cacgaagcaa cattaccgtc tttgacctcg gatcaggtag ggatacccgc
 481  tgaacttaag catatca
```

ITS 4

```
   1  gaaggatcat tatccatctc aactgagccg gcgcggcccc atttggtggt aacgcgaaag
  61  cgtgtacgcg ccgtcactct taccctttt ttacgagtac cttcgttctc cttcggtggg
 121  gcaacctgcc gctggaatca acaaaacctt ttttgcatct agcattacct gttctgatac
 181  aaataatcgt tacaactttc aacaatggat ctcttggctc tggcatcgat gaagaacgca
 241  gcgaaatgcg ataagtagtg tgaattgcag aattcagtga atcatcgaat ctttgaacgc
 301  acattgcgcc ccttggtatt ccatggggca tgcctgttcg agcgtcatct acaccctcaa
 361  gctctgcttg gtgttgggcg tctgtcccgc ctctgcgcgt ggactcgccc caaattcatt
 421  ggcagcggtc tttgcctcct ctcgcgcagc acaattgcgt ttcttggggg ggtgggtcgc
 481  atccacgaag caac
```

KP1-131AA

ITS 1

```
   1  atcgtacctt gttgcttcgg cgggcccgcN cNNtNgacgg ccgccgggga ggccttgcgc
  61  ccccgggccc gcgcccgccg aagaccccaa catgaacgct gttctgaaag tatgcagtct
 121  gagttgatta tcgtaatcag ttaaaacttt caacaacgga tctcttggtt ccggcatcga
 181  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgag
 241  tctttgaacg cacattgcgc cccctggtat tccggggggc atgcctgtcc gagcgtcatt
 301  gctgccctca agcacggctt gtgtgttggg cccccgtccc cctctcccgg gggacgggcc
 361  cgaaaggcag cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacctgctc
 421  tgtaggcccg gccggcgcca gccgacaccc aactttattt ttctaaggtt gacctcggat
 481  caggtaggga tacccgctga acttaagcat atca
```

ITS 4

```
   1  acctgcggaa ggatcattac cgagtgaggg ccctctgggt ccaacctccc acccgtgtct
  61  atcgtacctt gttgcttcgg cgggcccgcc gtttcgacgg ccgccgggga ggccttgcgc
 121  ccccgggccc gcgcccgccg aagaccccaa catgaacgct gttctgaaag tatgcagtct
 181  gagttgatta tcgtaatcag ttaaaacttt caacaacgga tctcttggtt ccggcatcga
 241  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgag
 301  tctttgaacg cacattgcgc cccctggtat tccgggggggc atgcctgtcc gagcgtcatt
 361  gctgccctca agcacggctt gtgtgttggg cccccgtccc cctctcccgg gggacgggcc
 421  cgaaaggcag cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacctgctc
 481  tNNNggcccg gccggcgcca gccgacaccc aactt
```

KP1-131B

ITS 1

```
   1  actcttgttg ctttggcagg ccgtggtctt ccactgtggg ctctgcctgc atgtgcctgc
  61  cagaggacca aactctgaat tttagtaatg tctgagtact atataatagt taaaactttc
 121  aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgcg ataagtaatg
 181  tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcacc cggtggtatt
 241  ccgccgggta tgcctgttcg agcgtctgta gaacaacaaa ttaccaggtc ttgggtttcg
 301  acccaggctt gattctgggg ttgcggcatc gtctgcagcc ctaaagtaat gtggcggcac
 361  cgataggttc taagcgtagt aatttctcct cgctacagag tctttcggcg cattgggtac
 421  tcactcccgg ccataaaacc cccaatttta gtttgacctc ggatcaagta gggatacccg
 481  ctgaacttaa gcatatc
```

ITS 4

```
   1  tgcggaagga tcattaataa aaggatgcct ccgggcatac cccatccgtg tctatatact
  61  cttgttgctt tggcaggccg tggtcttcca ctgtgggctc tgcctgcatg tgcctgccag
 121  aggaccaaac tctgaatttt agtaatgtct gagtactata taatagttaa aactttcaac
 181  aacggatctc ttggttctgg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga
 241  attgcagaat tcagtgaatc atcgaatctt tgaacgcaca ttgcacccgg tggtattccg
 301  ccgggtatgc ctgttcgagc gtctgtagaa caacaaatta ccaggtcttg ggtttcgacc
 361  caggcttgat tctggggttg cggcatcgtc tgcagcccta aagtaatgtg gcggcaccga
 421  taggttctaa gcgtagtaat ttctcctcgc tacagagtct ttcggcgcat tgggtactca
 481  ctcccggcca taaaaccccc aa
```

KP1-131BB

ITS 1

```
   1  tgtgggggta tatgacagga taccgcgaga cacctatagc gaggaatatt tactacgctc
  61  agggcctcgt ggcaccgcca ctaattttaa ggcccgccga gttaccggcg aagcccaaga
 121  ccaagcagtt atgcttgagg gttgtaatga cgctcgaaca ggcatgcccc ccggaatacc
 181  aaggggcgca atgtgcgttc aaagattcga tgattcactg aattctgcaa ttcacattac
 241  ttatcgcatt tcgctgcgtt cttcatcgat gccagaacca agagatccgt tgttgaaagt
 301  tttaactatt atatagtact cagacgacac caataatcag ggtttaagat cctctggcgg
 361  acgcgtacca gccgaaaccg a
```

ITS 4

```
   1  gatatgctta agttcagcgg gtatccctac ctgatccgag gtcaaactta gaatgtgggg
  61  gtatatgaca ggataccgcg agacacctat agcgaggaat atttactacg ctcagggcct
 121  cgtggcaccg ccactaattt taaggcccgc cgagttaccg gcgaagccca agaccaagca
 181  gttatgcttg agggttgtaa tgacgctcga acaggcatgc cccccggaat accaaggggc
 241  gcaatgtgcg ttcaaagatt cgatgattca ctgaattctg caattcacat tacttatcgc
 301  atttcgctgc gttcttcatc gatgccagaa ccaagagatc cgttgttgaa agttttaact
 361  attatatagt actcagacga caccaataat cagggtttaa gatcctctgg cggacgcgta
 421  ccagccgaaa ccgatagcct tgcggcggtc cgccaaagca acaatagta
```

KP1-131C

ITS 1

```
   1  tgaaaatagt ttttgggttg gtcggcgaag cgccggccgg gcctacagag cgggtgacaa
  61  agccccatac gctcgaggac cggacgcggt actgccattg cctttcgggc ccgtccctag
 121  ggacgaggac ccaacacaca agccgggctt gagggcagca atgacgctcg gacaggcatg
 181  ccccccggaa taccaggggg cgcaatgtgc gttcaaagac tcgatgattc actgaattct
 241  gcaattcaca ttatttatcg catttcgctg cgttcttcat cgataccgga accaagagat
 301  ccgttgttga aagttttaac taattaagtt atgttctcag actgcattat aaaacagagt
 361  tcaaatggcg tcctcggcgg cacggatgcc gccgaagcaa caagatgtaa tagacacggg
 421  tgggaggtta gctctgacga gcgtagactc agtaatgatc cttccgcagg t
```

KP1-131DA

ITS 1

```
   1  cccgtgtcta tacatcttgt tgcttcggcg gcatccgtgc cgccgaggac gccatttgaa
  61  ctctgtttta taatgcagtc tgagaacata acttaattag ttaaaacttt caacaacgga
 121  tctcttggtt ccggtatcga tgaagaacgc agcgaaatgc gataaataat gtgaattgca
 181  gaattcagtg aatcatcgag tctttgaacg cacattgcgc cccctggtat tccgggggggc
 241  atgcctgtcc gagcgtcatt gctgccctca agcccggctt gtgtgttggg tcctcgtccc
 301  tagggacggg cccgaaaggc aatggcagta ccgcgtccgg tcctcgagcg tatggggctt
 361  tgtcacccgc tctgtaggcc cggccggcgc ttcgccgacc aacccaaaaa ctatttttttc
 421  aggttgacct cggatcaggt agggataccc gctgaactta agcatatc
```

ITS 4

```
   1  aacctgcgga aggatcatta ctgagtctac gctcgtcaga gctaacctcc cacccgtgtc
  61  tattacatct tgttgcttcg gcggcatccg tgccgccgag gacgccattt gaactctgtt
 121  ttataatgca gtctgagaac ataacttaat tagttaaaac tttcaacaac ggatctcttg
 181  gttccggtat cgatgaagaa cgcagcgaaa tgcgataaat aatgtgaatt gcagaattca
 241  gtgaatcatc gagtctttga acgcacattg cgccccctgg tattccgggg ggcatgcctg
 301  tccgagcgtc attgctgccc tcaagcccgg cttgtgtgtt gggtcctcgt ccctagggac
 361  gggcccgaaa ggcaatggca gtaccgcgtc cggtcctcga gcgtatgggg ctttgtcacc
 421  cgctctgtag gcccggccgg cgcttcgccg accaacccaa aaac
```

KP1-131DB

ITS 1

```
   1  ataacttgtt gcctcggcat tggttggctt cgaatgaagt cccttatacc cttctgagtg
  61  taaggagcag accggccgac ggcccctata aactcttgtt tttgtaatat catctgagta
 121  aaacaactaa aatgaatcaa aactttcaac aacggatctc ttggttctgg catcgatgaa
 181  gaacgcagcg aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgaatctt
 241  tgaacgcaca ttgcgcccgg tggtattcca ccgggcatgc ctgttcgagc gtcatttcaa
 301  ccctcaaaaa tcttgtatta ttggtgttgg aggaatacct gtaacagggt accctctgaa
 361  atttagtggc gggctcgcta gaattttgag cgtagtaatt atacctcgtt tttaaagact
 421  agtgggactt cttgccgtaa aacccccaa ctttctgaaa tttgacctcg gatcaggtag
 481  gaatacccgc tgaacttaag catatca
```

ITS 4

```
   1  cggagggatc attgctggaa caaacgccct cacgggtgct acccagaaac cctttgtgaa
  61  cttataactt gttgcctcgg cattggttgg cttcgaatga agtcccttat acccttctga
 121  gtgtaaggag cagaccggcc gacggcccct ataaactctt gtttttgtaa tatcatctga
 181  gtaaaacaac taaaatgaat caaaactttc aacaacggat ctcttggttc tggcatcgat
 241  gaagaacgca gcgaaatgcg ataagtaatg tgaattgcag aattcagtga atcatcgaat
 301  ctttgaacgc acattgcgcc cggtggtatt ccaccgggca tgcctgttcg agcgtcattt
 361  caaccctcaa aaatcttgta ttattggtgt tggaggaata cctgtaacag ggtaccctct
 421  gaaatttagt ggcgggctcg ctagaatttt gagcgtagta attatacctc gtttttaaag
 481  actagtggga cttcttgccg taaaaccccc caact
```

KP1-131F

ITS 1

```
   1  actcttgttg ctttggcagg ccgtggtcac ccactgtggg ctatgcctgc atgcgcctgc
  61  cagaggacca aactctgaat attagtgatg tctgagtact atataatagt taaaactttc
 121  aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgcg ataagtaatg
 181  tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcacc cggtggtatt
 241  ccgccgggta tgcctgttcg agcgtcatta taaccactca agcctgtctt ggtgttgggg
 301  ttgcgaatct tttgcagccc tcgagtctcg tagcgccacc tgtgggttct aagcgtagta
 361  atttctcctc gctatagaac ctgctcgggg aaaagtataa ttcgtagcct ggttctatgt
 421  gcccgctata aaaccccccaa tttttaaagg ttgacctcgg atcaagtagg gatacccgct
 481  gaacttaagc atat
```

ITS 4

```
   1  tgcggaagga tcattaacca ttggatacct tcgggtatat cccatccgtg tctacatact
  61  cttgttgctt tggcaggccg tggtcaccca ctgtgggcta tgcctgcatg cgcctgccag
 121  aggaccaaac tctgaatatt agtgatgtct gagtactata taatagttaa aactttcaac
 181  aacggatctc ttggttctgg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga
 241  attgcagaat tcagtgaatc atcgaatctt tgaacgcaca ttgcacccgg tggtattccg
 301  ccgggtatgc ctgttcgagc gtcattataa ccactcaagc ctgtcttggt gttggggttg
 361  cgaatctttt gcagccctcg agtctcgtag cgccacctgt gggttctaag cgtagtaatt
 421  tctcctcgct atagaacctg ctcggggaaa agtataattc gtagcctggt tctatgtgcc
 481  cgctataaaa cccccaatt
```

KP1-131H

ITS 4

```
   1  ctccgcttat tgatatgctt aagttcagcg ggtattccta cctgatccga ggtcaaccat
  61  taaaaaagtg ccccccgaga gggtgcggtt tatggctgtt gtctgtacgg cttgcagaag
 121  cgagataaaa aattactacg ctcagagcac gaacagactc cgccactggt tttgaggagc
 181  tgcgtattag gcagtctccc aacactaagc taggcttaag ggttgaaatg acgctcgaac
 241  aggcatgccc actagaatac taatgggcgc aatgtgcgtt caaagattcg atgattcact
 301  gaattctgca attcacatta cttatcgcat ttcgctgcgt tcttcatcga tgccagaacc
 361  aagagatccg ttgttgaaag ttttaactta tttcttagtt ttgattcaga tttgacaaaa
 421  attaacaaga gtttagtagt ccaccggtgg cagcgctgtt tccagcaccg accaccgagg
 481  caacagtg
```

KP1-131I

ITS 1

```
   1  ctcggtgggg gctccagctt gtctgaatta tNNNccatgt cttttgcgca cttcttgttt
  61  cctgggcggg ttcgcccgcc accaggaccc aaccataaac ctttttttgt aattgcaatc
 121  agcgtcagta aacaatgtaa ttattacaac tttcaacaac ggatctcttg gttctggcat
 181  cgatgaagaa cgcagcgaaa tgcgatacgt agtgtgaatt gcagaattca gtgaatcatc
 241  gaatctttga acgcacattg cgccctttgg tattccaaag ggcatgcctg ttcgagcgtc
 301  atttgtaccc tcaagctttg cttggtgttg ggcgtctttg tctctcacga gactcgcctt
 361  aaaatgattg gcagccggcc tactggtttc ggagcgcagc acaatcttgc acttctgatc
 421  agccatggtt gagcatccat caagaccaca tttttctcac ttttgacctc ggatcaggta
 481  gggatacccg ctgaacttaa gcatat
```

ITS 4

```
   1  cggagggatc attacacaat atgaaagcgg gttgggacct catctcggtg ggggctccag
  61  cttgtctgaa ttattcaccc atgtcttttg cgcacttctt gtttcctggg cgggttcgcc
 121  cgccaccagg acccaaccat aaacctttttt ttgtaattgc aatcagcgtc agtaaacaat
 181  gtaattatta caactttcaa caacggatct cttggttctg gcatcgatga agaacgcagc
 241  gaaatgcgat acgtagtgtg aattgcagaa ttcagtgaat catcgaatct ttgaacgcac
 301  attgcgccct ttggtattcc aaagggcatg cctgttcgag cgtcatttgt accctcaagc
 361  tttgcttggt gttgggcgtc tttgtctctc acgagactcg ccttaaaatg attggcagcc
 421  ggcctactgg tttcggagcg cagcacaatc ttgcacttct gatcagccat gg
```

KP1-131J

ITS 1

```
   1  gaaccaggcg cccgccgcag gacccaaacc tctgtttttg ttaagatttc tcctctgagt
  61  ggattttaca aataaatcaa aactttcaac aacggatctc ttggctctgg catcgatgaa
 121  gaacgcagcg aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgaatctt
 181  tgaacgcaca ttgcgcccgc cagcattctg gcgggcatgc ctgtccgagc gtcatttcaa
 241  ccctcaggct cccgcgcctg gtgctgggga tcggccttca ccggccggcc ccgaaataca
 301  gtggcggccc cgcccgtgta cctctgcgta gtagcataca cctcgcagct ggaagcggcg
 361  gcggccacgc cggaaaaccc ccgacttctg aaagttgacc tcggatcagg taggaatacc
 421  cgctgaactt aagcatat
```

ITS 4

```
   1  acctgcggag ggatcattac cgagtttaca actcccaaac ccaatgtgaa catacctaca
  61  tgttgcctcg gcggaccgcc ccggcgccct cgggcaccgg aaccaggcgc ccgccgcagg
 121  acccaaacct ctgtttttgt taagatttct cctctgagtg gattttacaa ataaatcaaa
 181  actttcaaca acggatctct tggctctggc atcgatgaag aacgcagcga aatgcgataa
 241  gtaatgtgaa ttgcagaatt cagtgaatca tcgaatcttt gaacgcacat tgcgcccgcc
 301  agcattctgg cgggcatgcc tgtccgagcg tcatttcaac cctcaggctc ccgcgcctgg
 361  tgctggggat cggccttcac cggccggccc cgaaatacag tggcggcccc gcccgtgtac
 421  ctctgcgtag tagcatacac
```

KP1-131K

ITS 1

```
   1  cttacccatg tcttttgagt accttcgttt cctcggtggg ttcgcccgcc ggttggacaa
  61  cacttaaacc ctttgtaatt gaaatcagcg tctgaaaaaa cttaatagtt acaactttca
 121  acaacggatc tcttggttct ggcatcgatg aagaacgcag cgaaatgcga taagtagtgt
 181  gaattgcaga attcagtgaa tcatcgaatc tttgaacgca cattgcgccc cttggtattc
 241  catggggcat gcctgttcga gcgtcatttg taccttcaag ctctgcttgg tgttgggtgt
 301  ttgtctcgcc tttgcgcgca gactcgcctc aaaacgattg gcagccggcg tgttgacttc
 361  ggagcgcagt acatctcgcg ctttgcactc ataacgacga cgtccaaaaa gtacatttt
 421  acactcttga cctcggatca ggtagggata cccgctgaac ttaagcatat caataagcgg
 481  ag
```

ITS 4

```
   1  cttccgtagg tgaacctgcg gaaggatcat tacctagagt tgcgggcttt gcctgccatc
  61  tcttacccat gtctNNNgag taccttcgtt tcctcggtgg gttcgcccgc cggttggaca
 121  acacttaaac cctttgtaat tgaaatcagc gtctgaaaaa acttaatagt tacaactttc
 181  aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgcg ataagtagtg
 241  tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcgcc ccttggtatt
 301  ccatggggca tgcctgttcg agcgtcattt gtaccttcaa gctctgcttg gtgttgggtg
 361  tttgtctcgc ctttgcgcgc agactcgcct caaaacgatt ggcagccggc gtgttgactt
 421  cggagcgcag tacatctcgc gctttgcact cataacgacg acgtccaaaa ag
```

KP1-131L

ITS 1

```
   1  tgtaccttgt tgcttcggtg cgcccgcctc acggccgccg gggggcttct gccccccgggt
  61  ccgcgcgcac cggagacacc attgaactct gtctgaagat tgcagtctga gcataaacta
 121  aataagttaa aactttcaac aacggatctc ttggttccgg catcgatgaa gaacgcagcg
 181  aaatgcgata actaatgtga attgcagaat tcagtgaatc atcgagtctt tgaacgcaca
 241  ttgcgccccc tggtattccg gggggcatgc ctgtccgagc gtcattgctg ccctcaagca
 301  cggcttgtgt gttgggctcc gtccccccgg ggacgggccc gaaaggcagc ggcggcaccg
 361  agtccggtcc tcgagcgtat ggggctttgt cacccgctct gtaggcccgg ccggcgccag
 421  ccgacaacca atcatccttt tttcaggttg acctcggatc aggtagggat acccgctgaa
 481  cttaagcata t
```

ITS 4

```
   1  tgcggaagga tcattactga gtgagggccc tctgggtcca acctcccacc cgtgtttatt
  61  gtaccttgtt gcttcggtgc gcccgcctca cggccgccgg ggggcttctg ccccgggtc
 121  cgcgcgcacc ggagacacca ttgaactctg tctgaagatt gcagtctgag cataaactaa
 181  ataagttaaa actttcaaca acggatctct tggttccggc atcgatgaag aacgcagcga
 241  aatgcgataa ctaatgtgaa ttgcagaatt cagtgaatca tcgagtcttt gaacgcacat
 301  tgcgcccccc ggtattccgg ggggcatgcc tgtccgagcg tcattgctgc cctcaagcac
 361  ggcttgtgtg ttgggctccg tccccccggg gacgggcccg aaaggcagcg gcggcaccga
 421  gtccggtcct cgagcgtatg gggctttgtc acccgctctg taggcccggc cggcgccagc
 481  cgacaaccaa tcatccttt
```

KP1-131M

ITS 1

```
   1  tctatacatc ttgttgcttc ggcggcgcat tcgtgtgccg ccggggacac catttgaact
  61  ctgttttata atgcagtctg agaatataac ttaattagtt aaaactttca acaacggatc
 121  tcttggttcc ggtatcgatg aagaacgcag cgaaatgcga taaataatgt gaattgcaga
 181  attcagtgaa tcatcgagtc tttgaacgca cattgcgccc cctggtattc cgggggggcat
 241  gcctgtccga gcgtcattgc tgccctcaag cccggcttgt gtgttgggtc ctagtcctta
 301  gggacaggcc cgaaaggcaa tggcagtacc gcgtccggtc ctcgagcgta tggggctttg
 361  tcacccgctc tgtaggcccg gccggcgctc cgccgaccaa ccaaaaaact atttttcagg
 421  ttgacctcgg atcaggtagg gatacccgct gaacttaagc at
```

ITS 4

```
   1  acctgcggaa ggatcattac agagtttatg ctcgtcagag ctaacctccc acccgtgtct
  61  attacatctt gttgcttcgg cggcgcattc gtgtgccgcc ggggacacca tttgaactct
 121  gttttataat gcagtctgag aatataactt aattagttaa aactttcaac aacggatctc
 181  ttggttccgg tatcgatgaa gaacgcagcg aaatgcgata aataatgtga attgcagaat
 241  tcagtgaatc atcgagtctt tgaacgcaca ttgcgccccc tggtattccg gggggcatgc
 301  ctgtccgagc gtcattgctg ccctcaagcc cggcttgtgt gttgggtcct agtccttagg
 361  gacaggcccg aaaggcaatg gcagtaccgc gtccggtcct cgagcgtatg gggctttgtc
 421  acccgctctg taggcccggc cggcgctccg ccgaccaacc aaaaaact
```

KP1-131N

ITS 1

```
   1  tgttgcttcg tggacgcggg ccgcgcacct cgagaagcgc aatgtgctgc gcgagaggag
  61  gcaaggaccg ctgccaatgg atttggggcg agtccgcgcg cagaggcggg acagacgccc
 121  aacaccaagc agagcttgag ggtgtagatg acgctcgaac aggcatgccc catggaatac
 181  caaggggcgc aatgtgcgtt caaagattcg atgattcact gaattctgca attcacacta
 241  cttatcgcat ttcgctgcgt tcttcatcga tgccagagcc aagagatcca ttgttgaaag
 301  ttgtaacgat tgtttgtatc agaacaggta atgctagatg caaaaaaagg ttttgatagg
 361  ttccaacggc aggttgcccc gccgaaggag aacgaaaggt gctcgtaaaa aaaggattca
 421  gacatgcggc gcgtgaaagt gttacctcta ccgcccgacg gtagctgttg ctcccgccga
 481  gggccgcgac cgcacctcat ggaatagata atgatccttc cgc
```

ITS 4

```
   1  gatatgctta agttcagcgg gtatccctac ctgatccgag gtcaaagacg gtaatgttgc
  61  ttcgtggacg cgggccgcgc acctcgagaa gcgcaatgtg ctgcgcgaga ggaggcaagg
 121  accgctgcca atggatttgg ggcgagtccg cgcgcagagg cgggacagac gcccaacacc
 181  aagcagagct tgagggtgta gatgacgctc gaacaggcat gccccatgga ataccaaggg
 241  gcgcaatgtg cgttcaaaga ttcgatgatt cactgaattc tgcaattcac actacttatc
 301  gcatttcgct gcgttcttca tcgatgccag agccaagaga tccattgttg aaagttgtaa
 361  cgattgtttg tatcagaaca ggtaatgcta gatgcaaaaa aaggttttga taggttccaa
 421  cggcaggttg ccccgccgaa ggagaacgaa aggtgctcgt aaaaaaagga ttcagacatg
 481  cggcgcgtga aagtgttacc tctaccgccc gacggtagct gtgctcccgc cgag
```

KP1-131Q

ITS 1

```
   1  atcgtacctt gttgcttcgg cgggcccgcc gtttcgacgg ccgccgggga ggccttgcgc
  61  ccccgggccc gcgcccgccg aagaccccaa catgaacgct gttctgaaag tatgcagtct
 121  gagttgatta tcgtaatcag ttaaaacttt caacaacgga tctcttggtt ccggcatcga
 181  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgag
 241  tctttgaacg cacattgcgc cccctggtat tccgggggggc atgcctgtcc gagcgtcatt
 301  gctgccctca agcacggctt gtgtgttggg cccccgtccc cctctcccgg gggacgggcc
 361  cgaaaggcag cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacctgctc
 421  tgtaggcccg gccggcgcca gccgacaccc aactttattt ttctaaggtt gacctcggat
 481  caggtaggga tacccgctga acttaagcat atc
```

ITS 4

```
   1  tgcggaagga tcattaccga gtgagggccc tctgggtcca acctcccacc cgtgtctatc
  61  gtaccttgtt gcttcggcgg gcccgccgtt tcgacggccg ccggggaggc cttgcgcccc
 121  cgggcccgcg cccgccgaag accccaacat gaacgctgtt ctgaaagtat gcagtctgag
 181  ttgattatcg taatcagtta aaactttcaa caacggatct cttggttccg gcatcgatga
 241  agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgagtct
 301  ttgaacgcac attgcgcccc ctggtattcc gggggcatg cctgtccgag cgtcattgct
 361  gccctcaagc acggcttgtg tgttgggccc ccgtcccct ctcccgggg acgggcccga
 421  aaggcagcgg cggcaccgcg tccggtcctc gagcgtatgg ggctttgtca cctgctcNNN
 481  Nggcccggcc ggcgccagcc gacacccaac tt
```

KP1-131R

ITS 1

```
   1  actcttgttg ctttggcagg ccgtggtcac ccactgtggg ctatgcctgc atgcgcctgc
  61  cagaggacca aactctgaat attagtgatg tctgagtact atataatagt taaaactttc
 121  aacaacggat ctcttggttc tggcatcgat gaagaacgca gcgaaatgcg ataagtaatg
 181  tgaattgcag aattcagtga atcatcgaat ctttgaacgc acattgcacc cggtggtatt
 241  ccgccgggta tgcctgttcg agcgtcatta taaccactca agcctgtctt ggtgttgggg
 301  ttgcgaatct tttgcagccc tcgagtctcg tagcgccacc tgtgggttct aagcgtagta
 361  atttctcctc gctatagaac ctgctcgggg aaaagtataa ttcgtagcct ggttctatgt
 421  gcccgctata aaacccccaa tttttaaagg ttgacctcgg atcaagtagg gatacccgct
 481  gaacttaagc atatcat
```

ITS 4

```
   1  cggaaggatc attaaccatt ggataccttc gggtatatcc catccgtgtc tacatactct
  61  tgttgctttg gcaggccgtg gtcacccact gtgggctatg cctgcatgcg cctgccagag
 121  gaccaaactc tgaatattag tgatgtctga gtactatata atagttaaaa ctttcaacaa
 181  cggatctctt ggttctggca tcgatgaaga acgcagcgaa atgcgataag taatgtgaat
 241  tgcagaattc agtgaatcat cgaatctttg aacgcacatt gcacccggtg gtattccgcc
 301  gggtatgcct gttcgagcgt cattataacc actcaagcct gtcttggtgt tggggttgcg
 361  aatcttttgc agccctcgag tctcgtagcg ccacctgtgg gttctaagcg tagtaatttc
 421  tcctcgctat agaacctgct cggggaaaag tataattcgt agcctggttc tatgtgcccg
 481  ctataaaacc cccaa
```

KP1-131S

ITS 1

```
   1  catcttgttg cttcgggggc gaccctgcca ttcgtggcat tccccccgga ggtcatcaaa
  61  acactgcatt cttacgtcgg agtaaaaagt taatttaata aaactttcaa caacggatct
 121  cttggttctg gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa
 181  ttcagtgaat catcgaatct ttgaacgcac attgcgcccc ctggtattcc gggggggcatg
 241  cctgttcgag cgtcattaca ccactcaagc ctcgcttggt attgggcgtc gcgagtctct
 301  cgcgcgcctc aaagtctccg gctaggcagt tcgtctccca gcgttgtggc aactatttcg
 361  cagtggagtt cgagtcgtcg cggccgttaa atctttcaaa ggttgacctc ggatcaggta
 421  gggatacccg ctgaacttaa gcatatc
```

ITS 4

```
   1  tgcggaggga tcattactga gtttaggtgg aatccaccca actccaaccc tttgtgaaca
  61  catcttgttg cttcgggggc gaccctgcca ttcgtggcat tccccccgga ggtcatcaaa
 121  acactgcatt cttacgtcgg agtaaaaagt taatttaata aaactttcaa caacggatct
 181  cttggttctg gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa
 241  ttcagtgaat catcgaatct ttgaacgcac attgcgcccc ctggtattcc gggggggcatg
 301  cctgttcgag cgtcattaca ccactcaagc ctcgcttggt attgggcgtc gcgagtctct
 361  cgcgcgcctc aaagtctccg gctaggcagt tcgtctccca gcgttgtggc aactatttcg
 421  cagtggagtt cgagtcgtcg cggccg
```

KP1-131T

ITS 1

```
   1  acggccgccg gggaggcctt gcgcccccgg gcccgcgccc gccgaagacc ccaacatgaa
  61  cgctgttctg aaagtatgca gtctgagttg attatcgtaa tcagttaaaa ctttcaacaa
 121  cggatctctt ggttccggca tNNNtgaaga acgcagcgaa atgcgataag taatgtgaat
 181  tgcagaattc agtgaatcat NNNgtctttg aacgcacatt gcgccccctg gtattccggg
 241  gggcatgcct gtccgagcgt cattgctgcc ctcaagcacg gcttgtgtgt tgggcccccg
 301  tccccctctc ccgggggacg ggcccgaaag gcagcggcgg caccNNNNcc ggtcctcgag
 361  cgtatggggc tttgtcacct gctctgtagg cccggccggc gccagcNaNc acccaacttt
 421  atttttctaa ggttgacctN NNatcaggta gggatacccg ctg
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt accgagtgag ggccctctgg gtccaacctc
  61  ccacccgtgt ctatcgtacc ttgttgcttc ggcgggcccg ccgtttcgac ggccgccggg
 121  gaggccttgc gccccgggc ccgcgcccgc cgaagacccc aacatgaacg ctgttctgaa
 181  agtatgcagt ctgagttgat tatcgtaatc agttaaaact ttcaacaacg gatctcttgg
 241  ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag
 301  tgaatcatcg agtctttgaa cgcacattgc gccccctggt attccgggggg gcatgcctgt
 361  ccgagcgtca ttgctgccct caagcacggc ttgtgtgttg ggcccccgtc cccctctccc
 421  gggggacggg cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg tatggggctt
 481  tgtcacc
```

KP1-131V

ITS 1

```
   1  cttagaatgt gggggtggat gacaggatac cacgagacac ctatagcgag aagatttact
  61  acgctcaggg cctcgtggca ccgccactga ttttaaggcc cgccgggtaa gcggcgaagc
 121  ccaagaccaa gcagttatgc ttgagggttg taatgacgct cgaacaggca tgcccccggg
 181  aataccaagg ggcgcaatgt gcgttcaaag attcgatgat tcactgaatt ctgcaattca
 241  cattacttat cgcatttcgc tgcgttcttc atcgatgcca gaaccaagag atccgttgtt
 301  gaaagtttta actattatat agtactcaga cgacactaat attcagggtt tgagatcctc
 361  tggcgaacgc gtaccagtcg aaaccga
```

ITS 4

```
   1  tatgcttaag ttcagcgggt atccctacct gatccgaggt caaacttaga atgtgggggt
  61  ggatgacagg ataccacgag acacctatag cgagaagatt tactacgctc agggcctcgt
 121  ggcaccgcca ctgattttaa ggcccgccgg gtaagcggcg aagcccaaga ccaagcagtt
 181  atgcttgagg gttgtaatga cgctcgaaca ggcatgcccc ccggaatacc aaggggcgca
 241  atgtgcgttc aaagattcga tgattcactg aattctgcaa ttcacattac ttatcgcatt
 301  tcgctgcgtt cttcatcgat gccagaacca agagatccgt tgttgaaagt tttaactatt
 361  atatagtact cagacgacac taatattcag ggtttgagat cctctggcga acgcgtacca
 421  gtcgaaaccg atagccttgc ggcggttcgc caaagcaaca atggtaacaa taacatagg
```

KP1-131W

ITS 1

```
   1  ctttgtgact tataccttac tgttgcctcg gcgcatgccg gccccccggg gggcccctcc
  61  cccggaggag caggcacgcc ggcggccagc ccaactcttg tttttacact gaaactctga
 121  gaataaaaca taaatgaatc aaaactttca acaacggatc tcttggttct ggcatcgatg
 181  aagaacgcag cgaaatgcga taagtaatgt gaattgcaga attcagtgaa tcatcgaatc
 241  tttgaacgca cattgcgccc tctggtattc cggagggcat gcctgttcga gcgtcatttc
 301  aaccctcaag cctggcttgg tgatggggca ctgcttccta cccagggagc aggccctgaa
 361  attcagtggc aagctcgcca ggaccccgag cgcagtagtt aaaccctcgc tctggaaggc
 421  cctggcggtg ccctgccgtt aaacccccaa cttctgaaaa tttgacctcg gatcaggtag
 481  gaatacccgc tgaacttaag catat
```

ITS 4

```
   1  tgcggaggga tcattgctgg aacgcgcccc aggcgcaccc agaaaccctt tgtgaactta
  61  taccttactg ttgcctcggc gcatgccggc ccccccgggg gcccctcccc cggaggagca
 121  ggcacgccgg cggccagccc aactcttgtt tttacactga aactctgaga ataaaacata
 181  aatgaatcaa aactttcaac aacggatctc ttggttctgg catcgatgaa gaacgcagcg
 241  aaatgcgata agtaatgtga attgcagaat tcagtgaatc atcgaatctt tgaacgcaca
 301  ttgcgccctc tggtattccg gagggcatgc ctgttcgagc gtcatttcaa ccctcaagcc
 361  tggcttggtg atggggcact gcttcctacc cagggagcag gccctgaaat tcagtggcaa
 421  gctcgccagg accccgagcg cagtagttaa accctcgctc tggaaggccc tggcggtgcc
 481  ctgccgttaa acccccaac
```

KP1-131Y

ITS 1

```
   1  ccggggaggc cttgcgcccc cgggcccgcg cccgcNNaag accccaacat gaacgctgtt
  61  ctgaaagtat gcagtctgag ttgattatcg taatcagtta aaactttcaa caacggatct
 121  cttggttccg gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa
 181  ttcagtgaat catcgagtct ttgaacgcac attgcgcccc ctggtattcc gggggggcatg
 241  cctgtccgag cgtcattgct gccctcaagc acggcttgtg tgttgggccc ccgtcccccct
 301  ctcccggggg acgggcccga aaggcagcgg cggcaccgcg tccggtcctc gagcgtatgg
 361  ggctttgtca cctgctctgt NNNNccggcc ggcgccagcc gacacccaac tttatttttc
 421  taaggttgac ctcggatcNN Ntagggatac ccgctgaact taagcatatc aataagcgg
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt accgagtgag ggccctctgg gtccaacctc
  61  ccacccgtgt ctatcgtacc ttgttgcttc ggcgggcccg ccgtttcgac ggccgccggg
 121  gaggccttgc gccccccgggc ccgcgcccgc cgaagacccc aacatgaacg ctgttctgaa
 181  agtatgcagt ctgagttgat tatcgtaatc agttaaaact ttcaacaacg gatctcttgg
 241  ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag
 301  tgaatcatcg agtctttgaa cgcacattgc gccccctggt attccgggggg gcatgcctgt
 361  ccgagcgtca ttgctgccct caagcacggc ttgtgtgttg ggcccccgtc cccctctccc
 421  gggggacggg cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg tatggggctt
 481  tgtcacct
```

KP1-131Z

ITS 1

```
   1  gctgcccggc acgggatgtg ctcgtctgga tgcgtgtccc ttctctattc caccccactg
  61  tgaaccaagc gtgcgagccg aagagagatc ggaagctcgt atgcaaccct caatataccc
 121  catcatgtat cagaatgtac cttgcgttaa ctcgcacaaa tacaactttc aacaacggat
 181  ctcttggctc tcgcatcgat gaagaacgca gcgaaatgcg ataagtaatg tgaattgcag
 241  aattcagtga atcatcgaat ctttgaacgc accttgcgcc ccttggcatt ccgaggggca
 301  cgcctgtttg agtgtcgtga actcctccac cctctacctt tttcggaagg cactgggctg
 361  ggatttggga gcttgcgggt ccctggccga tccgctctcc ttgaatacat tagcgaagcc
 421  cttgcggcct tggtgtgata gtcatctacg cctcggctta gcgaacatac ggggacttgc
 481  ttctaaccgt ctcgcgagag acaactacta ccaacttgac ctcaaatcag gcgggactac
 541  ccgctgaact taagcatatc
```

ITS 4

```
   1  tgcggaagga tcattagcga agttcggaat gcgtgttcgg tactgacgct gcccggcaac
  61  gggatgtgct cgtctggatg cgtgtccctt ctctattcca ccccactgtg aaccaagcgt
 121  gcgagccgaa gagagatcgg aagctcgtat gcaaccctca atatacccca tcatgtatca
 181  gaatgtacct tgcgttaact cgcacaaata caactttcaa caacggatct cttggctctc
 241  gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat
 301  catcgaatct ttgaacgcac cttgcgcccc ttggcattcc gaggggcacg cctgtttgag
 361  tgtcgtgaac tcctccaccc tctacctttt tcggaaggca ctgggctggg atttgggagc
 421  ttgcgggtcc ctggccgatc cgctctcctt gaatacatta gcgaagccct tgcggccttg
 481  gtgtgatagt catctacgcc tcggcttagc gaacatacgg ggacttgctt ctaaccgtct
 541  cgcgagagac aactactacc aact
```

KP1-135B

ITS 1

```
   1  gttaccaaac tgttgcctcg gcggggtcac gccccgggtg cgtcgcagcc ccggaaccag
  61  gcgcccgccg gaggaaccaa ccaaactctt tctgtagtcc cctcgcggac gttatttctt
 121  acagctctga gcaaaaattc aaaatgaatc aaaactttca acaacggatc tcttggttct
 181  ggcatcgatg aagaacgcag cgaaatgcga taagtaatgt gaattgcaga attcagtgaa
 241  tcatcgaatc tttgaacgca cattgcgccc gccagtattc tggcgggcat gcctgtccga
 301  gcgtcatttc aaccctcgaa ccccctccggg ggatcggcgt tggggatcgg gacccctcac
 361  acgggtgccg gcccctaaat acagtggcgg tctcgccgca gcctctcctg cgcagtagtt
 421  tgcacaactc gcaccgggag cgcggcgcgt ccacgtccgt aaaacaccca actttctgaa
 481  atgttgacct cggatcaggt aggaataccc gctgaactta agcatatcaa
```

ITS 4

```
   1  aacctgcgga gggatcatta ccgagtttac aactcccaaa cccaatgtga acgttaccaa
  61  actgttgcct cggcggggtc acgccccggg tgcgtcgcag ccccggaacc aggcgcccgc
 121  cggaggaacc aaccaaactc tttctgtagt cccctcgcgg acgttatttc ttacagctct
 181  gagcaaaaat tcaaaatgaa tcaaaacttt caacaacgga tctcttggtt ctggcatcga
 241  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgaa
 301  tctttgaacg cacattgcgc ccgccagtat tctggcgggc atgcctgtcc gagcgtcatt
 361  tcaaccctcg aacccctccg ggggatcggc gttggggatc gggacccctc acacgggtgc
 421  cggcccctaa atacagtggc ggtctcgccg cagcctctcc tgcgcagtag tttgcacaac
 481  tcgcaccggg agcgcggcgc gtccacgtcc gtaaaacacc c
```

KP1-135C

ITS 1

```
   1  ggccgccggg gggcatccgc ccccgggccc gcgcccgccg aagacacctg tgaacactgt
  61  ctgaagttgc agtctgagaa actaNNNNNN Ntagttaaaa ctttcaacaa cggatctctt
 121  ggttccgNNN tcgatgaaga acgcagcgaa atgcgataaa taatgtgaat tgNNNaattc
 181  agtgaatcat cgagtctttg aacgcacatt gcgccctctg gtattccgga gggcatgcct
 241  gtccgagcgt cattgctgcc ctcaagcacg NNNtgtgtgt tgggcccccg tccccccccac
 301  cgggggggacg ggcccgaa
```

ITS 4

```
   1  gcggaaggat cattaccgag tgagggccct ctgggtccaa cctcccaccc gtgtttaacg
  61  aaccttgttg cttcggcggg cccgcctcac ggccgccggg gggcatccgc ccccgggccc
 121  gcgcccgccg aagacacctg tgaacactgt ctgaagttgc agtctgagaa actagctaaa
 181  ttagttaaaa ctttcaacaa cggatctctt ggttccggca tcgatgaaga acgcagcgaa
 241  atgcgataaa taatgtgaat tgcagaattc agtgaatcat cgagtctttg aacgcacatt
 301  gcgccctctg gtattccgga gggcatgcct gtccgagcgt cattgctgcc ctcaagcacg
 361  gcttgtgtgt tgggcccccg tccccccccac cgggggggacg ggcccgaaag gcagcggcgg
 421  caccgcgtcc ggtcctcgag cgtatggggc tctgtcaccc gctc
```

KP1-135D

ITS 1

```
   1  tgtggtcttg atggatgctc aaccatggct gatcagaagt gcaagattgt gctgcgctcc
  61  gaaaccagta ggccggctgc caatcatttt aaggcgagtc tcgtgagaga caaagacgcc
 121  caacaccaag caaagcttga gggtacaaat gacgctcgaa caggcatgcc ctttggaata
 181  ccaaagggcg caatgtgcgt tcaaagattc gatgattcac tgaattctgc aattcacact
 241  acgtatcgca tttcgctgcg ttcttcatcg atgccagaac caagagatcc gttgttgaaa
 301  gttgtaataa ttacattgtt tactgacgct gattgcaatt acaaaaaaag gtttatggtt
 361  gggtcctggt ggcgggcgaa cccgcccagg aaacaagaag tgcgcaaaag acatgggtga
 421  ataattcaga caagctggag cccccaccga gatgaggtcc caacccgctt tcatattgtg
 481  taatgatccc tccgca
```

ITS 4

```
   1  gatatgctta agttcagcgg gtatccctac ctgatccgag gtcaaaagtg agaaaaatgt
  61  ggtcttgatg gatgctcaac catggctgat cagaagtgca agattgtgct gcgctccgaa
 121  accagtaggc cggctgccaa tcattttaag gcgagtctcg tgagagacaa agacgcccaa
 181  caccaagcaa agcttgaggg tacaaatgac gctcgaacag gcatgccctt tggaatacca
 241  aagggcgcaa tgtgcgttca aagattcgat gattcactga attctgcaat tcacactacg
 301  tatcgcattt cgctgcgttc ttcatcgatg ccagaaccaa gagatccgtt gttgaaagtt
 361  gtaataatta cattgtttac tgacgctgat tgcaattaca aaaaaaggtt tatggttggg
 421  tcctggtggc gggcgaaccc gcccaggaaa caagaagtgc gcaaaagaca tgggtgaata
 481  attcagacaa g
```

KP1-135F

ITS 1

```
   1  gaaccttgtt gctttggcgg gcccgcctca NNNNcgccgg ggggcatctg cccccgggcc
  61  cgcgcccgcc gaagccacct gtgaactctg tctgaagtat gcagtctgag acaattatta
 121  aattaattaa aactttcaac aacggatctc ttggttccgg catcgatgaa gaacgcagcg
 181  aaatgcgata actaatgtga attgcagaat tcagtgaatc atcgagtctt tgaacgcaca
 241  ttgcgccctc tggtattccg gagggcatgc ctgtccgagc gtcattgctg ccctccagcc
 301  cggctggtgt gttgggcccc gccccccttc ccgggggggc gggcccgaaa ggcagcggcg
 361  gcaccgcgtc cggtcctcga gcgtatgggg ctttgtcacc cgctcttgta ggcccggccg
 421  gcgccagccg acccccctcaa tctatttttt caggttgacc tcggatcagg tagggatacc
 481  cgctgaactt aagcatatc
```

ITS 4

```
   1  cgttcaaaga ctcgatgatt cactgaattc tgcaattcac attagttatc gcatttcgct
  61  gcgttcttca tcgatgccgg aaccaagaga tccgttgttg aaagttttaa ttaatttaat
 121  aattgtctca gactgcatac ttcagacaga gttcacaggt ggcttcggcg ggcgcgggcc
 181  cgggggcaga tgccccccgg cggccgtgag gcgggcccgc caaagcaaca aggttcgtta
 241  aacacgggtg ggaggttgga ccccgagggg ccctcactca gtaatgatNN tNcNgNNNNN
 301  NNNNtgcgga aggatcatta ctgagtgagg gcccctcggg gtccaacctc ccacccgtgt
 361  ttaacgaacc ttgttgcttt ggcgggcccg cctcacggcc gccgggggggc atctgccccc
 421  gggcccgcgc ccgccgaagc cacctgtgaa ctctgtctga agtatgcagt ctgagacaat
 481  tattaaatta attaaaactt tcaacaacgg atctcttggt tccggcatcg atgaagaacg
 541  cagcgaaatg cgataactaa tgtgaattgc agaattcagt gaatcatcga gtctttgaac
 601  gcacattgcg ccctctggta ttccggaggg catgcctgtc cgagcgtcat tgctgccctc
 661  cagcccggct ggtgtgttgg gccccgcccc ccttcccggg ggggcgggcc cgaaaggcag
 721  cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacccgct
```

KP1-143A

ITS 4

```
   1  tttaaaaaaa aaaataaaaa actttcaaca acggatctct tggttctcgc atcgatgaag
  61  aacgcagcga attgcgatac gtaatatgac ttgcagacgt gaatcattga atttttgaac
 121  gcacattgcg ccttaaggta ttcctcaagg catgcgtgga tgagcgatat ttactctcaa
 181  accacttggt ttggtcttgg ccccccttt tttcataggg cctaaatatc aaatggtctc
 241  tagaataagt ttttagaaac tcaaaacctt aactc
```

KP1-143D

ITS 1

```
   1  gcatgtgcac gctctgctca tccactctac ccctgtgcac ttactgtagg ttggcgtggg
  61  ctccttaacg ggagcattct gccggcctat gtatactaca aacactttaa agtatcagaa
 121  tgtaaacgcg tctaacgcat ctataataca acttttagca acggatctct tggctctcgc
 181  atcgatgaag aacgcagcga aatgcgataa gtaatgtgaa ttgcagaatt cagtgaatca
 241  tcgaatcttt gaacgcacct tgcgctcctt ggtattccga ggagcatgcc tgtttgagtg
 301  tcatggaatt ctcaacttat aaatccttgt gatctataag cttggacttg gaggcttgct
 361  ggcccttgtt ggtcggctcc tcttgaatgc attagctcga ttccgtacgg atcggctctc
 421  agtgtgataa ttgtctacgc tgtgaccgtg aagtgttttg gcgagcttct aaccgtccat
 481  taggacaact ttttaacatc tgacctcaaa tcaggtagga ctacccgctg aacttaagca
 541  tatc
```

ITS 4

```
   1  ggaaggatca ttaacgagtt ttgaaacgag ttgtagctgg ccttccgagg catgtgcacg
  61  ctctgctcat ccactctacc cctgtgcact tactgtaggt tggcgtgggc tccttaacgg
 121  gagcattctg ccggcctatg tatactacaa acactttaaa gtatcagaat gtaaacgcgt
 181  ctaacgcatc tataatacaa cttttagcaa cggatctctt ggctctcgca tcgatgaaga
 241  acgcagcgaa atgcgataag taatgtgaat tgcagaattc agtgaatcat cgaatctttg
 301  aacgcacctt gcgctccttg gtattccgag gagcatgcct gtttgagtgt catggaattc
 361  tcaacttata aatccttgtg atctataagc ttggacttgg aggcttgctg gcccttgttg
 421  gtcggctcct cttgaatgca ttagctcgat tccgtacgga tcggctctca gtgtgataat
 481  tgtctacgct gtgaccgtga agtgttttgg cgagcttcta accgtccatt aggacaact
```

KP1-175G

ITS 1

```
   1  acccccggtc gccgggggcc actgcgcccc cgggcccgcg cccgccagag cgcctctgaa
  61  ccctaatgaa gaaggactgt ctgagtctac gatataatta tcaaaacttt caacaatgga
 121  tctcttggtt ccggcatcga tgaagaacgc agcgaaatgc gataagtaat gtgaattgca
 181  gaattccgtg aatcatcgaa tctttgaacg cacattgcgc cccctggcat tccgggggcc
 241  atgcctgtcc gagcgtcatt tctgccctca agcccggctt gtgtgttggg cgtggtcccc
 301  ccggtgtcgg ggggacctgc cccaaaggca gcggcgacgt tccgcctagg tcctcgagcg
 361  tatggggctt tgtcacccgc tcgggagggg cctacgggcg ttggccaccc accaattttt
 421  tttacggttg acctcggatc aggtaggagt tacccgctga acttaagcat atcaataagc
 481  ggag
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt actgagtgcg ggcccctcgc gggtccaacc
  61  tcccacccgt gtctcttgaa taccctgttg ctttggcggg cccaccgggc caccccccggt
 121  cgccgggggg cactgcgccc ccgggcccgc gcccgccaga gcgcctctga accctaatga
 181  agaaggactg tctgagtcta cgatataatt atcaaaactt tcaacaatgg atctcttggt
 241  tccggcatcg atgaagaacg cagcgaaatg cgataagtaa tgtgaattgc agaattccgt
 301  gaatcatcga atctttgaac gcacattgcg ccccctggca ttccgggggg catgcctgtc
 361  cgagcgtcat ttctgccctc aagcccggct tgtgtgttgg gcgtggtccc cccggtgtcg
 421  gggggacctg ccccaaaggc agcggcgacg ttccgcctag gtcctcgagc gtatggggct
 481  ttgtcacc
```

KP1-175L

ITS 1

```
   1  cgaaccttgt tgctttggcg ggcccgcctc acggccgccg gggggcatct gcccccgggc
  61  ccgcgcccgc cgaagccacc tgtgaactct gtctgaagta tgcagtctga gacaattatt
 121  aaattaatta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 181  gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 241  attgcgccct ctggtattcc ggagggcatg cctgtccgag cgtcattgct gccctccagc
 301  ccggctggtg tgttgggccc cgccccccctt cccgggggggg cgggcccgaa aggcagcggc
 361  ggcaccgcgt ccggtcctcg agcgtatggg gctttgtcac ccgctcttgt aggcccggcc
 421  ggcgccagcc gacccccctca atctatttttt tcaggttgac ctcggatcag gtagggatac
 481  ccgctgaact taagcatat
```

ITS 4

```
   1  tgcggaagga tcattactga gtgagggccc ctcggggtcc aacctcccac ccgtgtttaa
  61  cgaaccttgt tgctttggcg ggcccgcctc acggccgccg gggggcatct gccccgggc
 121  ccgcgcccgc cgaagccacc tgtgaactct gtctgaagta tgcagtctga gacaattatt
 181  aaattaatta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 241  gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 301  attgcgccct ctggtattcc ggagggcatg cctgtccgag cgtcattgct gccctccagc
 361  ccggctggtg tgttgggccc cgccccccctt cccgggggggg cgggcccgaa aggcagcggc
 421  ggcaccgcgt ccggtcctcg agcgtatggg gctttgtcac ccgctcNNta ggcccggccg
 481  gcgccagccg acccccctcaa tc
```

KP1-175M

ITS 1

```
   1  cgaaccttgt tgctttggcg ggcccgcctc acggccgccg gggggcatct gccccgggc
  61  ccgcgcccgc cgaagccacc tgtgaactct gtctgaagta tgcagtctga gacaattatt
 121  aaattaatta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 181  gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 241  attgcgccct ctggtattcc ggagggcatg cctgtccgag cgtcattgct gccctccagc
 301  ccggctggtg tgttgggccc cgccccccctt cccgggggggg cgggcccgaa aggcagcggc
 361  ggcaccgcgt ccggtcctcg agcgtatggg gctttgtcac ccgctcttgt aggcccggcc
 421  ggcgccagcc gacccccctca atctatttttt tcaggttgac ctcggatcag gtagggatac
 481  ccgctgaact taagcatat
```

ITS 4

```
   1  tgcggaagga tcattactga gtgagggccc ctcggggtcc aacctcccac ccgtgtttaa
  61  cgaaccttgt tgctttggcg ggcccgcctc acggccgccg gggggcatct gccccgggc
 121  ccgcgcccgc cgaagccacc tgtgaactct gtctgaagta tgcagtctga gacaattatt
 181  aaattaatta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 241  gaaatgcgat aactaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 301  attgcgccct ctggtattcc ggagggcatg cctgtccgag cgtcattgct gccctccagc
 361  ccggctggtg tgttgggccc cgccccccctt cccgggggggg cgggcccgaa aggcagcggc
 421  ggcaccgcgt ccggtcctcg agcgtatggg gctttgtcac ccgct
```

KP2-001C

ITS 1

```
   1  ctatcgtacc ttgttgcttc ggcgggcccg ccgtttcgac ggccgccggg gaggccttgc
  61  gccccgggc ccgcgcccgc cgaagacccc aacatgaacg ctgttctgaa agtatgcagt
 121  ctgagttgat tatcgtaatc agttaaaact ttcaacaacg gatctcttgg ttccggcatc
 181  gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag tgaatcatcg
 241  agtctttgaa cgcacattgc gccccctggt attccgggggg gcatgcctgt ccgagcgtca
 301  ttgctgccct caagcacggc ttgtgtgttg ggcccccgtc cccctctccc gggggacggg
 361  cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg tatggggctt tgtcacctgc
 421  tctgtaggcc cggccggcgc cagccgacac ccaactttat ttttctaagg ttgacctcgg
 481  atcaggtagg gatacccgct gaacttaagc atatc
```

ITS 4

```
   1  tgcggaagga tcattaccga gtgagggccc tctgggtcca acctcccacc cgtgtctatc
  61  gtaccttgtt gcttcggcgg gcccgccgtt tcgacggccg ccggggaggc cttgcgcccc
 121  cgggcccgcg cccgccgaag accccaacat gaacgctgtt ctgaaagtat gcagtctgag
 181  ttgattatcg taatcagtta aaactttcaa caacggatct cttggttccg gcatcgatga
 241  agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgagtct
 301  ttgaacgcac attgcgcccc ctggtattcc gggggggcatg cctgtccgag cgtcattgct
 361  gccctcaagc acggcttgtg tgttgggccc ccgtcccccct ctcccgggggg acgggcccga
 421  aaggcagcgg cggcaccgcg tccggtcctc gagcgtatgg ggctttgtca cctgc
```

KP2-001F

ITS 1

```
   1  cgccgggggg catctgcccc cgggcccgcg cccgccgaag ccacctgtga actctgtctg
  61  aagtatgcag tctgagacaa ttattaaatt aattaaaact ttcaacaacg gatctcttgg
 121  ttccggcatc gatgaagaac gcagcgaaat gcgataacta atgtgaattg cagaattcag
 181  tgaatcatcg agtctttgaa cgcacattgc gccctctggt attccggagg gcatgcctgt
 241  ccgagcgtca ttgctgccct ccagcccggc tggtgtgttg ggccccgccc cccttcccgg
 301  gggggcgggc ccgaaaggca gcggcggcac cgcgtccggt cctcgagcgt atggggcttt
 361  gtcacccgct cttgtaggcc cggccggcgc cagccgaccc cctcaatcta ttttttcagg
 421  ttgacctcgg atcaggtagg gatacccgct gaacttaagc atatc
```

ITS 4

```
   1  acctgcggaa ggatcattac tgagtgaggg cccctcgggg tccaacctcc cacccgtgtt
  61  taacgaacct tgttgctttg gcgggcccgc ctcacggccg ccgggggggca tctgcccccg
 121  ggcccgcgcc cgccgaagcc acctgtgaac tctgtctgaa gtatgcagtc tgagacaatt
 181  attaaattaa ttaaaacttt caacaacgga tctcttggtt ccggcatcga tgaagaacgc
 241  agcgaaatgc gataactaat gtgaattgca gaattcagtg aatcatcgag tctttgaacg
 301  cacattgcgc cctctggtat tccggagggc atgcctgtcc gagcgtcatt gctgccctcc
 361  agcccggctg gtgtgttggg ccccgccccc cttcccgggg gggcgggccc gaaaggcagc
 421  ggcggcaccg cgtccggtcc tcgagcgtat ggggctttgt cacccgctct NNtaggcccg
 481  gccggcgcca gccgaccccc tcaat
```

KP2-005A

ITS 1

```
   1  ttgtaggtcg gcagaagggc gagccttaaa acagctcgct tggaagcctt cctatgtttt
  61  accacaaacg cttcagttta agaatgtaac ctgcgtataa cgcaactata tacaactttc
 121  agcaacggat ctcttggctc tcgcatcgat gaagaacgca gcgaaatgcg ataagtaatg
 181  tgaattgcag aattcagtga atcatcgaat ctttgaacgc accttgcgct ccctggtatt
 241  ccggggagca tgcctgtttg agtgtcatgg tatcctcatc cttcataact ttttgttatc
 301  gaaggcatgg acttggaggt cgtgctggtt cctcgttgaa tcggctcctc ttaaatgtat
 361  tagcgtgagt gtaacggatc gcttcggtgt gataattatc tgcgccgtgg tcgtgaagta
 421  acataagctt gcgcttctaa ccgtccttaa gctggacaac ataactttga catctgacct
 481  caaatcaggt aggactaccc gctgaactta agcatatcaa
```

ITS 4

```
   1  cgttcttcat cgatgcgaga gccaagagat ccgttgcNNa aagttgtata tagttgcgtt
  61  atacgcaggt tacattctta aaNNNaagcg tttgtggtaa aacataggaa ggcttccaag
 121  cgagctgttt taaggctcgc ccttctgccg acctacaaca agtgcacaga ggttgaagag
 181  tggatgagcc aggtgtgcac atgccccgag aggccagcta caacccgttc agttactcgt
 241  taatgatcct tccgtaggtg aacctgcgga aggatcatta acgagtaact gaacgggttg
 301  tagctggcct ctcggggcat gtgcacacct ggctcatcca ctcttcaacc tctgtgcact
 361  tgttgtaggt cggcagaagg gcgagcctta aaacagctcg cttggaagcc ttcctatgtt
 421  ttaccacaaa cgcttcagtt taagaatgta acctgcgtat aacgcaacta tatacaactt
 481  tcagcaacgg atctcttggc tctcgcatcg atgaagaacg cagcgaaatg cgataagtaa
 541  tgtgaattgc agaattcagt gaatcatcga atctttgaac gcaccttgcg ctccctggta
 601  ttccggggag catgcctgtt tgagtgtcat ggtatcctca tccttcataa ctttttgtta
 661  tcgaaggcat ggacttggag gtcgtgctgg ttcctcgttg aatcggctcc tcttaaatgt
 721  attagcgtga gtgtaacgga tcgcttcggt gtgataatta tctgcgccgt ggtcgtgaag
 781  taacataagN NNNcgcttct aaccgtcctt aagctggaca acataa
```

KP2-009A

ITS 1

```
   1  ggttggcgtg ggctccttaa cgggagcatt ctgccggcct atgtatacta caaacacttt
  61  aaagtatcag aatgtaaacg cgtctaacgc atctataata caacttttag caacggatct
 121  cttggctctc gcatcgatga agaacgcagc gaaatgcgat aagtaatgtg aattgcagaa
 181  ttcagtgaat catcgaatct ttgaacgcac cttgcgctcc ttggtattcc gaggagcatg
 241  cctgtttgag tgtcatggaa ttctcaactt ataaatcctt gtgatctata agcttggact
 301  tggaggcttg ctggcccttg cggtcggctc ctcttgaatg cattagctcg attccgtacg
 361  gatcggctct cagtgtgata attgtctacg ctgtgaccgt gaagtgtttt ggcgagcttc
 421  taaccgtcca ttaggacaac tttttaacat ctgacctcaa atcaggtagg actacccgct
 481  gaacttaagc atatcaataa gc
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt aacgagtttt gaaacgagtt gtagctggcc
  61  ttccgaggca tgtgcacgct ctgctcatcc actctacccc tgtgcactta ctgtaggttg
 121  gcgtgggctc cttaacggga gcattctgcc ggcctatgta tactacaaac actttaaagt
 181  atcagaatgt aaacgcgtct aacgcatcta taatacaact tttagcaacg gatctcttgg
 241  ctctcgcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag
 301  tgaatcatcg aatctttgaa cgcaccttgc gctccttggt attccgagga gcatgcctgt
 361  ttgagtgtca tggaattctc aacttataaa tccttgtgat ctataagctt ggacttggag
 421  gcttgctggc ccttgcggtc ggctcctctt gaatgcatta gctcgattcc gtacggatcg
 481  gctctcagtg tgataattgt ctacgctgtg a
```

KP2-009B

ITS 1

```
   1  tgtgcacgct ctgctcatcc actctacccc tgtgNNctta Ntgtaggttg gcgtgggctc
  61  cttaacggga gcattctgcc ggcctatgta tactacaaac actttaaagt atcagaatgt
 121  aaacgcgtct aacgcatcta taatacaact tttagcaacg gatctcttgg ctctcgcatc
 181  gatgaagaac gcagcgaaat gcgataagta atgtgaattg cagaattcag tgaatcatcg
 241  aatctttgaa cgcaccttgc gctccttggt attccgagga gcatgcctgt ttgagtgtca
 301  tggaattctc aacttataaa tccttgtgat ctataagctt ggacttggag gcttgctggc
 361  ccttgcggtc ggctcctctc gaatgcatta gctcgattcc gtacggatcg gctctcagtg
 421  tgataattgt ctacgctgtg accgtgaagt gttttggcga gcttctaacc gtccattagg
 481  acaacttttt aacatctgac ctcaaatcag gtaggactac ccgctgaact taagcatatc
 541  aa
```

ITS 4

```
   1  acctgcggaa ggatcattaa cgagttttga aatgagttgt agctggcctt ccgaggcatg
  61  tgcacgctct gctcatccac tctacccctg tgcacttact gtaggttggc gtgggctcct
 121  taacgggagc attctgccgg cctatgtata ctacaaacac tttaaagtat cagaatgtaa
 181  acgcgtctaa cgcatctata atacaacttt tagcaacgga tctcttggct ctcgcatcga
 241  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgaa
 301  tctttgaacg caccttgcgc tccttggtat tccgaggagc atgcctgttt gagtgtcatg
 361  gaattctcaa cttataaatc cttgtgatct ataagcttgg acttggaggc ttgctggccc
 421  ttgcggtcgg ctcctctcga atgcattagc tcgattccgt acggatcggc tctcagtgtg
 481  ataattgtct acgctgtgac cgtgaagtgt ttggcgagct tctaaccgtc cattaggaca
 541  act
```

KP2-013F

ITS 1

```
   1  aaaacacaat ttaattattt ttattgatag tcaaattttg aattaatctt caaaactttc
  61  aacaacggat ctcttggttc tcgcatcgat gaagaacgca NNgaaatgcg ataagtaata
 121  tgaattgcag attttcgtga atcatcgaat ctttgaacgc acattgcgcc ctctggtatt
 181  ccagagggca tgcctgtttg agcgtcattt ctctctcaaa ccccgggtt tggtattgag
 241  tgatactctt agtcgaacta gNcgtttgct tgaaaagtat tggcatgggt agtactggat
 301  agtgctgtcg acctctcaat gtattaggtt tatccaactc gttgaatggt gtggcgggat
 361  atttctggta ttgttggccc ggccttacaa caaccaaaca agtttgacct caaatcaagt
 421  aggaataccc gctgaactta agcatatcaa taagcggag
```

ITS 4

```
   1  cttccgtagg tgaacctgcg gaaggatcat tacagtattc ttttgccagc gcttaactgc
  61  gcggcgaaaa accttacaca cagtgtcttt ttgatacaga actcttgctt tggtttggcc
 121  tagagatagg ttgggccaga ggtttaacaa aacacaattt aattattttt attgatagtc
 181  aaattttgaa ttaatcttca aaactttcaa caacggatct cttggttctc gcatcgatga
 241  agaacgcagc gaaatgcgat aagtaatatg aattgcagat tttcgtgaat catcgaatct
 301  ttgaacgcac attgcgccct ctggtattcc agagggcatg cctgtttgag cgtcatttct
 361  ctctcaaacc cccgggtttg gtattgagtg atactcttag tcgaactagg cgtttgcttg
 421  aaaagtattg gcatgggtag tactggatag tgctgtcgac ctctcaatgt attaggttta
 481  tccaactcgt tgaatggt
```

KP2-025D

ITS 1

```
   1  ggccgccggg gaggccttgc gccccgggc ccgcgcccgc cgaagacccc aacatgaacg
  61  ctgttctgaa agtatgcagt ctgagttgat tatcgtaatc agttaaaact ttcaacaacg
 121  gatctcttgg ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg
 181  cagaattcag tgaatcatcg agtctttgaa cgcacattgc gccccctggt attccggggg
 241  gcatgcctgt ccgagcgtca ttgctgccct caagcacggc ttgtgtgttg ggcccccgtc
 301  cccctctccc gggggacggg cccgaaaggc agcggcggca ccgcgtccgg tcctcgagcg
 361  tatggggctt tgtcacctgc tctgtaggcc cggccggcgc cagccgacac ccaactttat
 421  ttttctaagg ttgacctcgg atcaggtagg gatacccgct gaacttaagc atatc
```

ITS 4

```
   1  acctgcggaa ggatcattac cgagtgaggg ccctctgggt ccaacctccc acccgtgtct
  61  atcgtacctt gttgcttcgg cgggcccgcc gtttcgacgg ccgccgggga ggccttgcgc
 121  ccccgggccc gcgcccgccg aagaccccaa catgaacgct gttctgaaag tatgcagtct
 181  gagttgatta tcgtaatcag ttaaaacttt caacaacgga tctcttggtt ccggcatcga
 241  tgaagaacgc agcgaaatgc gataagtaat gtgaattgca gaattcagtg aatcatcgag
 301  tctttgaacg cacattgcgc cccctggtat tccggggggc atgcctgtcc gagcgtcatt
 361  gctgccctca agcacggctt gtgtgttggg cccccgtccc cctctcccgg gggacgggcc
 421  cgaaaggcag cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacctgc
```

KP2-033B

ITS 1

```
   1  gccgggggggc tcacgccccc gggcccgcgc ccgccgaaga caccccgaa ctctgcctga
  61  agattgtcgt ctgagtgaaa atataaatta tttaaaactt tcaacaacgg atctcttggt
 121  tccggcatcg atgaagaacg cagcgaaatg cgatacgtaa tgtgaattgc aaattcagtg
 181  aatcatcgag tctttgaacg cacattgcgc cccctggtat tccgggggggc atgcctgtcc
 241  gagcgtcatt gctgccctca agcccggctt gtgtgttggg ccccgtcctc cgattccggg
 301  ggacgggccc gaaaggcagc ggcggcaccg cgtccggtcc tcgagcgtat ggggctttgt
 361  cacccgctct gtaggcccgg ccggcgcttg ccgatcaacc caaattttta tccaggttga
 421  cctcggatca ggtagggata cccgctgaac ttaagcatat caataagcgg ag
```

ITS 4

```
   1  ttccgtaggt gaacctgcgg aaggatcatt accgagtgag ggcccttttgg gtccaacctc
  61  ccacccgtgt ttatttacct cgttgcttcg gcgggcccgc cttaactggc cgccgggggg
 121  ctcacgcccc cgggcccgcg cccgccgaag acacccccga actctgcctg aagattgtcg
 181  tctgagtgaa aatataaatt atttaaaact ttcaacaacg gatctcttgg ttccggcatc
 241  gatgaagaac gcagcgaaat gcgatacgta atgtgaattg caaattcagt gaatcatcga
 301  gtctttgaac gcacattgcg ccccctggta ttccgggggg catgcctgtc cgagcgtcat
 361  tgctgccctc aagcccggct tgtgtgttgg gccccgtcct ccgattccgg gggacgggcc
 421  cgaaaggcag cggcggcacc gcgtccggtc ctcgagcgta tggggctttg tcacc
```

KP2-033D

ITS 4

```
   1  gatccttccg taggtgaacc tgcggaagga tcattgNNtN NNccgcgctc gtccgcgccc
  61  gcggtaNNNg gggcccgccc ttcggggccg gccctgtctg caccctctgc cattgtcgca
 121  cctcgcgttt cctcggcggg cccgcccgcc aatggggacc ccaaaccaaa cccatttgca
 181  gtgcctgcag taaacgtctc aaaacaatgg aaatcaaaac tttcaacaac ggatctcttg
 241  gttctggcat cgatgaagaa cgcagcgaaa tgcgataagt agtgtgaatt gcagaattca
 301  gtgaatcatc gaatctttga acgcacattg cgccctttgg tattccttag ggcatgcctg
 361  ttcgagcgtc atctaacccc tcaagcaccg cttgatgttg ggcgcttgtc cccgcccccg
 421  cgcgcggact cgcctcgaag acattggcgg cctgtgtatt ggctacgagc gcagcagacc
```

KP2-033E

ITS 4

```
   1  tcgcatttcg ctgcgttctt catcgatgcc agaaccaaga gatccgttgt tgaaagtttt
  61  gatttccatt gttttgagac gtttactgca ggcactgcaa atgggtttgg tttggggtcc
 121  ccattggcgg gcgggcccgc cgaggaaacg cgaggtgcga caatggcaga gggtgcagac
 181  agggccggcc ccgaagggcg ggcccccgat accgcgggcg cggacgagcg cggtgtagac
 241  aatgatcctt ccgtaggtga acctgcggaa ggatcattgt ctacaccgcg ctcgtccgcg
 301  cccgcggtat cgggggcccg cccttcgggg ccggccctgt ctgcaccctc tgccattgtc
 361  gcacctcgcg tttcctcggc gggcccgccc gccaatgggg accccaaacc aaacccattt
 421  gcagtgcctg cagtaaacgt ctcaaaacaa tggaaatcaa aactttcaac aacggatctc
 481  ttggttctgg catcgatgaa gaacgcagcg aaatgcgata agtagtgtga attgcagaat
 541  tcagtgaatc atcgaatctt tgaacgcaca ttgcgccctt tggtattcct tagggcatgc
 601  ctgttcgagc gtcatctaac ccctcaagca ccgcttgatg ttgggcgctt gtccccgccc
 661  ccgcgcgcgg actcgcctcg aagacattgg cggcctgtgt attggctacg agcgcagcag
 721  acc
```

KP2-033H

ITS 1

```
   1  cgtaccttgt tgcttcggcg ggcccgcctc acggccgccg gggggcacct gcccccgggc
  61  ccgcgcccgc cgaagacacc attgaactct gtctgaagat tgcagtctga gcgattaact
 121  aaatcagtta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 181  gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 241  attgcgcccc ctggtattcc gggggggcatg cctgtccgag cgtcattgct gccctcaagc
 301  acggcttgtg tgttgggctc cgccccccctc ccgggggggcg ggcccgaaag gcagcggcgg
 361  caccgcgtcc ggtcctcgag cgtatggggc tttgtcaccc gctctgtagg cccggccggc
 421  gcccgccggc gaccccaatc aatctttcca ggttgacctc ggatcaggta gggatacccg
 481  ctgaacttaa gcatatc
```

ITS 4

```
   1  ctgcggaagg atcattaccg agtgagggcc ctctgggtcc aacctcccac ccgtgtttat
  61  cgtaccttgt tgcttcggcg ggcccgcctc acggccgccg gggggcacct gcccccgggc
 121  ccgcgcccgc cgaagacacc attgaactct gtctgaagat tgcagtctga gcgattaact
 181  aaatcagtta aaactttcaa caacggatct cttggttccg gcatcgatga agaacgcagc
 241  gaaatgcgat aagtaatgtg aattgcagaa ttcagtgaat catcgagtct ttgaacgcac
 301  attgcgcccc ctggtattcc gggggggcatg cctgtccgag cgtcattgct gccctcaagc
 361  acggcttgtg tgttgggctc cgccccccctc ccgggggggcg ggcccgaaag gcagcggcgg
 421  caccgcgtcc ggtcctcgag cgtatggggc tttgtcaccc gctctgtagg cccggccggc
 481  gcccgccggc gaccccaatc aatcttcca
```

Appendix 4: Morphological description of isolates

KP1-009A
Sterile Beige I

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate white margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-009B
Sterile brown filamentous I

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation, media pigmented light brown. **Potato dextrose agar**: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation, media pigmented dark brown. **Cornmeal agar**: Filamentous white colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white/grey colony, flat on surface, rough surface, filiform margin, lack of spore formation, media pigmented dark brown. **Microscopy of isolate**: Sterile, septate, brown pigmented.

KP1-009C
Sterile beige II

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, wrinkled surface, undulating white margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Czapek-dox agar**: Circular beige colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-013B
***Penicillium* sp. I**

Malt extract agar: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, entire white margin, spore green formation, and media pigmented orange, with liquid secretion on surface of colony. **Cornmeal agar**: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous beige colony, crateriform on surface, filiform margin, white spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-017A
Sterile hyaline ascomycete I

Malt extract agar: Filamentous white colony, raised on surface, rough surface, filiform margin, lack of spore formation, media pigmented dark brown. **Czapek-dox agar**: Filamentous brown colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-017C
Coprinellus micaceus

Malt extract agar: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-017D
Sterile beige III

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, wrinkled surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-017E
***Penicillium* sp. II**

Malt extract agar: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, flat on surface with white floccose growth, rough surface, entire margin, green spore formation. **Cornmeal agar**: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: White floccose, filamentous colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-021A
Sterile beige IV

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, wrinkled surface, undulating beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod, unpigmented.

KP1-025B
***Penicillium* sp. III**

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, white entire margin, green spore formation. **Cornmeal agar**: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface with white floccose growth, smooth surface, white entire margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-025C
Yellow yeast I

Malt extract agar: Irregular yellow colony, flat on surface, wrinkled glistening surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular yellow colony, crateriform on surface, smooth surface, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-045A
***Penicillium* sp. IV**

Malt extract agar: Filamentous beige colony, flat on surface with floccose growth, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, white entire margin, green spore formation. **Cornmeal agar**: Filamentous green colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous yellow colony, flat on surface with white floccose growth, rough surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-045B
Orange yeast

Malt extract agar: Circular orange colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Irregular pale yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular pale yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, wrinkled surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-045C
***Penicillium* sp. V**

Malt extract agar: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous yellow colony, flat on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface with some floccose elevations, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-045D
Sterile beige filamentous

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin lack of spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, white entire margin, green spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, smooth surface, white entire margin, green spore formation, and media pigmented yellow. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-045G
Sterile beige V

Malt extract agar: Irregular beige colony, flat on surface, wrinkled surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, wrinkled surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-045I
Sterile white I

Malt extract agar: Irregular white colony, raised on surface, wrinkled surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, light beige undulated margin. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-045J
Sterile white II

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, light beige undulated margin. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-045K
***Dendryphiella* sp. I**

Malt extract agar: Filamentous black/dark green colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous black/dark green colony, flat on surface, rough surface, entire margin, brown spore formation. **Cornmeal agar**: Filamentous unpigmented colony, flat on surface, smooth surface, entire margin, brown spore formation. **Czapek-dox agar**: Filamentous black/dark green colony, flat on surface with white floccose growth, rough surface, entire margin, brown spore formation. **Microscopy of isolate**: Septate, spores, pigmented green/brown.

KP1-063A
Sterile white III

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular orange/yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular orange/yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular orange/yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063B
Septate beige filamentous I

Malt extract agar: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous white colony, crateriform and floccose on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, yellow entire margin, lack of spore formation. **Microscopy of isolate**: Filamentous, septate, hyaline, unpigmented, spores (loose).

KP1-063C
Sterile beige VI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, wrinkled surface, light beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063E
Sterile white IV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating margin, margin more opaque than centre of colony, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, entire margin, margin lighter beige, dark beige ring in centre of colony, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063F
Sterile beige VII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular dark beige colony, flat on surface, wrinkled surface, white entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod, unpigmented.

KP1-063J
***Penicillium* sp. VI**

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface, wrinkled surface, entire margin, green spore formation, and media pigmented yellow. **Cornmeal agar**: Filamentous beige colony, flat on surface with white floccose growth, rough surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous white colony, raised on surface, wrinkled surface, entire margin, green spore formation, and media pigmented yellow. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-063L
Sterile beige VIII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, lighter beige entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063M
Sterile beige IX

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, lighter beige undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063N
***Aspergillus fumigatus* I**

Malt extract agar: Filamentous colourless colony, flat on surface, rough surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous green colony, flat on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, rough surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous beige colony, flat on surface, rough surface, filiform margin, grey/green spore formation, and media pigmented green/grey. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-063O
Sterile white V

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, lighter beige undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-063P
Sterile white VI

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-069A
Sterile beige X

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, entire white margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, lobate white margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-069B
Sterile beige XI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, undulating lighter beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod, unpigmented.

KP1-069C
Sterile beige XII

Malt extract agar: Irregular dark beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, entire beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular dark beige colony, flat on surface, smooth surface, entire light beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-069D
Sterile beige XIII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-069E
Sterile white VII

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular dark beige colony, flat on surface, smooth surface, lighter beige undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-075A
Sterile beige XIV

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular beige/orange colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-075B
***Penicillium* sp. VII**

Malt extract agar: Filamentous beige colony, flat of surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, entire margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface with white floccose growth, rough surface, green spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, green spore formation, and media pigmented yellow. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-081A
Sterile beige XV

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating beige margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-089A
Sterile pigmented ascomycete I

Malt extract agar: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface, rough surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, raised on surface, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Sterile, septate, brown pigmented.

KP1-091A
***Penicillium* sp. VIII**

Malt extract agar: Filamentous beige colony, flat of surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, entire margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, rough surface, green spore formation. **Czapek-dox agar**: Floccose white colony, raised on surface, rough surface, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-095A
Sterile beige XVI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular orange colony, raised on surface, wrinkled surface, undulating margin, lack of spore formation and media pigmented yellow. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular orange colony, raised on surface, wrinkled white surface, undulating margin, lack of spore formation and media pigmented yellow. **Microscopy of isolate**: Hyaline, filamentous and yeast-like, unpigmented, sterile.

KP1-099A
Sterile white VIII

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-115A
Sterile white IX

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Circular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-119A
Yellow yeast II

Malt extract agar: Circular yellow colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-119B
Sterile white X

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating lighter beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod/oval, unpigmented.

KP1-119C
***Paraconiothyrium* sp.**

Malt extract agar: Filamentous orange colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous grey colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-119D
Sterile orange filamentous

Malt extract agar: Filamentous orange colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous orange colony, raised on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous orange colony, raised on surface, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-119E
***Aureobasidium* sp.**

Malt extract agar: Filamentous brown colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface, rough surface, entire margin, lack of spore formation, and media pigmented yellow. **Cornmeal agar**: Filamentous orange colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, black pigmented.

KP1-123A
***Penicillium* sp. IX**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, colourless spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface with white floccose growth, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, entire margin, brown spore formation. **Czapek-dox agar**: Filamentous white colony, raised and floccose on surface, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-123B
***Penicillium* sp. X**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, colourless spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, green spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous white colony, raised and floccose on surface, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-123C
***Chrysosporium* sp.**

Malt extract agar: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, sterile, green pigmented.

KP1-131A
Sterile brown filamentous II

Malt extract agar: Filamentous brown colony, flat on surface with white floccose growth, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous colony, flat on surface with white floccose growth, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131B
Sterile hyaline ascomycete II

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131C
***Thysanophora* sp. I**

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, flat on surface with white floccose growth, entire white margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, entire white margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131DA
***Thysanophora* sp. II**

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131DB
Ophiognomonia intermedia

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous beige colony, crateriform on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131E
Sterile grey filamentous I

Potato dextrose agar: Filamentous grey colony, raised on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar:** Filamentous grey colony, flat on surface, filiform margin, grey spore formation. **Czapek-dox agar:** Filamentous grey colony, raised on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate:** Septate, sterile, brown pigmented.

KP1-131F
***Mollisia* sp. I**

Malt extract agar: Filamentous brown colony, flat on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, flat on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous orange colony, flat on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131G
Septate brown filamentous

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, entire margin, brown spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, brown spore formation. **Microscopy of isolate**: Septate, spores (loose), brown pigmented.

KP1-131H
Microdochium bolleyi

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, grey spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface, rough surface, entire margin, brown spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131I
***Dendryphiella* sp. II**

Malt extract agar: Filamentous green colony, raised/floccose on surface, rough surface, entire margin, brown spore formation. **Potato dextrose agar**: Filamentous grey colony, raised on surface, rough surface, entire white margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, brown spore formation. **Czapek-dox agar**: Filamentous grey colony, raised on surface, rough surface, filiform margin, grey spore formation. **Microscopy of isolate**: Septate, spores, brown pigmented.

KP1-131J
***Myrothecium* sp.**

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, flat on surface with white floccose growth, smooth surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131K
Didymella bryoniae

Malt extract agar: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous orange colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131L
***Penicillium* sp. XII**

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, pink spore formation. **Potato dextrose agar**: Filamentous beige colony, flat on surface, rough surface, filiform margin, grey spore formation, and media pigmented orange. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, filiform margin, pink spore formation. **Czapek-dox agar**: Filamentous orange colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-131M
Thysanophora penicillioides

Malt extract agar: Filamentous brown colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Potato dextrose agar**: Filamentous brown colony, flat on surface, rough surface, entire margin, brown spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, brown spore formation. **Microscopy of isolate**: Septate, spores, brown pigmented.

KP1-131N
Sterile pigmented ascomycete II

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous orange colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous orange colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131O
Sterile beige XVI

Malt extract agar: Irregular beige colony, flat on surface, wrinkled surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular pale yellow colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular pale yellow colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-131Q
***Aspergillus fumigatus* III**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous grey colony, raised on surface with white floccose growth, rough surface, undulating white margin, grey spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, grey spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-131R
***Mollisia* sp. II**

Malt extract agar: Filamentous beige colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131S
Mycosphaerella nyssicola

Malt extract agar: Filamentous orange colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous orange colony, raised on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131T
***Aspergillus fumigatus* IV**

Malt extract agar: Filamentous colourless colony, flat on surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, flat on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface with white floccose growth, rough surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-131U
Sterile green filamentous

Malt extract agar: Filamentous green colony, raised on surface with floccose growth, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous green colony, flat on surface, rough surface, entire white margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, slight green pigment.

KP1-131V
Sterile hyaline ascomycete III

Malt extract agar: Filamentous brown colony, flat on surface with white floccose growth, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface with white floccose growth, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-131W
***Diaporthe* sp.**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, green filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, raised on surface, rough surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, raised on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, slight brown pigment.

KP1-131Y
***Aspergillus fumigatus* V**

Malt extract agar: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface, rough surface, filiform white margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-131Z
Sterile hyaline ascomycete IV

Malt extract agar: Filamentous orange colony, flat on surface with white floccose growth, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous orange colony, flat on surface with white floccose growth, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Sterile, septate, hyaline, unpigmented.

KP1-131AA
***Aspergillus fumigatus* II**

Malt extract agar: Filamentous green colony, flat on surface, smooth surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-131BB
Septate ascomycete

Malt extract agar: Filamentous green colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-131CC
***Penicillium* sp. XI**

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, grey spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, yellow entire margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, rough surface, filiform margin, brown spore formation. **Czapek-dox agar**: Filamentous colourless colony, flat on surface, rough surface, white filiform margin, brown spore formation. **Microscopy of isolate**: Septate, spores, brown pigmented.

KP1-131DD
Sterile grey filamentous II

Malt extract agar: Filamentous grey colony, flat on surface, rough surface, undulating margin, lack of spore formation, and media pigmented orange. **Potato dextrose agar**: Filamentous grey colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous grey colony, flat on surface, rough surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP1-135B
***Trichoderma* sp.**

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous beige colony, raised on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-135C
Penicillium roseopurpureum

Malt extract agar: Filamentous orange colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Potato dextrose agar**: Filamentous grey colony, flat on surface, smooth surface, filiform margin, grey spore formation, and media pigmented red. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Czapek-dox agar**: Filamentous grey colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Microscopy of isolate**: Septate, spores, orange/brown pigment.

KP1-135D
***Dendryphiella* sp. III**

Malt extract agar: Filamentous green colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous black colony, flat on surface, rough surface, filiform margin, grey spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, grey spore formation. **Czapek-dox agar**: Filamentous black colony, flat on surface, rough surface, filiform margin, grey spore formation. **Microscopy of isolate**: Septate, spores, brown pigmented.

KP1-135E
Sterile white XI

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-135E2
Sterile beige XVIII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Circular beige colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-135F
***Penicillium* sp. XIII**

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, beige spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, undulating yellow margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, rough surface, filiform margin, brown spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface, rough surface, undulating pale orange margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-139A
Yellow yeast III

Malt extract agar: Irregular yellow colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Irregular yellow colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular yellow colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-139B
Sterile beige XIX

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, smooth surface, flat on surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-143A
***Metschnikowia* sp.**

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, yeast/filamentous like, sterile, oval/rod, unpigmented.

KP1-143B
Sterile white XII

Malt extract agar: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod/oval, unpigmented.

KP1-143C
Sterile white XIII

Malt extract agar: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Circular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Circular yellow colony, raised on surface, wrinkled surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-143D
***Trametes versicolor* I**

Malt extract agar: Filamentous white colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP1-171A
Sterile white XIV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175A
Sterile white XV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175C
Sterile beige XX

Potato dextrose agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating lighter beige margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175D
Sterile white XVI

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Circular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175E
***Tolypocladium* sp.**

Malt extract agar: Filamentous white colony, flat on surface, rough surface, filiform margin, white spore formation. **Potato dextrose agar**: Filamentous white colony, crateriform on surface, smooth surface, undulating margin, white spore formation. **Cornmeal agar**: Filamentous white colony, raised on surface, rough surface, beige entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface with white floccose growth, rough surface, undulating margin, brown spore formation. **Water agar**: Filamentous white colony, colonies form a flat, white surface, reverse white; aerial hyphae white, filiform margin, white spore formation. **Microscopy of isolate**: Hyphae septate and hyaline, branching and phialidic. Phialides were ovoid to obclavate and solitary. Conidia were one celled, hyaline and subglobose to ellipsoidal.

KP1-175F
Sterile beige XXI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175G
***Penicillium* sp. XIV**

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, brown spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface, rough surface, filiform margin, green spore formation, and media pigmented yellow. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-175H
Sterile white XVII

Malt extract agar: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-175J
Septate white filamentous I

Malt extract agar: Filamentous white colony, flat of surface with white floccose growth, rough surface, entire margin, green spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface with white floccose growth, rough surface, entire margin, green spore formation. **Cornmeal agar**: Filamentous green colony, flat on surface, smooth surface, entire margin, green spore formation. **Czapek-dox agar**: Filamentous white colony, raised on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-175K
Septate white filamentous II

Malt extract agar: Filamentous white growth, flat on surface, rough surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, flat on surface, rough surface, undulating white margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous yellow colony, raised on surface, rough surface, filiform margin, green spore formation, and media pigmented orange. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-175L
***Penicillium* sp. XV**

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, filiform margin, white spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, entire margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, brown spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-175M
***Penicillium* sp. XVI**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, white spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, entire margin, green spore formation, and media pigmented yellow. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, entire margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP1-179A
Sterile beige XXII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular orange colony, flat on surface, wrinkled surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, raised on surface, wrinkled surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-179B
Sterile beige XXIII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, wrinkled surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP1-179C
Sterile white XXIV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-001A
Sterile beige XXIV

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-001B
Sterile beige XXV

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, raised on surface, wrinkled surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular transparent colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-001C
***Aspergillus fumigatus* VI**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface with white floccose growth, rough surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-001D
Sterile beige XXVI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, lobate margin, dark centre, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-001E
Sterile beige XXVII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, undulating light beige margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod/oval, unpigmented.

KP2-001F
***Penicillium* sp. XVII**

Malt extract agar: Filamentous beige colony, flat on surface, smooth surface, lobate margin, green spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface, rough surface, undulating margin, green spore production, and media pigmented yellow. **Cornmeal agar**: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous white colony, raised on surface, rough surface, undulating margin, green spore production, and media pigmented yellow. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-001G
Sterile white XIX

Malt extract agar: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-005A
***Phanerochaete* sp.**

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP2-009A
***Trametes versicolor* II**

Malt extract agar: Filamentous white colony, flat on surface with white floccose growth, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous beige colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP2-009B
Septate hyaline ascomycete

Malt extract agar: Filamentous white colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Potato dextrose agar**: Filamentous beige colony, flat on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Cornmeal agar**: Filamentous white colony, flat on surface, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-013A
Sterile beige XXVIII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, white lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular pale yellow colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-013E
Sterile white XX

Malt extract agar: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-013F
Yeast endophyte

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Circular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod/oval, unpigmented.

KP2-013G
Sterile white XXI

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, rod/oval, unpigmented.

KP2-017A
Sterile white XXII

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-025A
Sterile white XXIII

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony with red centre, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-025B
***Penicillium* sp. XVIII**

Malt extract agar: Filamentous brown colony, flat on surface, rough surface, filiform margin, brown spore formation. **Microscopy of isolate**: Septate, spores, green pigmented.

KP2-025C
***Penicillium* sp. XIX**

Malt extract agar: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface with white floccose growth, rough surface, undulating white margin, green spore formation, and media pigmented yellow. **Cornmeal agar**: Filamentous beige colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-025D
***Aspergillus fumigatus* VII**

Malt extract agar: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation, and media pigmented yellow. **Potato dextrose agar**: Filamentous white colony, flat on surface, rough surface, filiform white margin, green spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous green colony, flat on surface, rough surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-025E
Sterile beige XXIX

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-029B
Sterile white XXIV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-029C
Sterile beige XXX

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony with dark centre, flat on surface, smooth surface, white lobate margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-029D
Sterile beige XXXI

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony with dark centre, flat on surface, smooth surface, white lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular beige colony, flat on surface, wrinkled surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-029E
Sterile white XXV

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony with dark centre, flat on surface, smooth surface, white lobate margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-029F
Sterile beige XXXII

Malt extract agar: Irregular beige colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Potato dextrose agar**: Irregular beige colony with dark centre, flat on surface, smooth surface, undulating margin, lack of spore formation. **Cornmeal agar**: Irregular beige colony, flat on surface, smooth surface, lobate margin, lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, undulating margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-033A
***Penicillium* sp. XX**

Malt extract agar: Filamentous colourless colony, flat on surface, smooths surface, filiform margin, and colourless spore formation. **Potato dextrose agar**: Filamentous yellow colony, raised on surface with white floccose growth, rough surface, entire white margin, lack of spore formation. **Cornmeal agar**: Filamentous colourless colony, flat on surface, smooths surface, filiform margin, and colourless spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-033B
***Penicillium* sp. XXI**

Malt extract agar: Filamentous beige colony, flat on surface, smooth surface, filiform margin, green spore formation. **Potato dextrose agar**: Filamentous green colony, raised on surface, rough surface, filiform margin, green spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, smooth surface, filiform margin, green spore formation. **Czapek-dox agar**: Filamentous beige colony, flat on surface with white floccose growth, smooth surface, filiform margin, green spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

KP2-033C
Sterile white XXVI

Malt extract agar: Irregular white colony, flat on surface, smooth surface, lobate margin and lack of spore formation. **Potato dextrose agar**: Irregular beige colony with dark centre, flat on surface, smooth surface, undulating white margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, lobate margin and lack of spore formation. **Czapek-dox agar**: Irregular white colony, flat on surface, smooth surface, lobate margin and lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-033D
Sterile hyaline ascomycete V

Malt extract agar: Filamentous beige colony, flat on surface, rough surface, entire margin, lack of spore formation. **Potato dextrose agar**: Filamentous white colony, raised on surface, rough surface, filiform margin, yellow spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, entire margin, lack of spore formation. **Czapek-dox agar**: Filamentous white colony, raised on surface, rough surface, filiform margin, yellow spore formation. **Microscopy of isolate**: Septate, sterile, hyaline, unpigmented.

KP2-033E
Sterile pigmented ascomycete III

Malt extract agar: Filamentous black colony, raised on surface, rough surface, entire white margin, lack of spore formation. **Potato dextrose agar**: Filamentous black colony, raised on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Czapek-dox agar**: Filamentous brown colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, brown pigmented.

KP2-033F
Sterile white XXVII

Malt extract agar: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Potato dextrose agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Cornmeal agar**: Irregular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Czapek-dox agar**: Circular white colony, flat on surface, smooth surface, entire margin, lack of spore formation. **Microscopy of isolate**: Non-septate, unicellular, sterile, round, unpigmented.

KP2-033G
Sterile black filamentous

Malt extract agar: Filamentous black/dark green colony, flat on surface, smooth surface, curled margin, lack of spore formation. **Potato dextrose agar**: Filamentous black colony, raised on surface, rough surface, entire margin, lack of spore formation. **Cornmeal agar**: Filamentous black/dark green colony, flat on surface, smooth surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, sterile, black pigmented.

KP2-033H
***Penicillium* sp. XXII**

Malt extract agar: Filamentous beige colony, flat on surface with white floccose growth, rough surface, filiform margin, white spore formation. **Potato dextrose agar**: Filamentous white colony, crateriform on surface with white floccose growth, rough surface, entire margin, white spore formation. **Cornmeal agar**: Filamentous beige colony, flat on surface, rough surface, filiform margin, grey spore formation. **Czapek-dox agar**: Filamentous beige colony, crateriform on surface with white floccose growth, rough surface, filiform margin, lack of spore formation. **Microscopy of isolate**: Septate, spores, hyaline, unpigmented.

Appendix 5: Extract antimicrobial raw data

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF10-001-01	KP2-005A	0.0	0.0	7.4	1.0	2.0	0.0	74.0	26.5	25.6
AF10-009-01	KP2-029E	0.0	0.0	8.1	1.2	0.0	0.0	19.8	65.1	12.0
AF10-013-01	KP2-029F	0.0	0.0	11.2	16.0	0.0	0.0	69.4	60.9	78.2
AF10-025-01	KP1-009A	0.0	0.0	7.4	1.2	2.1	0.0	72.5	67.7	25.8
AF10-029-01	KP2-013G	0.0	0.0	13.4	10.4	1.3	0.0	74.1	58.7	82.9
AF10-033-01	KP1-063E	4.3	0.0	7.9	0.0	0.0	0.0	25.3	30.9	43.8
AF10-037-01	KP1-063F	8.2	0.0	1.3	3.9	0.0	0.0	73.7	55.4	24.3
AF10-041-01	KP1-063L	17.2	0.0	5.5	1.5	0.0	1.0	52.0	22.5	13.7
AF10-045-01	KP1-063M	19.1	0.0	4.1	0.0	0.0	0.0	61.7	32.9	0.0
AF10-049-01	KP1-063N	23.0	0.0	10.5	0.0	0.0	0.0	66.4	27.0	0.0
AF10-053-01	KP1-063P	21.8	0.0	9.9	0.0	0.0	5.2	55.8	27.8	0.0
AF10-057-01	KP1-095A	10.4	0.0	8.9	0.0	0.0	0.4	58.4	30.6	0.0
AF10-061-01	KP1-123A	100.0	0.0	0.0	6.4	99.3	10.6	18.4	71.0	0.0
AF10-065-01	KP1-135E	0.8	0.0	5.8	0.0	0.0	8.2	62.0	57.2	3.5
AF10-069-01	KP1-135E2	0.0	0.0	0.0	0.3	0.0	13.3	53.6	30.1	7.8
AF10-073-01	KP1-135F	0.0	0.0	0.0	0.0	0.0	1.0	62.6	30.7	1.2
AF10-077-01	KP1-139A	0.0	0.0	0.0	0.0	0.0	8.3	60.1	29.7	5.0
AF10-081-01	KP1-139B	0.0	0.0	0.0	4.0	0.0	7.0	62.7	0.0	35.2
AF10-085-01	KP2-001A	8.2	0.0	5.8	1.1	0.0	7.0	64.9	25.8	0.0
AF10-089-01	KP2-001B	0.0	0.0	3.1	0.0	0.0	2.8	22.7	0.0	37.3
AF10-093-01	KP2-013A	0.0	0.0	2.6	0.0	0.0	2.9	0.0	0.0	44.1
AF10-097-01	KP2-013E	1.2	0.0	0.0	4.4	0.0	0.0	52.4	51.5	59.2

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization, continued[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF10-101-01	KP2-013F	0.0	0.0	12.9	7.8	0.0	0.0	51.1	107.8	82.7
AF10-105-01	KP1-131M	0.0	0.0	11.5	0.0	0.0	4.6	49.5	32.8	53.3
AF10-109-01	KP1-131N	0.0	0.0	16.0	1.5	3.7	0.0	56.1	8.0	1.9
AF10-113-01	KP1-131O	0.0	0.0	21.1	0.0	0.0	0.0	16.9	30.5	30.4
AF10-121-01	KP1-131Q	50.8	0.0	10.5	0.0	8.9	0.0	36.5	64.4	63.5
AF10-125-01	KP1-131R	0.0	0.0	6.5	0.0	2.0	0.0	37.2	71.5	57.5
AF10-129-01	KP1-131S	0.0	0.0	6.5	0.0	46.9	8.3	31.9	33.1	63.4
AF10-133-01	KP1-131T	24.4	0.0	7.0	3.1	15.1	100.3	40.2	84.0	42.1
AF10-137-01	KP1-131U	22.0	0.0	0.0	0.0	3.9	5.7	64.0	0.0	69.0
AF10-141-01	KP1-131V	28.1	0.0	0.0	2.8	2.8	5.4	70.0	36.4	72.9
AF10-145-01	KP1-131Y	0.0	0.0	13.7	0.1	3.5	3.0	65.6	10.9	80.5
AF10-149-01	KP1-131Z	0.0	0.0	12.0	0.0	0.4	5.4	63.9	0.0	76.3
AF10-153-01	KP1-131AA	0.0	0.0	20.4	0.0	3.4	5.3	65.3	43.5	80.4
AF10-157-01	KP1-131BB	0.0	0.0	9.8	0.0	0.0	5.6	71.4	26.1	0.0
AF10-161-01	KP1-131CC	6.6	0.0	3.5	0.0	0.0	4.6	53.9	33.7	38.2
AF10-165-01	KP1-131DD	79.8	5.4	20.7	17.3	5.3	1.5	6.5	60.5	64.5
AF10-169-01	KP1-131DB	22.3	11.3	17.9	9.7	0.0	0.0	64.1	36.7	81.2
AF10-173-01	KP1-175A	19.6	0.0	17.9	4.4	0.0	0.0	57.6	25.5	77.4
AF10-177-01	KP1-175C	47.4	4.8	11.7	13.4	0.0	0.0	59.7	32.1	81.2
AF10-181-01	KP1-175D	0.2	5.4	0.0	30.5	98.2	0.0	63.5	41.6	76.6
AF11-001-01	KP2-009B	0.0	10.2	13.8	0.0	1.1	0.0	26.6	35.8	0.0
AF11-005-01	KP1-175F	0.0	0.0	12.4	6.7	1.7	0.0	51.4	40.6	78.6
AF11-009-01	KP1-175H	0.0	0.0	14.2	0.0	0.0	10.2	50.3	0.0	60.5
AF11-013-01	KP1-175K	0.0	0.0	14.6	6.6	4.3	0.0	15.4	6.0	0.0

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization, continued[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF11-017-01	KP1-175L	18.3	0.1	10.6	11.1	1.3	9.4	0.0	40.7	0.0
AF11-021-01	KP1-175M	0.0	0.0	7.5	0.0	1.1	7.0	0.0	23.5	0.0
AF11-025-01	KP1-131A	99.9	0.1	1.7	92.6	89.5	1.8	96.6	87.4	0.0
AF11-029-01	KP1-131B	10.2	0.0	12.7	0.0	0.0	0.0	47.3	52.0	71.2
AF11-033-01	KP1-131DA	61.1	0.0	0.0	7.3	1.3	0.0	55.4	63.9	79.6
AF11-037-01	KP2-025E	18.4	8.3	2.9	8.5	3.3	3.0	40.7	3.4	8.1
AF11-041-01	KP1-131C	50.6	9.6	3.4	5.1	0.2	0.0	47.9	50.7	0.0
AF11-045-01	KP1-131E	13.9	9.3	10.0	0.0	1.2	0.0	26.5	0.9	0.0
AF11-049-01	KP1-175E	3.1	1.1	7.7	31.1	96.9	14.4	72.8	0.0	55.1
AF11-053-01	KP1-175G	19.8	7.3	2.9	1.3	0.3	0.0	35.1	0.0	64.3
AF11-057-01	KP1-175J	14.0	6.6	4.4	0.0	0.0	1.8	16.5	0.0	60.0
AF11-061-01	KP2-009A	3.3	9.2	14.3	4.8	6.2	3.0	54.8	0.0	51.5
AF11-065-01	KP2-025A	0.0	0.0	13.7	8.4	3.4	0.0	54.6	0.0	0.0
AF11-069-01	KP2-025B	0.0	0.0	9.3	0.0	0.0	0.0	12.2	2.3	9.7
AF11-073-01	KP2-025C	0.0	0.0	5.6	0.0	0.3	11.4	15.0	0.0	37.6
AF11-077-01	KP2-025D	0.0	0.0	8.8	1.2	2.2	3.4	16.3	36.2	68.1
AF11-085-01	KP1-009C	0.0	0.0	20.9	13.2	2.5	0.0	65.6	67.0	68.4
AF11-089-01	KP2-017A	0.0	0.0	11.2	17.8	2.8	0.0	65.1	44.0	66.9
AF11-093-01	KP1-017C	76.4	0.0	12.4	3.5	5.8	0.5	56.4	69.2	63.7
AF11-097-01	KP1-017D	0.0	0.0	16.1	0.2	1.1	0.0	8.8	0.0	53.7
AF11-101-01	KP1-063J	0.0	0.9	19.6	0.0	5.4	0.0	21.3	0.0	0.0
AF11-105-01	KP1-017E	72.8	4.0	20.0	18.9	5.5	6.4	83.4	39.1	10.8
AF11-109-01	KP1-091A	0.0	17.0	4.6	2.4	1.7	1.9	58.6	55.7	76.9
AF11-113-01	KP2-033G	0.0	10.6	15.9	0.0	2.6	0.0	63.2	50.0	3.9

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization, continued[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF11-117-01	KP2-033H	0.0	0.0	13.2	8.9	0.0	0.0	63.4	0.0	10.8
AF11-125-01	KP1-063O	0.0	9.0	3.7	0.6	2.5	0.0	60.8	0.0	16.0
AF11-129-01	KP1-075A	0.0	0.0	4.3	16.8	1.0	0.0	81.0	82.0	90.4
AF11-133-01	KP1-075B	8.6	0.0	17.7	1.2	4.7	19.6	39.8	0.0	36.0
AF11-137-01	KP1-131F	0.0	0.0	12.8	0.0	0.6	10.2	56.7	33.9	58.9
AF11-141-01	KP1-131G	0.0	0.0	26.6	16.8	3.2	8.0	52.2	0.0	37.3
AF11-145-01	KP1-131H	52.1	0.0	23.1	6.2	2.4	0.4	58.1	0.0	33.4
AF11-149-01	KP1-131I	0.0	0.0	20.8	6.0	0.0	6.9	63.6	20.1	27.8
AF11-153-01	KP1-131J	0.0	0.0	11.0	0.0	0.0	4.2	55.7	42.2	7.9
AF11-157-01	KP1-131L	4.6	0.0	11.7	0.0	0.0	5.2	54.2	57.9	13.8
AF11-165-01	KP1-171A	0.0	5.7	19.2	9.1	0.0	0.0	38.1	16.6	56.9
AF11-169-01	KP1-179B	0.0	2.7	18.8	7.9	0.0	0.0	53.5	74.4	21.6
AF11-173-01	KP2-001C	0.0	7.5	4.4	6.3	0.0	8.6	44.1	36.0	0.0
AF11-177-01	KP2-001D	0.0	4.3	15.9	6.6	0.0	14.7	35.4	33.9	0.0
AF11-181-01	KP2-001E	0.0	6.8	12.9	0.4	0.0	0.0	48.4	43.7	7.7
AF12-001-01	KP2-001F	3.2	0.0	3.5	0.4	0.0	15.3	41.2	80.5	10.1
AF12-005-01	KP2-001G	0.0	7.8	4.1	0.5	0.0	0.0	23.8	73.9	56.2
AF12-009-01	KP2-029C	0.0	0.0	17.6	5.7	0.0	0.0	12.6	56.3	42.2
AF12-013-01	KP2-029D	25.8	0.0	13.1	0.0	0.0	4.6	0.0	67.2	41.3
AF9-041-01	KP1-045C	3.1	2.0	9.3	1.4	0.0	0.0	4.3	0.0	16.4
AF9-045-01	KP1-045I	6.2	0.0	12.7	0.3	0.1	0.0	6.8	0.0	18.3
AF9-049-01	KP2-033B	25.5	2.0	0.0	41.2	12.0	15.5	99.2	89.7	65.0
AF9-053-01	KP1-045J	2.7	0.0	11.8	0.0	2.0	0.0	21.7	10.8	35.3
AF9-057-01	KP2-033E	3.9	4.6	0.0	0.9	0.2	5.2	6.9	0.5	23.6

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization, continued[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF9-061-01	KP1-045A	0.0	0.0	0.0	5.8	3.7	12.3	19.8	0.0	0.0
AF9-065-01	KP2-033A	0.0	0.0	10.2	2.3	99.6	17.3	0.5	10.6	0.0
AF9-069-01	KP2-033D	0.0	0.0	3.3	3.9	7.4	2.9	0.0	0.0	0.0
AF9-073-01	KP1-119C	0.0	0.0	8.2	1.1	1.1	6.5	50.8	6.5	27.1
AF9-077-01	KP1-115A	0.0	0.0	17.9	17.8	0.6	0.0	84.1	99.9	72.6
AF9-081-01	KP1-081A	0.0	0.0	13.0	5.7	1.1	0.0	80.6	104.3	71.3
AF9-085-01	KP2-029B	0.0	0.0	9.4	0.0	0.0	0.0	8.2	0.0	44.2
AF9-089-01	KP1-045G	3.5	0.0	0.0	0.2	0.0	0.0	44.2	65.5	39.5
AF9-093-01	KP2-033F	0.0	0.0	0.0	0.2	0.0	0.0	12.8	44.0	19.5
AF9-097-01	KP1-179C	0.0	0.0	5.1	0.0	0.0	0.0	45.6	46.7	61.5
AF9-101-01	KP1-119E	38.7	0.0	5.1	3.8	0.0	0.0	55.7	13.8	59.0
AF9-105-01	KP1-119A	0.0	0.0	16.8	0.0	0.8	0.0	24.4	0.0	57.3
AF9-109-01	KP2-033C	0.0	2.1	11.1	0.4	1.8	0.0	41.3	56.0	61.9
AF9-113-01	KP1-119B	0.0	0.9	11.8	0.9	8.9	0.0	63.5	70.2	66.1
AF9-117-01	KP1-179A	5.5	5.0	3.2	0.4	2.4	0.0	59.7	0.0	65.3
AF9-121-01	KP1-021A	0.0	0.0	1.4	2.3	2.7	0.0	0.0	92.3	44.8
AF9-125-01	KP1-025B	0.0	0.0	8.8	5.0	4.7	0.0	50.2	0.0	57.8
AF9-129-01	KP1-025C	0.0	0.0	6.0	0.0	0.0	0.0	0.0	56.4	59.0
AF9-133-01	KP1-063A	0.0	0.0	18.6	3.4	5.4	0.0	35.7	43.9	65.9
AF9-137-01	KP1-063B	0.0	0.0	10.8	1.6	2.6	0.0	59.2	70.5	71.1
AF9-141-01	KP1-063C	0.0	0.0	12.7	2.5	3.5	4.4	4.0	97.7	74.0
AF9-145-01	KP1-069A	0.0	0.0	7.7	0.0	0.0	0.0	28.5	0.0	67.2
AF9-149-01	KP1-069B	0.0	0.0	2.4	0.0	0.3	1.7	61.7	29.3	56.5
AF9-153-01	KP1-069C	1.0	0.8	4.9	1.2	1.7	0.0	11.1	0.0	60.2

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A5.1. Antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada before normalization, continued[a].

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF9-157-01	KP1-069D	20.9	0.0	7.4	0.0	0.0	0.0	0.0	0.0	66.6
AF9-161-01	KP1-069E	24.6	0.0	11.8	3.4	0.0	0.0	55.8	44.5	72.7
AF9-165-01	KP1-099A	30.9	0.0	15.0	2.7	0.0	0.0	38.0	64.5	55.8
AF9-169-01	KP1-143A	0.0	0.0	9.3	2.3	0.0	0.0	56.8	34.9	9.0
AF9-173-01	KP1-143B	0.0	0.0	7.6	4.3	0.0	0.0	70.5	72.6	41.6
AF9-177-01	KP1-143C	8.8	0.0	4.3	1.0	0.0	0.0	64.9	0.0	34.9
AF9-181-01	KP1-143D	0.0	0.0	10.0	11.1	3.4	0.0	78.9	89.5	48.7
KP2-037-01	KP1-017A	0.0	8.3	6.8	0.5	0.0	0.0	25.7	0.0	10.5
KP2-041-01	KP1-123C	2.5	12.2	9.5	0.0	0.0	1.3	19.6	0.0	16.9
KP2-045-01	KP1-045D	0.0	6.7	5.9	2.1	0.6	9.3	51.0	0.0	0.0
KP2-049-01	KP1-131W	0.0	5.1	6.1	6.1	0.0	2.6	64.6	3.0	5.9
KP2-057-01	KP1-009B	51.6	0.0	0.0	11.4	0.0	0.0	50.5	12.5	21.0
KP2-061-01	KP1-013B	0.0	0.0	5.6	6.2	4.3	12.9	59.2	7.5	1.3
KP2-065-01	KP1-131K	0.0	0.0	9.5	0.0	2.6	15.6	18.3	0.0	17.4
KP2-069-01	KP1-089A	0.0	0.0	4.4	0.7	0.0	2.3	38.9	0.0	20.3
KP2-073-01	KP1-135C	63.4	0.0	0.0	0.2	4.5	11.6	98.8	77.5	0.0
KP2-077-01	KP1-045B	0.0	0.0	2.5	3.8	2.9	8.2	55.6	0.0	0.0
KP2-081-01	KP1-135D	0.0	0.0	12.4	0.9	0.0	3.5	29.6	0.0	3.5
KP2-085-01	KP1-045K	0.0	0.0	11.2	6.2	0.0	0.0	53.9	0.0	8.0
KP2-089-01	KP1-119D	0.0	0.0	5.6	3.4	0.0	0.0	47.0	1.5	7.8
KP2-093-01	KP1-123B	100.3	2.3	0.5	0.7	99.8	0.0	92.4	5.9	2.2
KP2-097-01	KP1-135B	0.0	0.0	0.0	2.5	6.5	0.0	27.5	0.0	2.5

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Appendix 6: Extract normalized data

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF10-001-01	KP2-005A	0.00	0.00	0.10	0.01	0.03	0.00	1.00	0.36	0.35
AF10-009-01	KP2-029E	0.00	0.00	0.12	0.02	0.00	0.00	0.30	1.00	0.18
AF10-013-01	KP2-029F	0.00	0.00	0.14	0.20	0.00	0.00	0.89	0.78	1.00
AF10-025-01	KP1-009A	0.00	0.00	0.10	0.02	0.03	0.00	1.00	0.93	0.36
AF10-029-01	KP2-013G	0.00	0.00	0.16	0.12	0.02	0.00	0.89	0.71	1.00
AF10-033-01	KP1-063E	0.10	0.00	0.18	0.00	0.00	0.00	0.58	0.71	1.00
AF10-037-01	KP1-063F	0.11	0.00	0.02	0.05	0.00	0.00	1.00	0.75	0.33
AF10-041-01	KP1-063L	0.33	0.00	0.11	0.03	0.00	0.02	1.00	0.43	0.26
AF10-045-01	KP1-063M	0.31	0.00	0.07	0.00	0.00	0.00	1.00	0.53	0.00
AF10-049-01	KP1-063N	0.35	0.00	0.16	0.00	0.00	0.00	1.00	0.41	0.00
AF10-053-01	KP1-063P	0.39	0.00	0.18	0.00	0.00	0.09	1.00	0.50	0.00
AF10-057-01	KP1-095A	0.18	0.00	0.15	0.00	0.00	0.01	1.00	0.52	0.00
AF10-061-01	KP1-123A	1.00	0.00	0.00	0.06	0.99	0.11	0.18	0.71	0.00
AF10-065-01	KP1-135E	0.01	0.00	0.09	0.00	0.00	0.13	1.00	0.92	0.06
AF10-069-01	KP1-135E2	0.00	0.00	0.00	0.01	0.00	0.25	1.00	0.56	0.14
AF10-073-01	KP1-135F	0.00	0.00	0.00	0.00	0.00	0.02	1.00	0.49	0.02
AF10-077-01	KP1-139A	0.00	0.00	0.00	0.00	0.00	0.14	1.00	0.49	0.08
AF10-081-01	KP1-139B	0.00	0.00	0.00	0.06	0.00	0.11	1.00	0.00	0.56
AF10-085-01	KP2-001A	0.13	0.00	0.09	0.02	0.00	0.11	1.00	0.40	0.00
AF10-089-01	KP2-001B	0.00	0.00	0.08	0.00	0.00	0.07	0.61	0.00	1.00
AF10-093-01	KP2-013A	0.00	0.00	0.06	0.00	0.00	0.07	0.00	0.00	1.00
AF10-097-01	KP2-013E	0.02	0.00	0.00	0.07	0.00	0.00	0.88	0.87	1.00

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada, continued.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF10-101-01	KP2-013F	0.00	0.00	0.12	0.07	0.00	0.00	0.47	1.00	0.77
AF10-105-01	KP1-131M	0.00	0.00	0.22	0.00	0.00	0.09	0.93	0.62	1.00
AF10-109-01	KP1-131N	0.00	0.00	0.28	0.03	0.07	0.00	1.00	0.14	0.03
AF10-113-01	KP1-131O	0.00	0.00	0.69	0.00	0.00	0.00	0.55	1.00	0.99
AF10-121-01	KP1-131Q	0.79	0.00	0.16	0.00	0.14	0.00	0.57	1.00	0.99
AF10-125-01	KP1-131R	0.00	0.00	0.09	0.00	0.03	0.00	0.52	1.00	0.80
AF10-129-01	KP1-131S	0.00	0.00	0.10	0.00	0.74	0.13	0.50	0.52	1.00
AF10-133-01	KP1-131T	0.24	0.00	0.07	0.03	0.15	1.00	0.40	0.84	0.42
AF10-137-01	KP1-131U	0.32	0.00	0.00	0.00	0.06	0.08	0.93	0.00	1.00
AF10-141-01	KP1-131V	0.39	0.00	0.00	0.04	0.04	0.07	0.96	0.50	1.00
AF10-145-01	KP1-131Y	0.00	0.00	0.17	0.00	0.04	0.04	0.82	0.14	1.00
AF10-149-01	KP1-131Z	0.00	0.00	0.16	0.00	0.00	0.07	0.84	0.00	1.00
AF10-153-01	KP1-131AA	0.00	0.00	0.25	0.00	0.04	0.07	0.81	0.54	1.00
AF10-157-01	KP1-131BB	0.00	0.00	0.14	0.00	0.00	0.08	1.00	0.37	0.00
AF10-161-01	KP1-131CC	0.12	0.00	0.07	0.00	0.00	0.09	1.00	0.63	0.71
AF10-165-01	KP1-131DD	1.00	0.07	0.26	0.22	0.07	0.02	0.08	0.76	0.81
AF10-169-01	KP1-131DB	0.28	0.14	0.22	0.12	0.00	0.00	0.79	0.45	1.00
AF10-173-01	KP1-175A	0.25	0.00	0.23	0.06	0.00	0.00	0.74	0.33	1.00
AF10-177-01	KP1-175C	0.58	0.06	0.14	0.17	0.00	0.00	0.73	0.39	1.00
AF10-181-01	KP1-175D	0.00	0.06	0.00	0.31	1.00	0.00	0.65	0.42	0.78
AF11-001-01	KP2-009B	0.00	0.28	0.39	0.00	0.03	0.00	0.74	1.00	0.00
AF11-005-01	KP1-175F	0.00	0.00	0.16	0.09	0.02	0.00	0.65	0.52	1.00
AF11-009-01	KP1-175H	0.00	0.00	0.23	0.00	0.00	0.17	0.83	0.00	1.00
AF11-013-01	KP1-175K	0.00	0.00	0.95	0.43	0.28	0.00	1.00	0.39	0.00
AF11-017-01	KP1-175L	0.45	0.00	0.26	0.27	0.03	0.23	0.00	1.00	0.00

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada, continued.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF11-021-01	KP1-175M	0.00	0.00	0.32	0.00	0.05	0.30	0.00	1.00	0.00
AF11-025-01	KP1-131A	1.00	0.00	0.02	0.93	0.90	0.02	0.97	0.87	0.00
AF11-029-01	KP1-131B	0.14	0.00	0.18	0.00	0.00	0.00	0.66	0.73	1.00
AF11-033-01	KP1-131DA	0.77	0.00	0.00	0.09	0.02	0.00	0.70	0.80	1.00
AF11-037-01	KP2-025E	0.45	0.20	0.07	0.21	0.08	0.07	1.00	0.08	0.20
AF11-041-01	KP1-131C	1.00	0.19	0.07	0.10	0.00	0.00	0.94	1.00	0.00
AF11-045-01	KP1-131E	0.52	0.35	0.38	0.00	0.05	0.00	1.00	0.03	0.00
AF11-049-01	KP1-175E	0.03	0.01	0.08	0.32	1.00	0.15	0.75	0.00	0.57
AF11-053-01	KP1-175G	0.31	0.11	0.04	0.02	0.00	0.00	0.55	0.00	1.00
AF11-057-01	KP1-175J	0.23	0.11	0.07	0.00	0.00	0.03	0.28	0.00	1.00
AF11-061-01	KP2-009A	0.06	0.17	0.26	0.09	0.11	0.05	1.00	0.00	0.94
AF11-065-01	KP2-025A	0.00	0.00	0.25	0.15	0.06	0.00	1.00	0.00	0.00
AF11-069-01	KP2-025B	0.00	0.00	0.76	0.00	0.00	0.00	1.00	0.19	0.80
AF11-073-01	KP2-025C	0.00	0.00	0.15	0.00	0.01	0.30	0.40	0.00	1.00
AF11-077-01	KP2-025D	0.00	0.00	0.13	0.02	0.03	0.05	0.24	0.53	1.00
AF11-085-01	KP1-009C	0.00	0.00	0.31	0.19	0.04	0.00	0.96	0.98	1.00
AF11-089-01	KP2-017A	0.00	0.00	0.17	0.27	0.04	0.00	0.97	0.66	1.00
AF11-093-01	KP1-017C	1.00	0.00	0.16	0.05	0.08	0.01	0.74	0.91	0.83
AF11-097-01	KP1-017D	0.00	0.00	0.30	0.00	0.02	0.00	0.16	0.00	1.00
AF11-101-01	KP1-063J	0.00	0.04	0.92	0.00	0.25	0.00	1.00	0.00	0.00
AF11-105-01	KP1-017E	0.87	0.05	0.24	0.23	0.07	0.08	1.00	0.47	0.13
AF11-109-01	KP1-091A	0.00	0.22	0.06	0.03	0.02	0.02	0.76	0.72	1.00
AF11-113-01	KP2-033G	0.00	0.17	0.25	0.00	0.04	0.00	1.00	0.79	0.06
AF11-117-01	KP2-033H	0.00	0.00	0.21	0.14	0.00	0.00	1.00	0.00	0.17
AF11-125-01	KP1-063O	0.00	0.15	0.06	0.01	0.04	0.00	1.00	0.00	0.26

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada, continued.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF11-129-01	KP1-075A	0.00	0.00	0.05	0.19	0.01	0.00	0.90	0.91	1.00
AF11-133-01	KP1-075B	0.22	0.00	0.45	0.03	0.12	0.49	1.00	0.00	0.90
AF11-137-01	KP1-131F	0.00	0.00	0.22	0.00	0.01	0.17	0.96	0.58	1.00
AF11-141-01	KP1-131G	0.00	0.00	0.51	0.32	0.06	0.15	1.00	0.00	0.72
AF11-145-01	KP1-131H	0.90	0.00	0.40	0.11	0.04	0.01	1.00	0.00	0.57
AF11-149-01	KP1-131I	0.00	0.00	0.33	0.09	0.00	0.11	1.00	0.32	0.44
AF11-153-01	KP1-131J	0.00	0.00	0.20	0.00	0.00	0.08	1.00	0.76	0.14
AF11-157-01	KP1-131L	0.08	0.00	0.20	0.00	0.00	0.09	0.94	1.00	0.24
AF11-165-01	KP1-171A	0.00	0.10	0.34	0.16	0.00	0.00	0.67	0.29	1.00
AF11-169-01	KP1-179B	0.00	0.04	0.25	0.11	0.00	0.00	0.72	1.00	0.29
AF11-173-01	KP2-001C	0.00	0.17	0.10	0.14	0.00	0.19	1.00	0.82	0.00
AF11-177-01	KP2-001D	0.00	0.12	0.45	0.19	0.00	0.42	1.00	0.96	0.00
AF11-181-01	KP2-001E	0.00	0.14	0.27	0.01	0.00	0.00	1.00	0.90	0.16
AF12-001-01	KP2-001F	0.04	0.00	0.04	0.00	0.00	0.19	0.51	1.00	0.13
AF12-005-01	KP2-001G	0.00	0.11	0.06	0.01	0.00	0.00	0.32	1.00	0.76
AF12-009-01	KP2-029C	0.00	0.00	0.31	0.10	0.00	0.00	0.22	1.00	0.75
AF12-013-01	KP2-029D	0.38	0.00	0.19	0.00	0.00	0.07	0.00	1.00	0.61
AF9-041-01	KP1-045C	0.19	0.12	0.57	0.09	0.00	0.00	0.26	0.00	1.00
AF9-045-01	KP1-045I	0.34	0.00	0.69	0.01	0.01	0.00	0.37	0.00	1.00
AF9-049-01	KP2-033B	0.26	0.02	0.00	0.42	0.12	0.16	1.00	0.90	0.65
AF9-053-01	KP1-045J	0.08	0.00	0.33	0.00	0.06	0.00	0.61	0.31	1.00
AF9-057-01	KP2-033E	0.17	0.19	0.00	0.04	0.01	0.22	0.29	0.02	1.00
AF9-061-01	KP1-045A	0.00	0.00	0.00	0.30	0.19	0.62	1.00	0.00	0.00
AF9-065-01	KP2-033A	0.00	0.00	0.10	0.02	1.00	0.17	0.00	0.11	0.00
AF9-069-01	KP2-033D	0.00	0.00	0.45	0.53	1.00	0.39	0.00	0.00	0.00

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada, continued.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF9-073-01	KP1-119C	0.00	0.00	0.16	0.02	0.02	0.13	1.00	0.13	0.53
AF9-077-01	KP1-115A	0.00	0.00	0.18	0.18	0.01	0.00	0.84	1.00	0.73
AF9-081-01	KP1-081A	0.00	0.00	0.12	0.05	0.01	0.00	0.77	1.00	0.68
AF9-085-01	KP2-029B	0.00	0.00	0.21	0.00	0.00	0.00	0.18	0.00	1.00
AF9-089-01	KP1-045G	0.05	0.00	0.00	0.00	0.00	0.00	0.67	1.00	0.60
AF9-093-01	KP2-033F	0.00	0.00	0.00	0.00	0.00	0.00	0.29	1.00	0.44
AF9-097-01	KP1-179C	0.00	0.00	0.08	0.00	0.00	0.00	0.74	0.76	1.00
AF9-101-01	KP1-119E	0.65	0.00	0.09	0.06	0.00	0.00	0.94	0.23	1.00
AF9-105-01	KP1-119A	0.00	0.00	0.29	0.00	0.01	0.00	0.43	0.00	1.00
AF9-109-01	KP2-033C	0.00	0.03	0.18	0.01	0.03	0.00	0.67	0.90	1.00
AF9-113-01	KP1-119B	0.00	0.01	0.17	0.01	0.13	0.00	0.91	1.00	0.94
AF9-117-01	KP1-179A	0.08	0.08	0.05	0.01	0.04	0.00	0.91	0.00	1.00
AF9-121-01	KP1-021A	0.00	0.00	0.01	0.03	0.03	0.00	0.00	1.00	0.49
AF9-125-01	KP1-025B	0.00	0.00	0.15	0.09	0.08	0.00	0.87	0.00	1.00
AF9-129-01	KP1-025C	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.95	1.00
AF9-133-01	KP1-063A	0.00	0.00	0.28	0.05	0.08	0.00	0.54	0.67	1.00
AF9-137-01	KP1-063B	0.00	0.00	0.15	0.02	0.04	0.00	0.83	0.99	1.00
AF9-141-01	KP1-063C	0.00	0.00	0.13	0.03	0.04	0.04	0.04	1.00	0.76
AF9-145-01	KP1-069A	0.00	0.00	0.11	0.00	0.00	0.00	0.42	0.00	1.00
AF9-149-01	KP1-069B	0.00	0.00	0.04	0.00	0.01	0.03	1.00	0.47	0.92
AF9-153-01	KP1-069C	0.02	0.01	0.08	0.02	0.03	0.00	0.18	0.00	1.00
AF9-157-01	KP1-069D	0.31	0.00	0.11	0.00	0.00	0.00	0.00	0.00	1.00
AF9-161-01	KP1-069E	0.34	0.00	0.16	0.05	0.00	0.00	0.77	0.61	1.00
AF9-165-01	KP1-099A	0.48	0.00	0.23	0.04	0.00	0.00	0.59	1.00	0.87
AF9-169-01	KP1-143A	0.00	0.00	0.16	0.04	0.00	0.00	1.00	0.61	0.16

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Table A6.1. Normalized antimicrobial activity of extracts obtained from endophytes of marine macroalgae of the Bay of Fundy, New Brunswick, Canada, continued.

Extract	Endophyte	SA	EF	PA	EC	CA	SC	MT	MA	MS
AF9-173-01	KP1-143B	0.00	0.00	0.10	0.06	0.00	0.00	0.97	1.00	0.57
AF9-177-01	KP1-143C	0.13	0.00	0.07	0.02	0.00	0.00	1.00	0.00	0.54
AF9-181-01	KP1-143D	0.00	0.00	0.11	0.12	0.04	0.00	0.88	1.00	0.54
KP2-037-01	KP1-017A	0.00	0.32	0.26	0.02	0.00	0.00	1.00	0.00	0.41
KP2-041-01	KP1-123C	0.13	0.62	0.49	0.00	0.00	0.07	1.00	0.00	0.86
KP2-045-01	KP1-045D	0.00	0.13	0.12	0.04	0.01	0.18	1.00	0.00	0.00
KP2-049-01	KP1-131W	0.00	0.08	0.09	0.09	0.00	0.04	1.00	0.05	0.09
KP2-057-01	KP1-009B	1.00	0.00	0.00	0.22	0.00	0.00	0.98	0.24	0.41
KP2-061-01	KP1-013B	0.00	0.00	0.09	0.10	0.07	0.22	1.00	0.13	0.02
KP2-065-01	KP1-131K	0.00	0.00	0.52	0.00	0.14	0.86	1.00	0.00	0.95
KP2-069-01	KP1-089A	0.00	0.00	0.11	0.02	0.00	0.06	1.00	0.00	0.52
KP2-073-01	KP1-135C	0.64	0.00	0.00	0.00	0.05	0.12	1.00	0.78	0.00
KP2-077-01	KP1-045B	0.00	0.00	0.05	0.07	0.05	0.15	1.00	0.00	0.00
KP2-081-01	KP1-135D	0.00	0.00	0.42	0.03	0.00	0.12	1.00	0.00	0.12
KP2-085-01	KP1-045K	0.00	0.00	0.21	0.11	0.00	0.00	1.00	0.00	0.15
KP2-089-01	KP1-119D	0.00	0.00	0.12	0.07	0.00	0.00	1.00	0.03	0.17
KP2-093-01	KP1-123B	1.00	0.02	0.01	0.01	1.00	0.00	0.92	0.06	0.02
KP2-097-01	KP1-135B	0.00	0.00	0.00	0.09	0.24	0.00	1.00	0.00	0.09

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Appendix 7: Antimicrobial standard raw data

Table A7.1. Antimicrobial activity of antimicrobial standards before normalization[a].

Antimicrobial	SA	EF	PA	EC	CA	SC	MT	MA	MS
Actinomycin D	100.1	99.8	17.0	0.0	0.0	1.7	95.3	71.0	0.0
Amoxicillin	100.1	27.3	12.7	1.1	1.3	0.0	8.4	0.0	2.5
Amphotericin B	1.9	0.0	0.0	0.0	100.1	0.0	23.2	1.5	5.4
Chloramphenicol	14.9	6.2	16.4	50.0	0.0	0.0	54.0	51.2	6.5
Ciprofloxacin	99.8	8.8	100.1	101.6	4.0	0.0	93.9	76.2	54.3
Erythromycin	99.9	0.0	39.3	5.4	0.0	1.2	71.9	76.8	0.0
Ethambutol	0.0	99.9	0.0	0.0	1.6	0.0	0.0	0.0	0.0
Gentamicin	96.4	0.0	1.2	101.7	3.3	0.0	57.6	49.4	0.0
Isoniazid	0.0	0.0	0.0	0.0	5.9	0.0	52.0	0.0	0.0
Kanamycin	21.8	0.0	3.0	32.7	0.0	1.6	66.5	56.2	87.4
Miconazole	12.2	0.0	17.8	0.0	30.2	78.4	68.8	21.1	5.8
Nystatin	0.0	0.0	0.0	0.0	75.2	0.0	19.8	0.0	2.9
Rifampin	99.9	13.9	26.9	9.8	7.0	1.6	98.0	86.4	0.0
Streptomycin	1.7	0.0	7.4	27.2	3.5	2.3	81.9	79.1	87.6
Sulfamethazine	0.0	4.0	0.0	0.0	0.0	8.0	11.6	1.8	3.5
Tetracycline	99.9	99.8	27.9	101.6	4.7	5.3	80.0	49.4	84.9
Vancomycin	100.2	0.0	21.6	0.0	0.0	1.0	79.4	54.5	50.6

[a] Mean percentage inhibition (n=3); All negative values removed and represented as zero.

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Appendix 8: Antimicrobial standard normalized data

Table A8.1. Normalized antimicrobial activity of antimicrobial standards.

Antimicrobial	SA	EF	PA	EC	CA	SC	MT	MA	MS
Actinomycin D	1.00	1.00	0.17	0.00	0.00	0.02	0.95	0.71	0.00
Amoxicillin	1.00	0.27	0.13	0.01	0.01	0.00	0.08	0.00	0.03
Amphotericin B	0.02	0.00	0.00	0.00	1.00	0.00	0.23	0.02	0.05
Chloramphenicol	0.28	0.11	0.30	0.93	0.00	0.00	1.00	0.95	0.12
Ciprofloxacin	0.98	0.09	0.98	1.00	0.04	0.00	0.92	0.75	0.53
Erythromycin	1.00	0.00	0.39	0.05	0.00	0.01	0.72	0.77	0.00
Ethambutol	0.00	1.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Gentamicin	0.95	0.00	0.01	1.00	0.03	0.00	0.57	0.49	0.00
Isoniazid	0.00	0.00	0.00	0.00	0.11	0.00	1.00	0.00	0.00
Kanamycin	0.25	0.00	0.03	0.37	0.00	0.02	0.76	0.64	1.00
Miconazole	0.16	0.00	0.23	0.00	0.39	1.00	0.88	0.27	0.07
Nystatin	0.00	0.00	0.00	0.00	1.00	0.00	0.26	0.00	0.04
Rifampin	1.00	0.14	0.27	0.10	0.07	0.02	0.98	0.86	0.00
Streptomycin	0.02	0.00	0.08	0.31	0.04	0.03	0.94	0.90	1.00
Sulfamethazine	0.00	0.35	0.00	0.00	0.00	0.69	1.00	0.15	0.30
Tetracycline	0.98	0.98	0.27	1.00	0.05	0.05	0.79	0.49	0.84
Vancomycin	1.00	0.00	0.22	0.00	0.00	0.01	0.79	0.54	0.50

SA: *Staphylococcus aureus*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; CA: *Candida albicans*; SC: *Saccharomyces cerevisiae*; MT: *Mycobacterium tuberculosis*; MA; *Mycobacterium avium*; MS: *Mycobacterium smegmatis*

Appendix 9: Principal component analysis plots

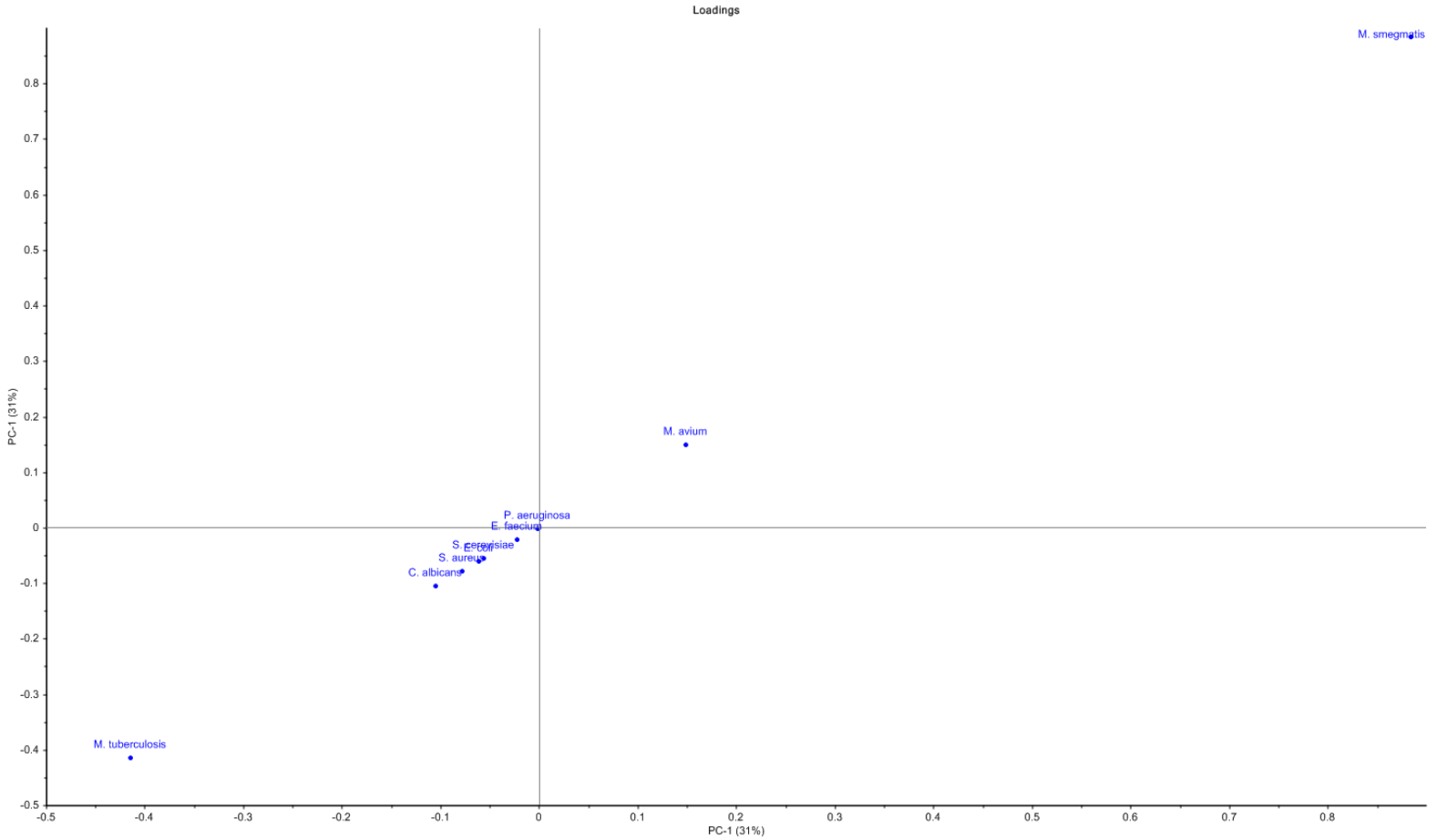


Figure A9.1. Loadings plot for principal component 1 plotted against principal component 1 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

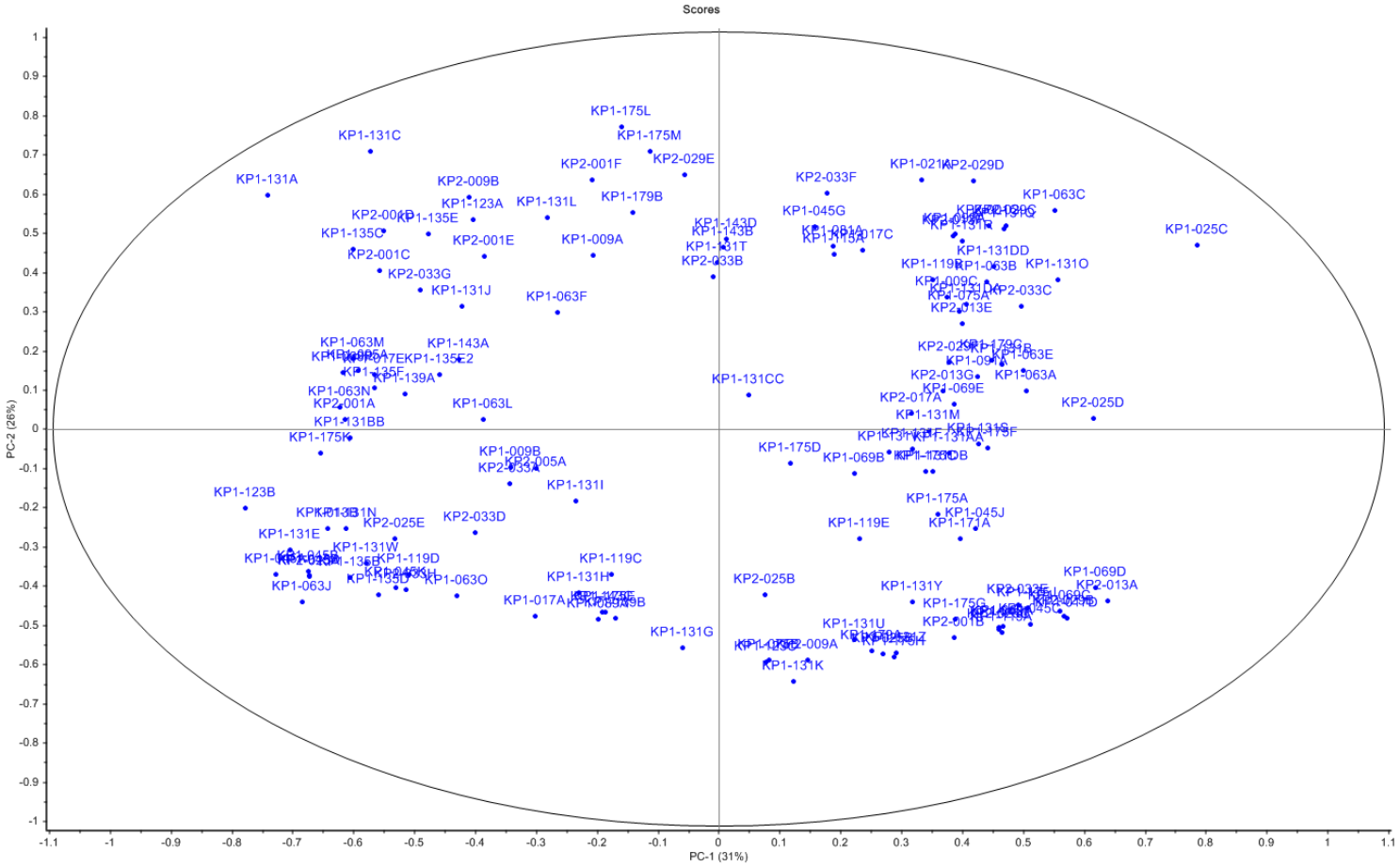


Figure A9.2. Principal component 1 plotted against principal component 2 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

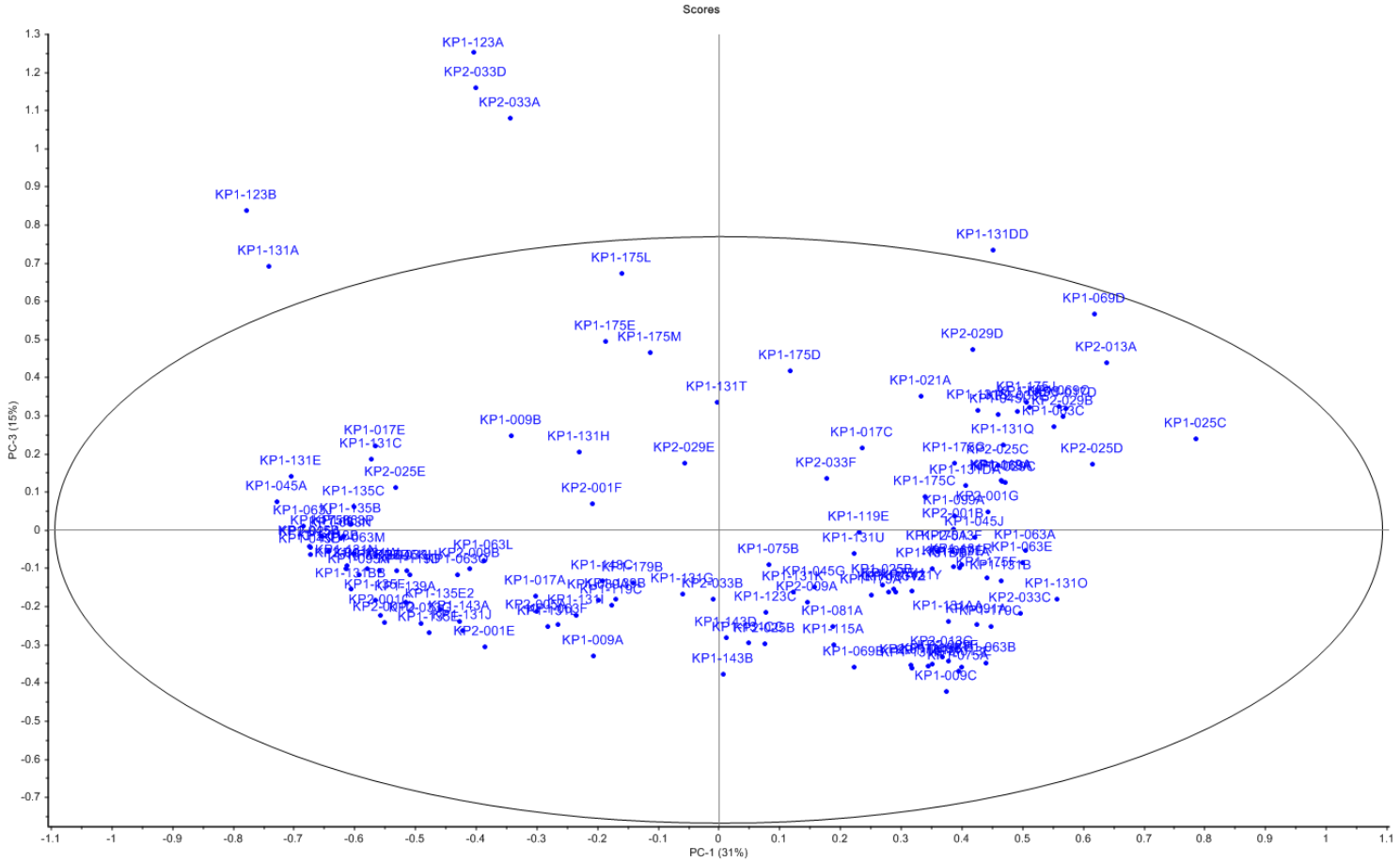


Figure A9.3. Principal component 1 plotted against principal component 3 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

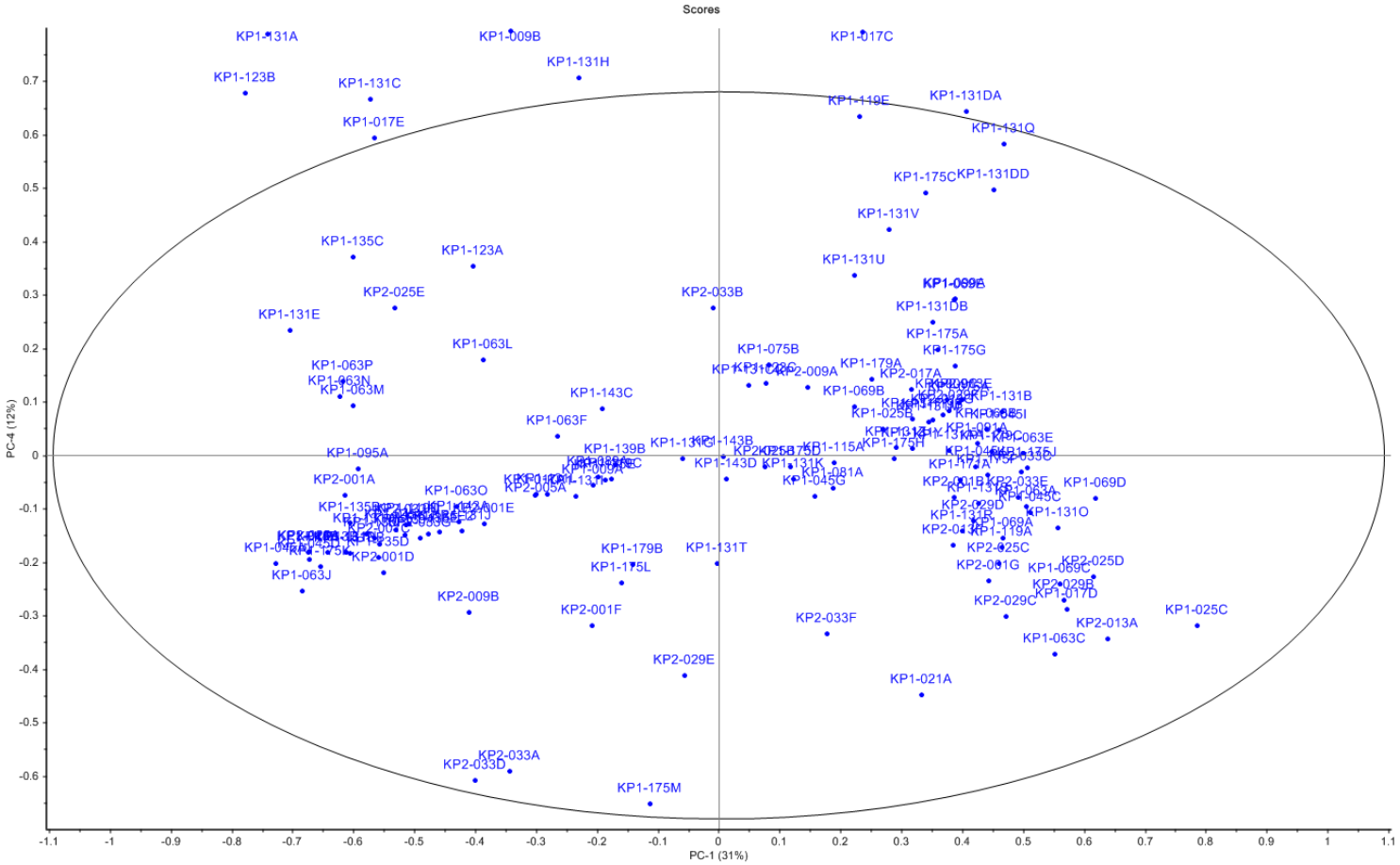


Figure A9.4. Principal component 1 plotted against principal component 4 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

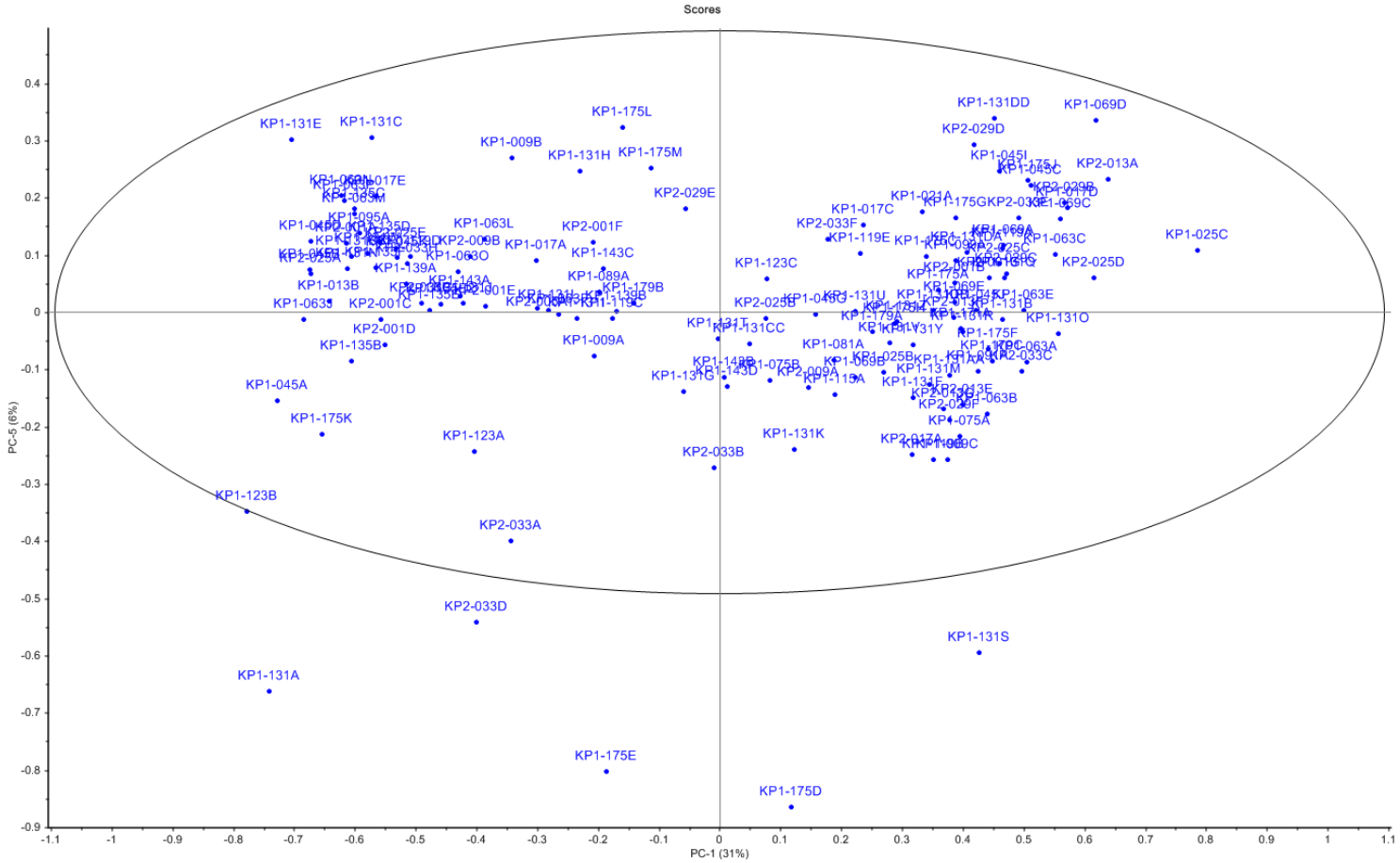


Figure A9.5. Principal component 1 plotted against principal component 5 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

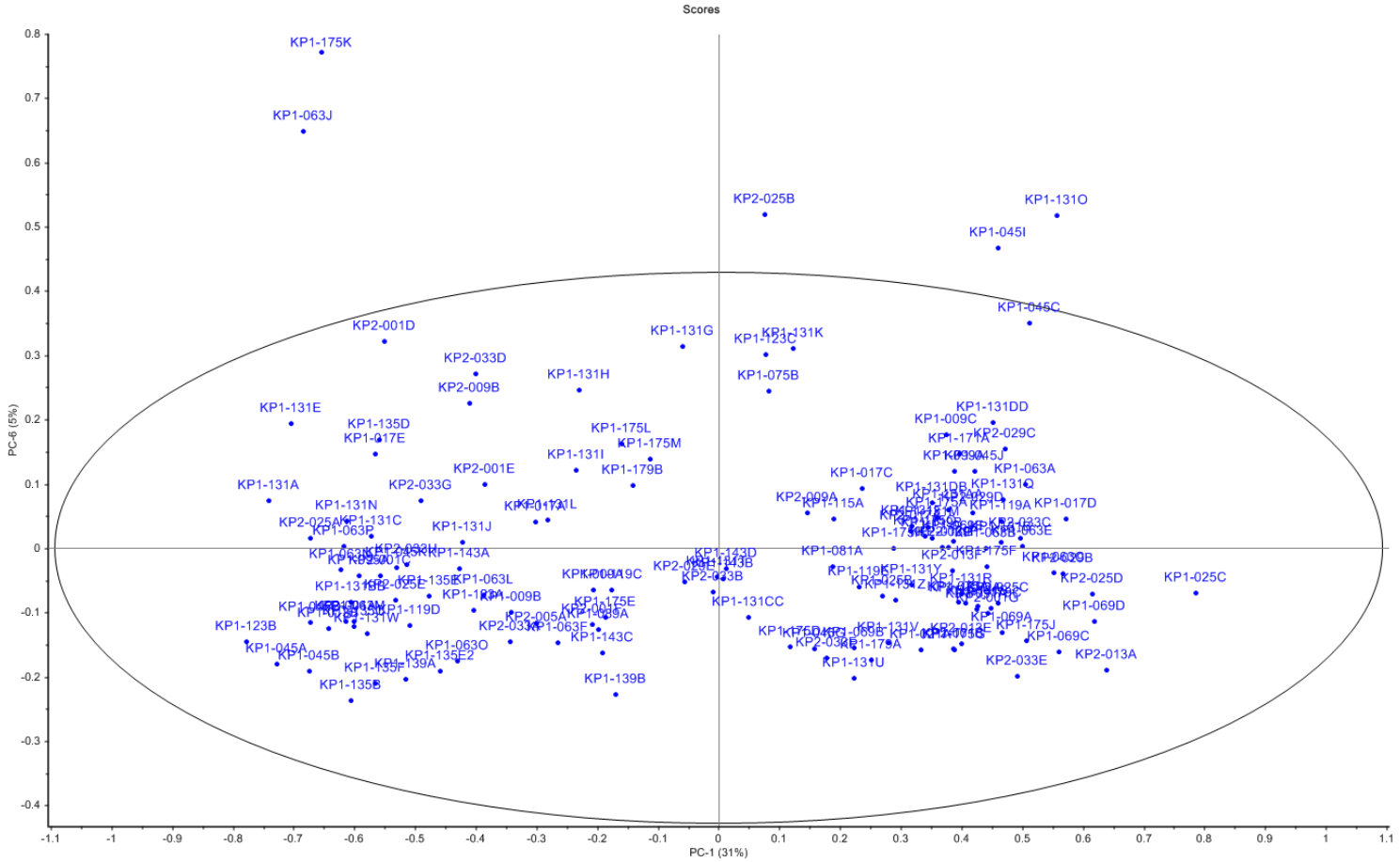


Figure A9.6. Principal component 1 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

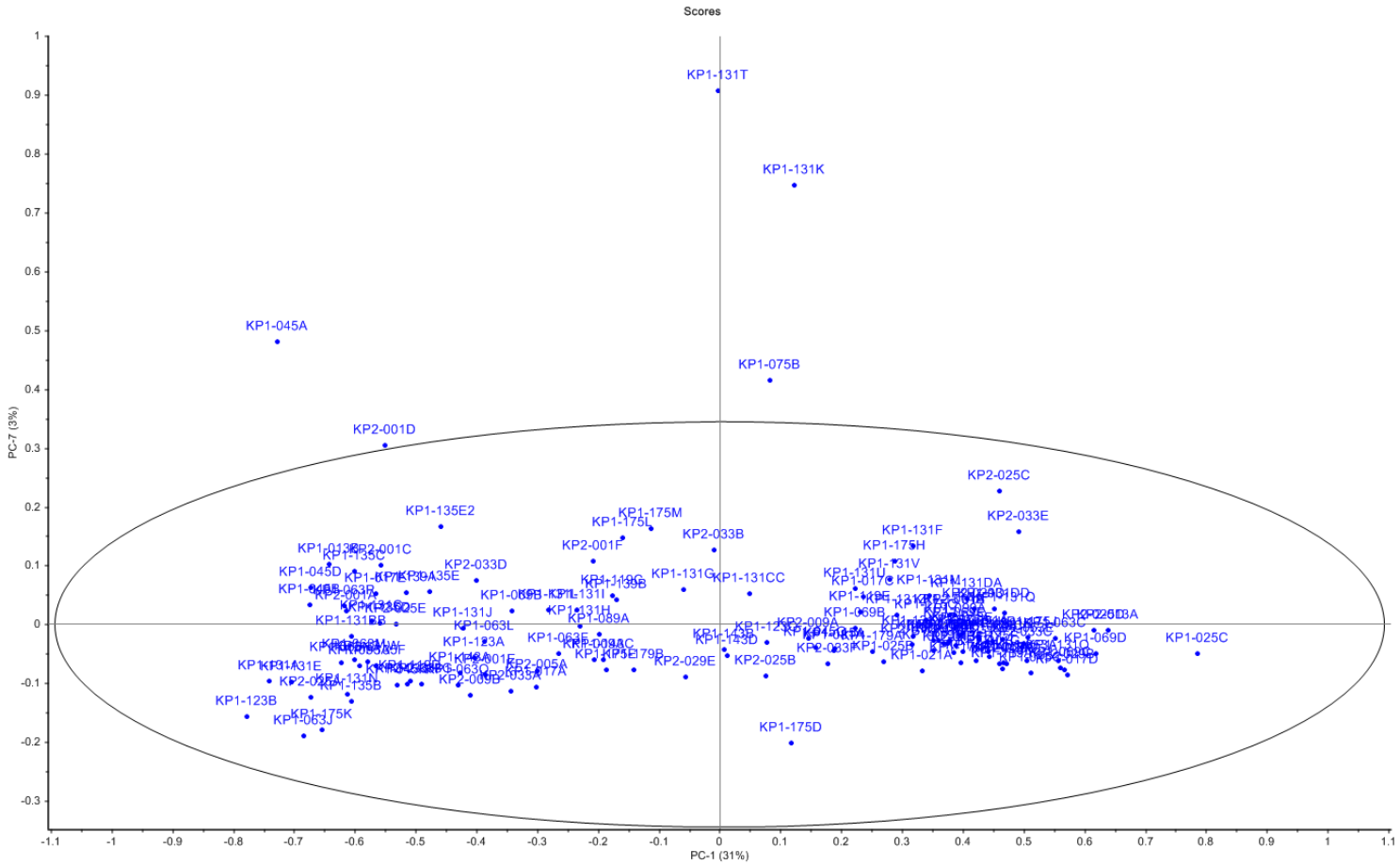


Figure A9.7. Principal component 1 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

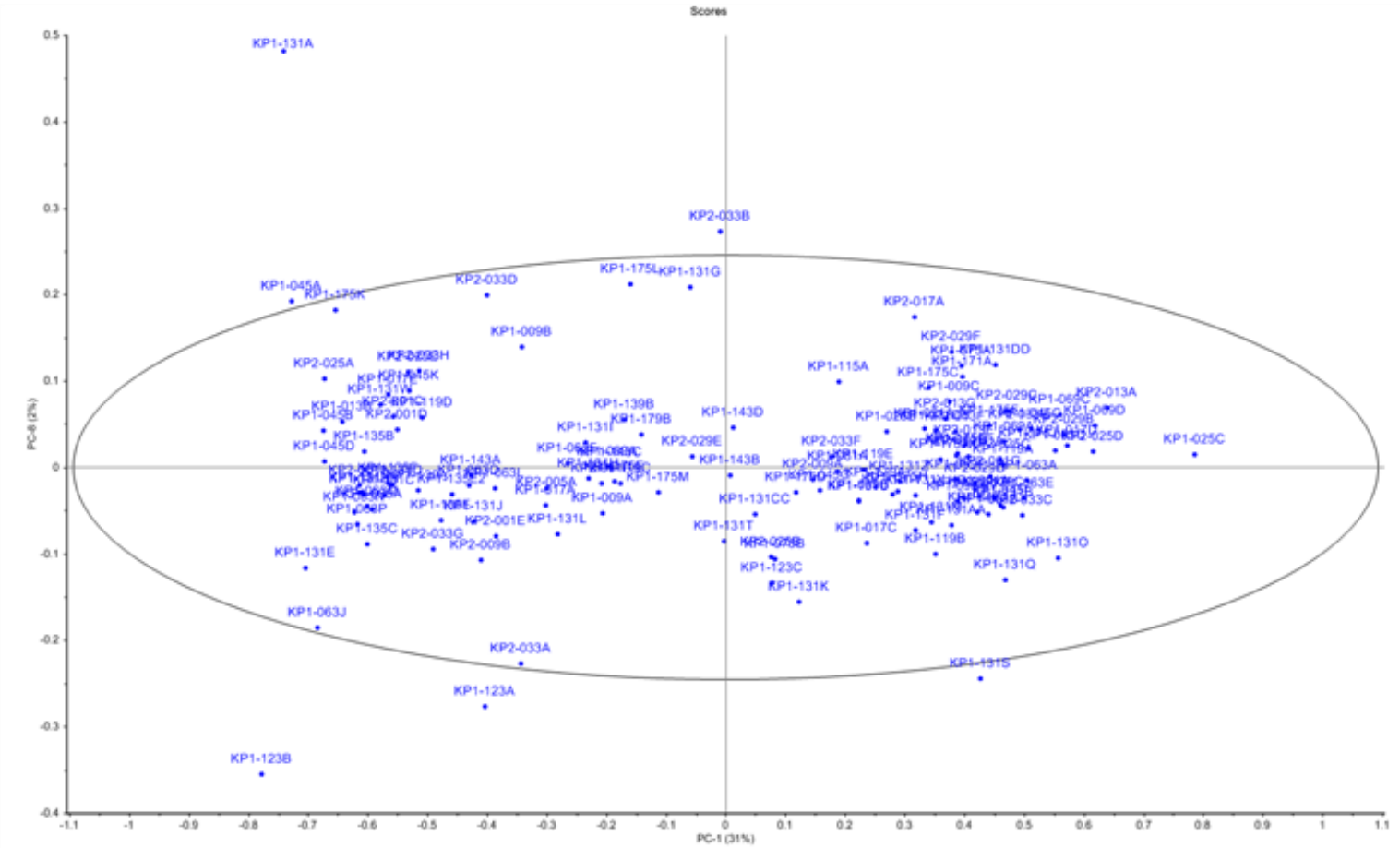


Figure A9.8. Principal component 1 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

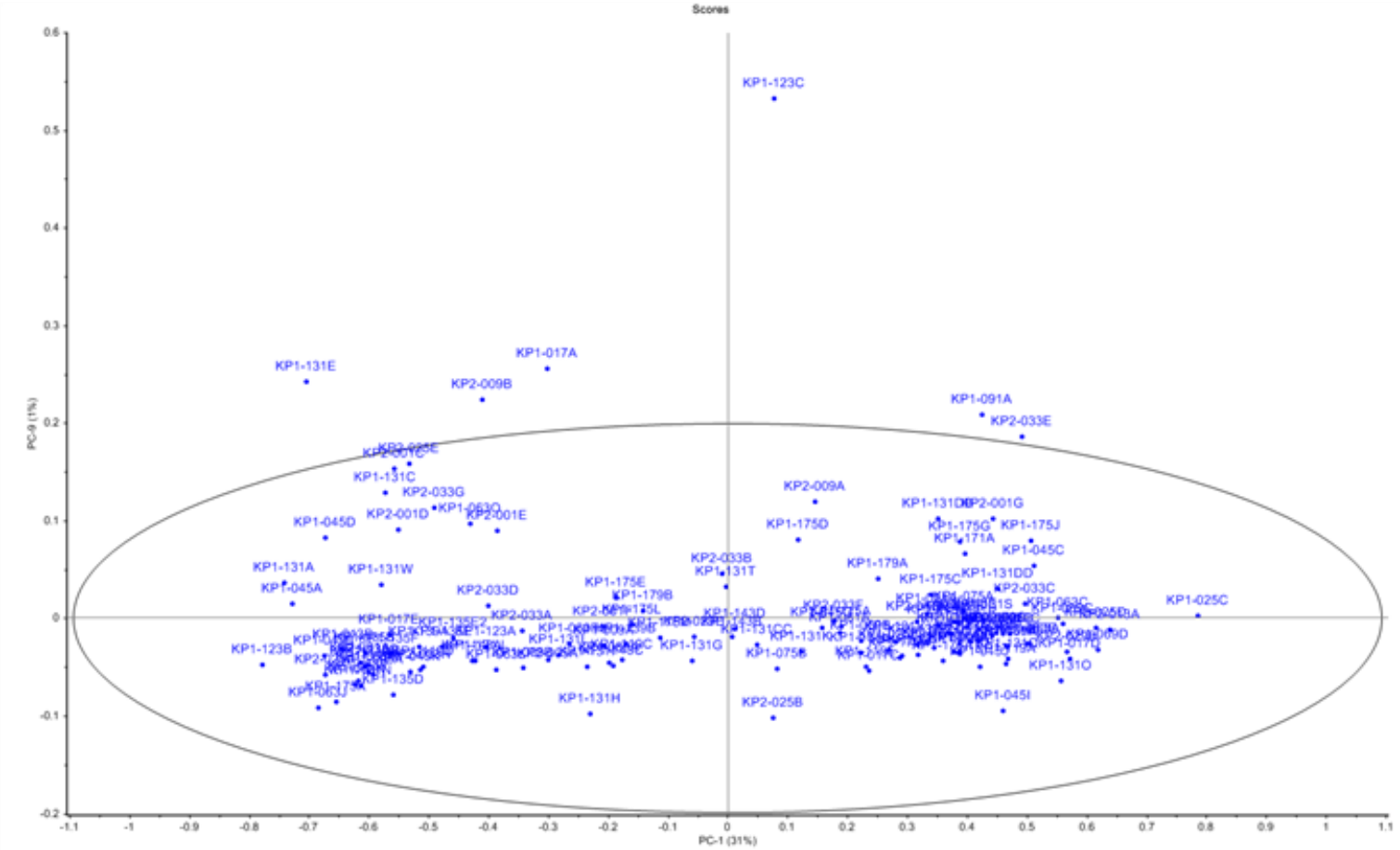


Figure A9.9. Principal component 1 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

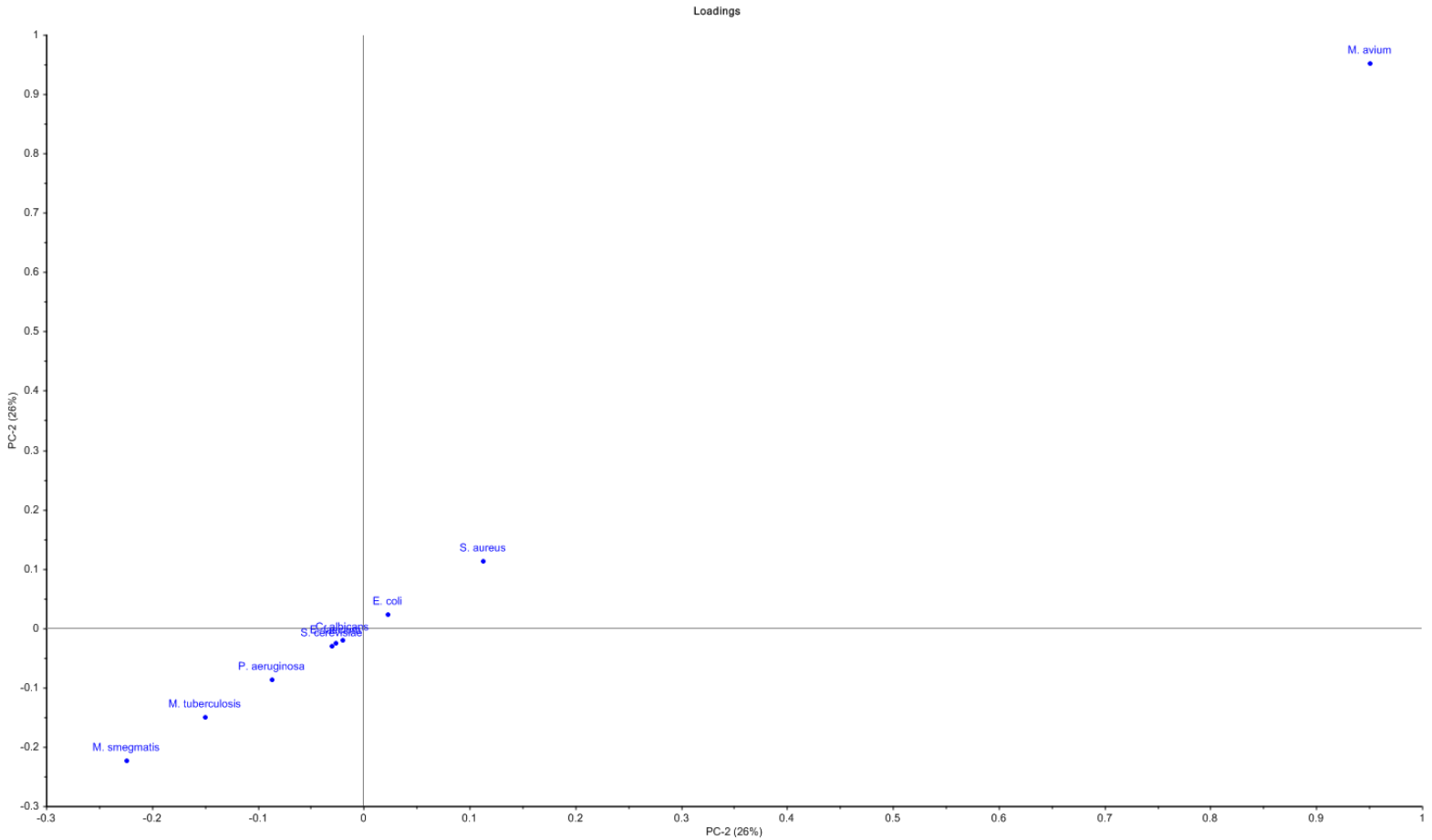


Figure A9.10. Loadings plot for principal component 2 plotted against principal component 2 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

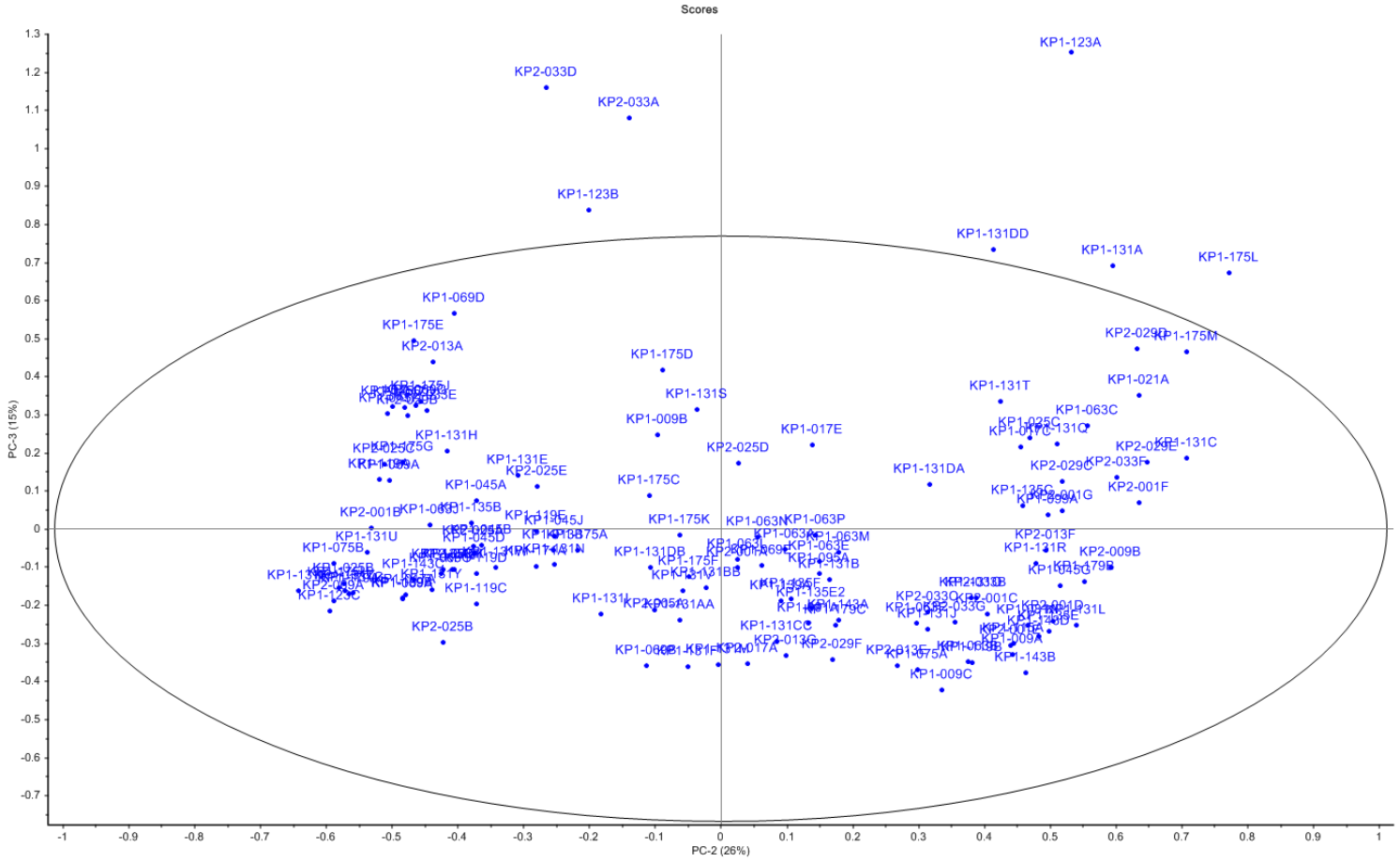


Figure A9.11. Principal component 2 plotted against principal component 3 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

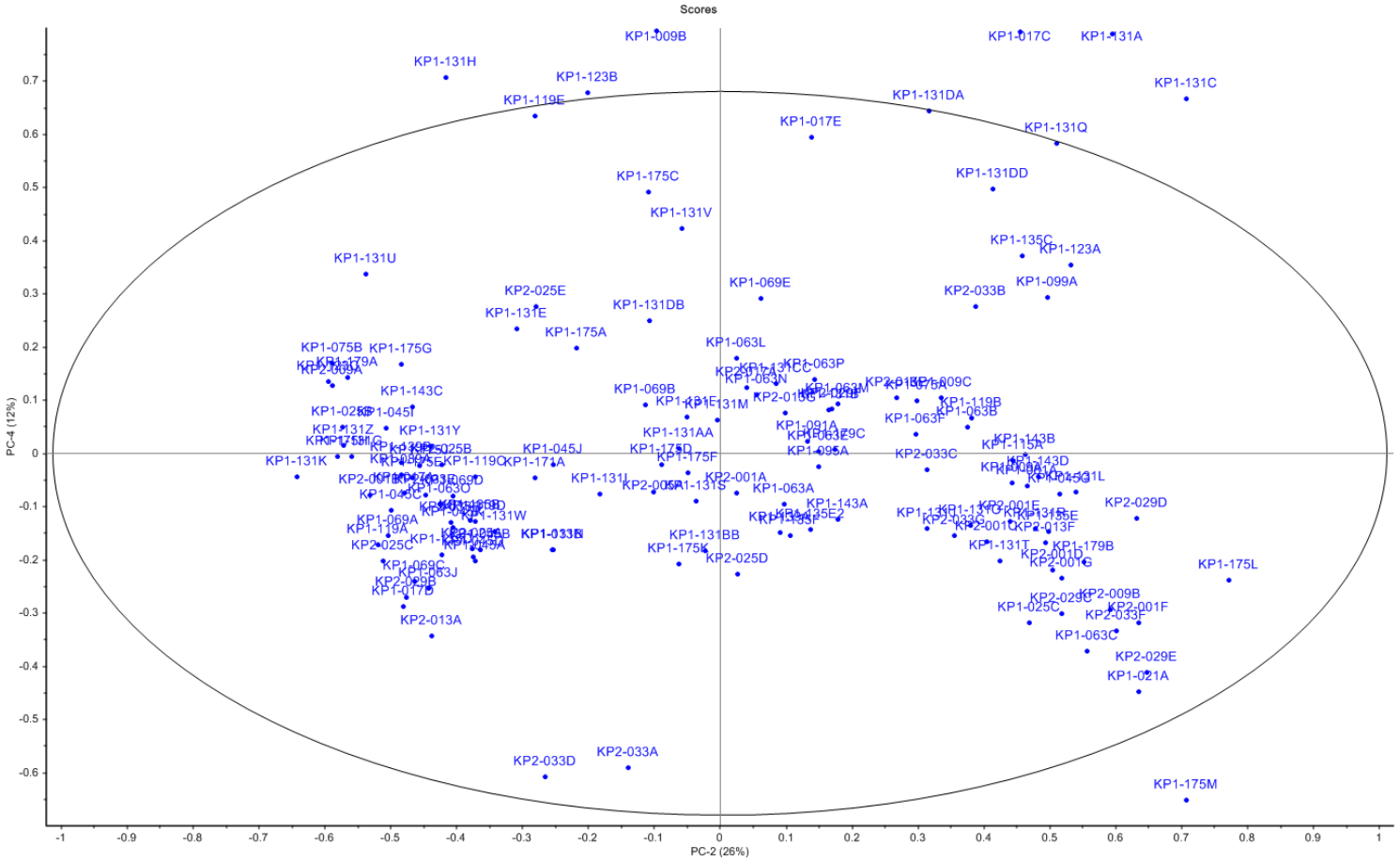


Figure A9.12. Principal component 2 plotted against principal component 4 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

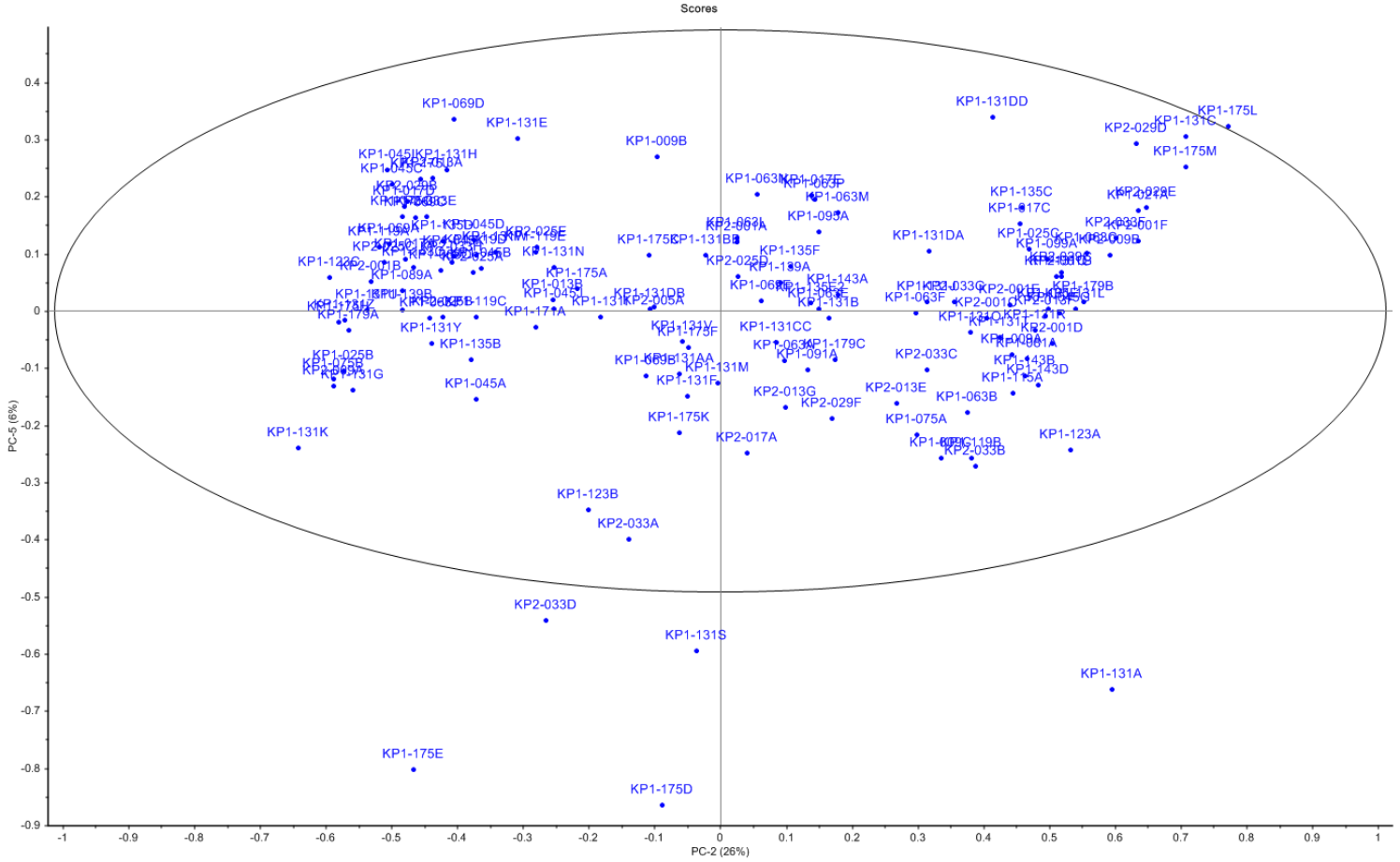


Figure A9.13. Principal component 2 plotted against principal component 5 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

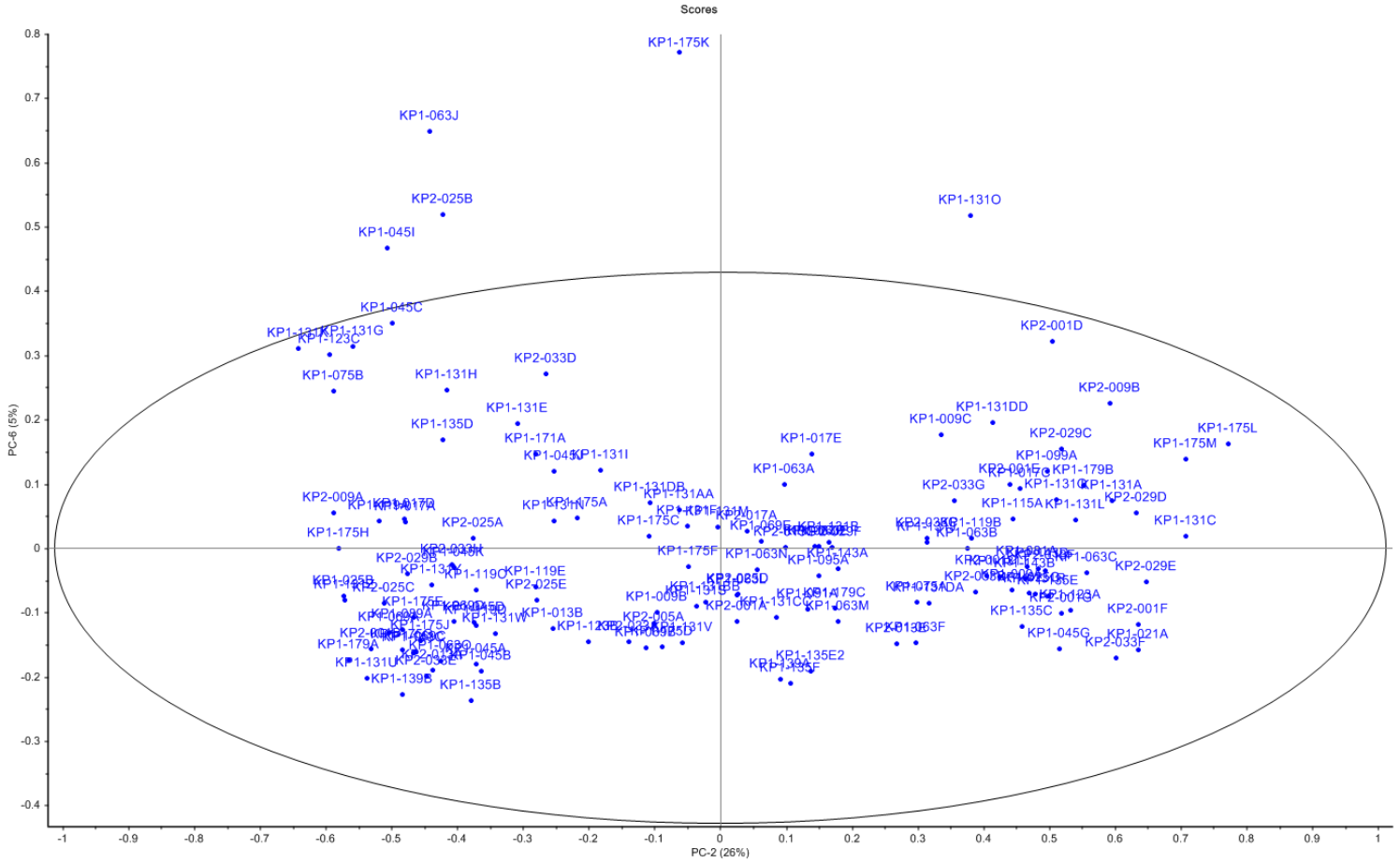


Figure A9.14. Principal component 2 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

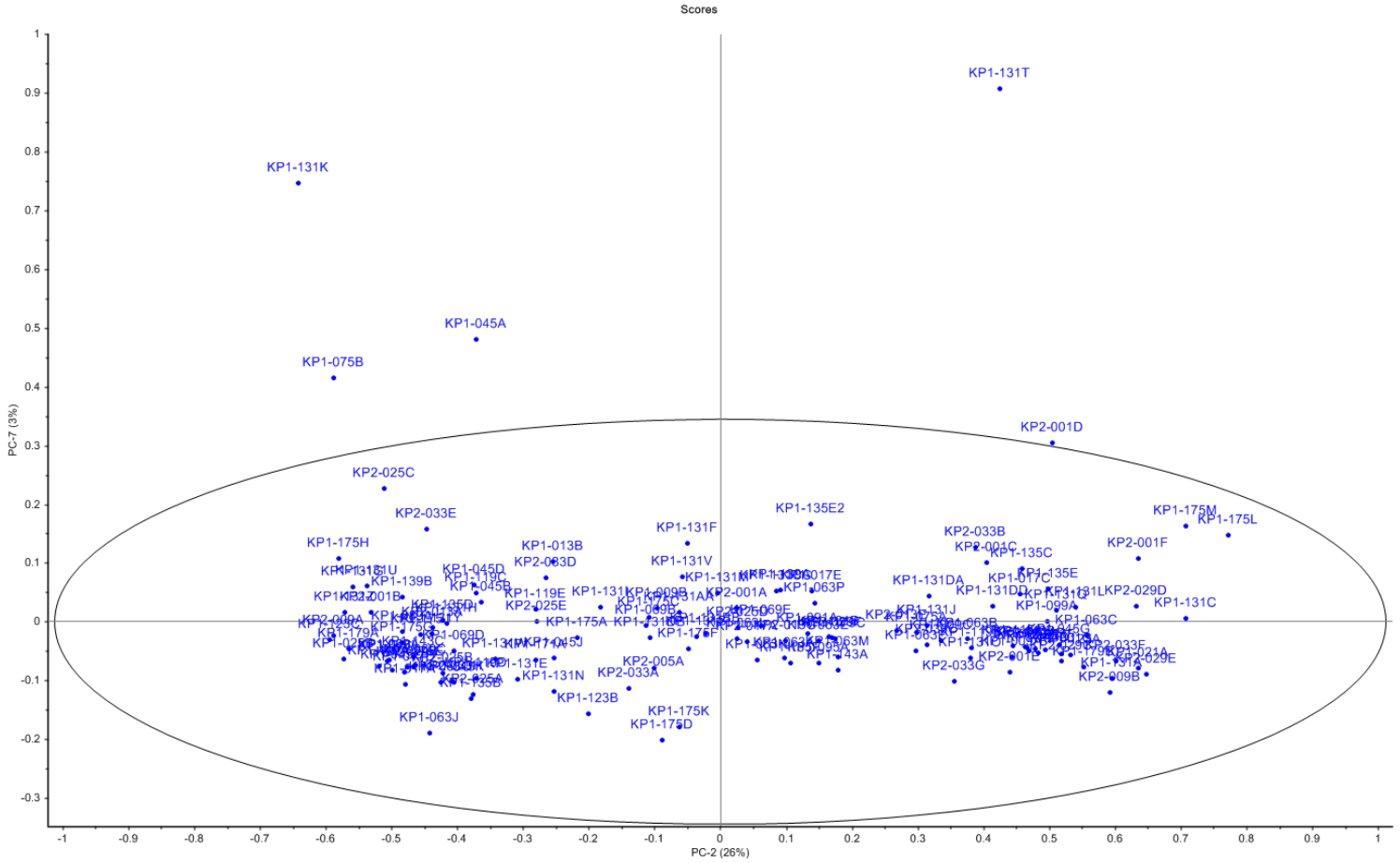


Figure A9.15. Principal component 2 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

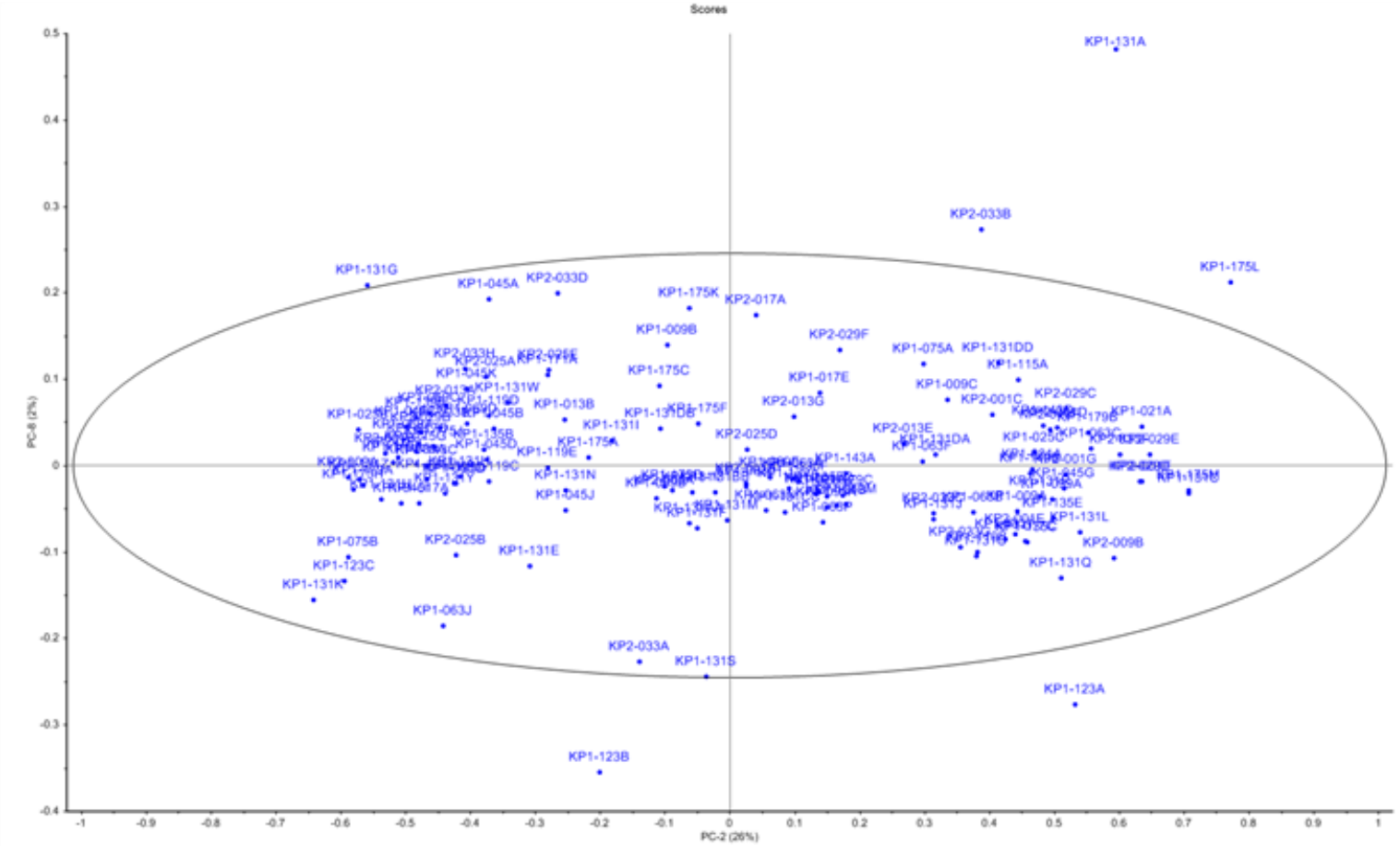


Figure A9.16. Principal component 2 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

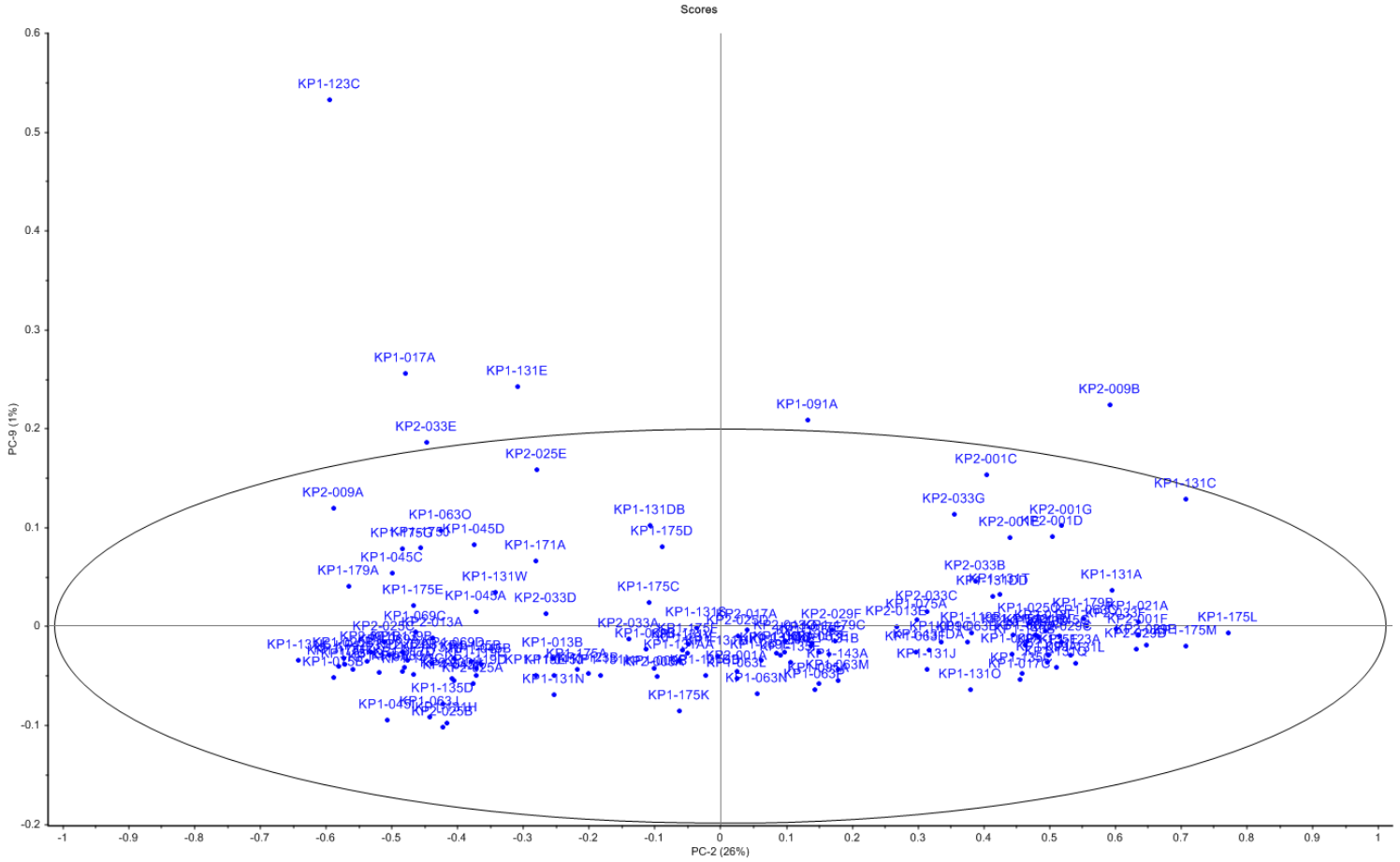


Figure A9.17. Principal component 2 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

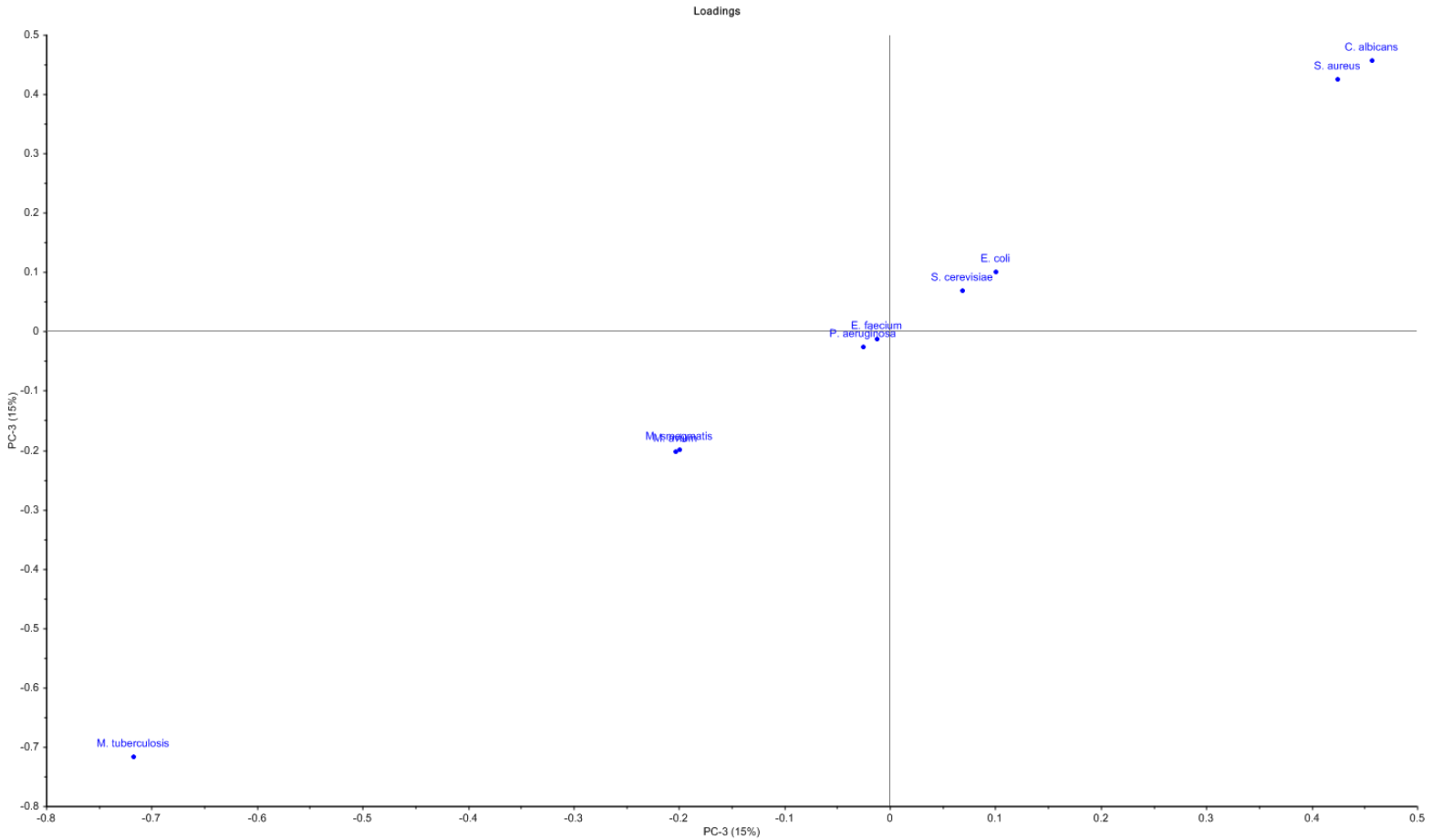


Figure A9.18. Loadings plot for principal component 3 plotted against principal component 3 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

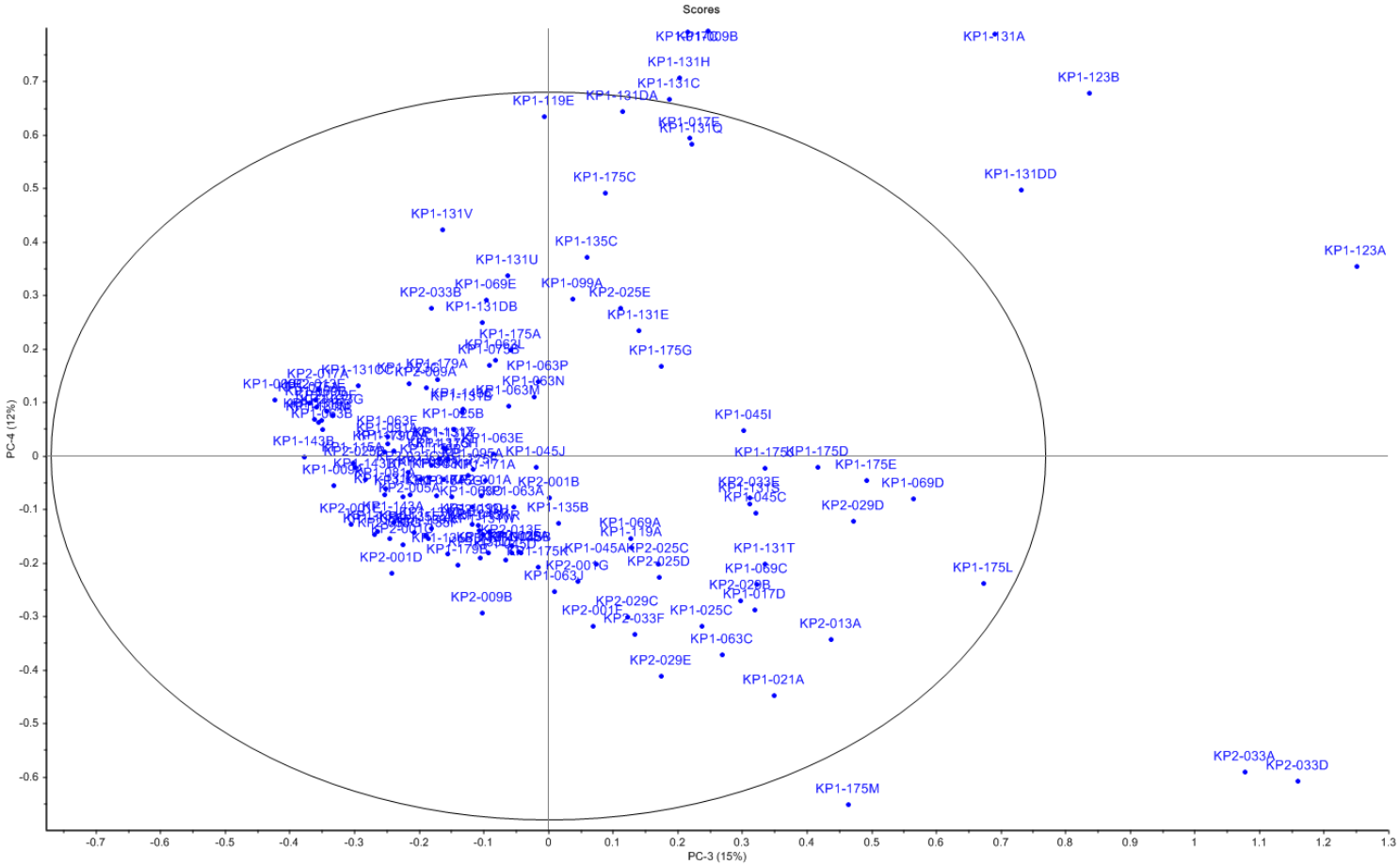


Figure A9.19. Principal component 3 plotted against principal component 4 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

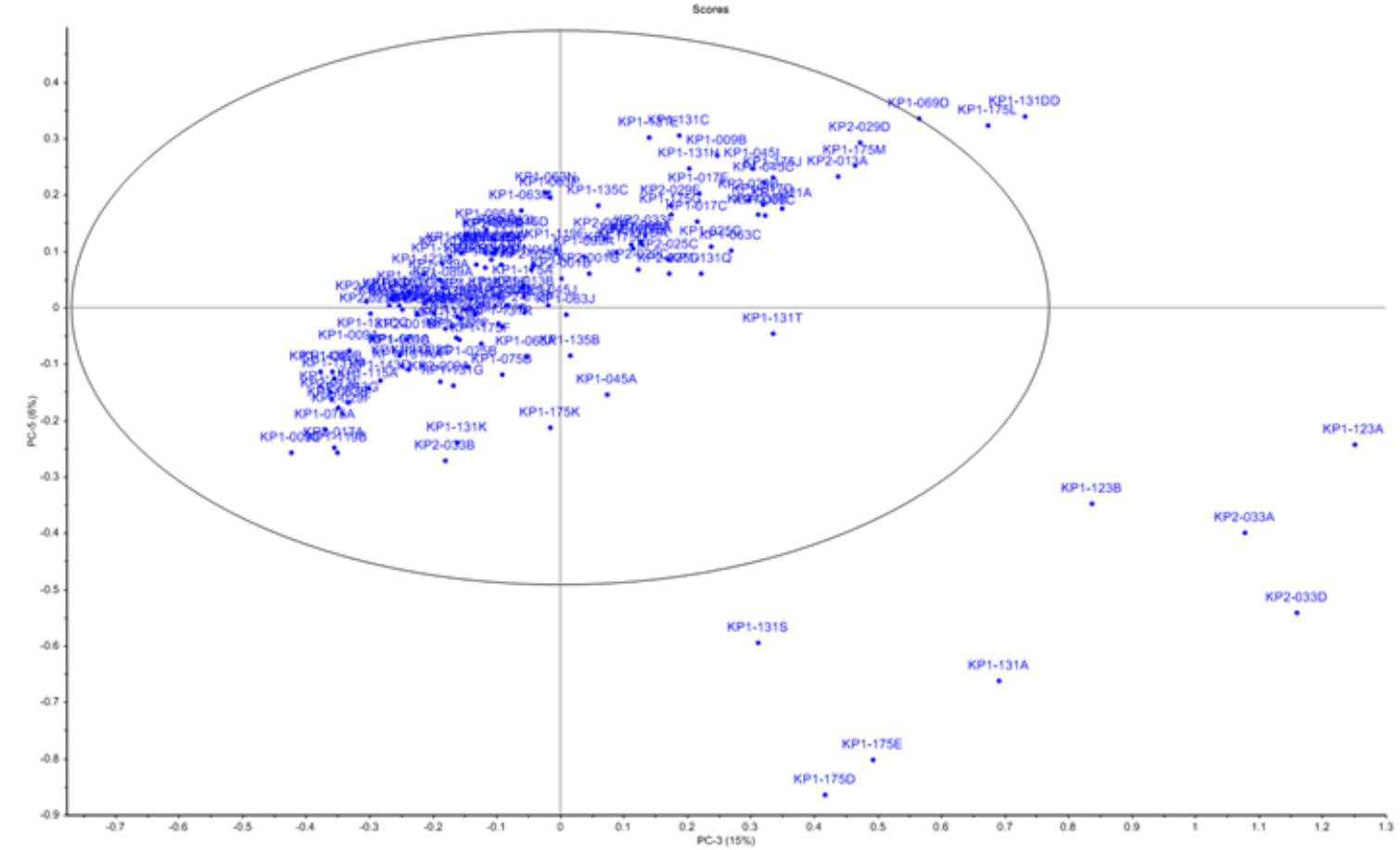


Figure A9.20. Principal component 3 plotted against principal component 5 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

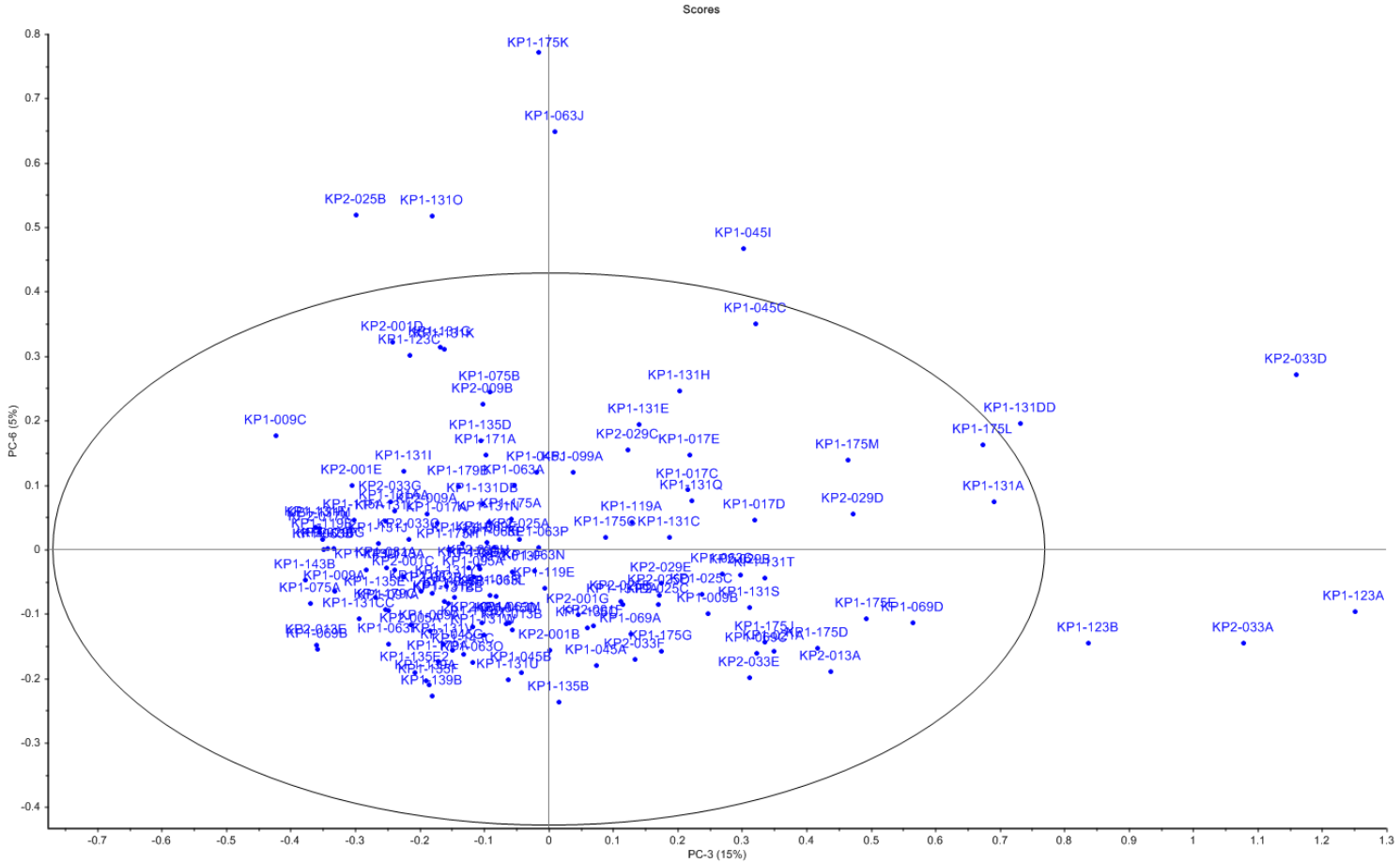


Figure A9.21. Principal component 3 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

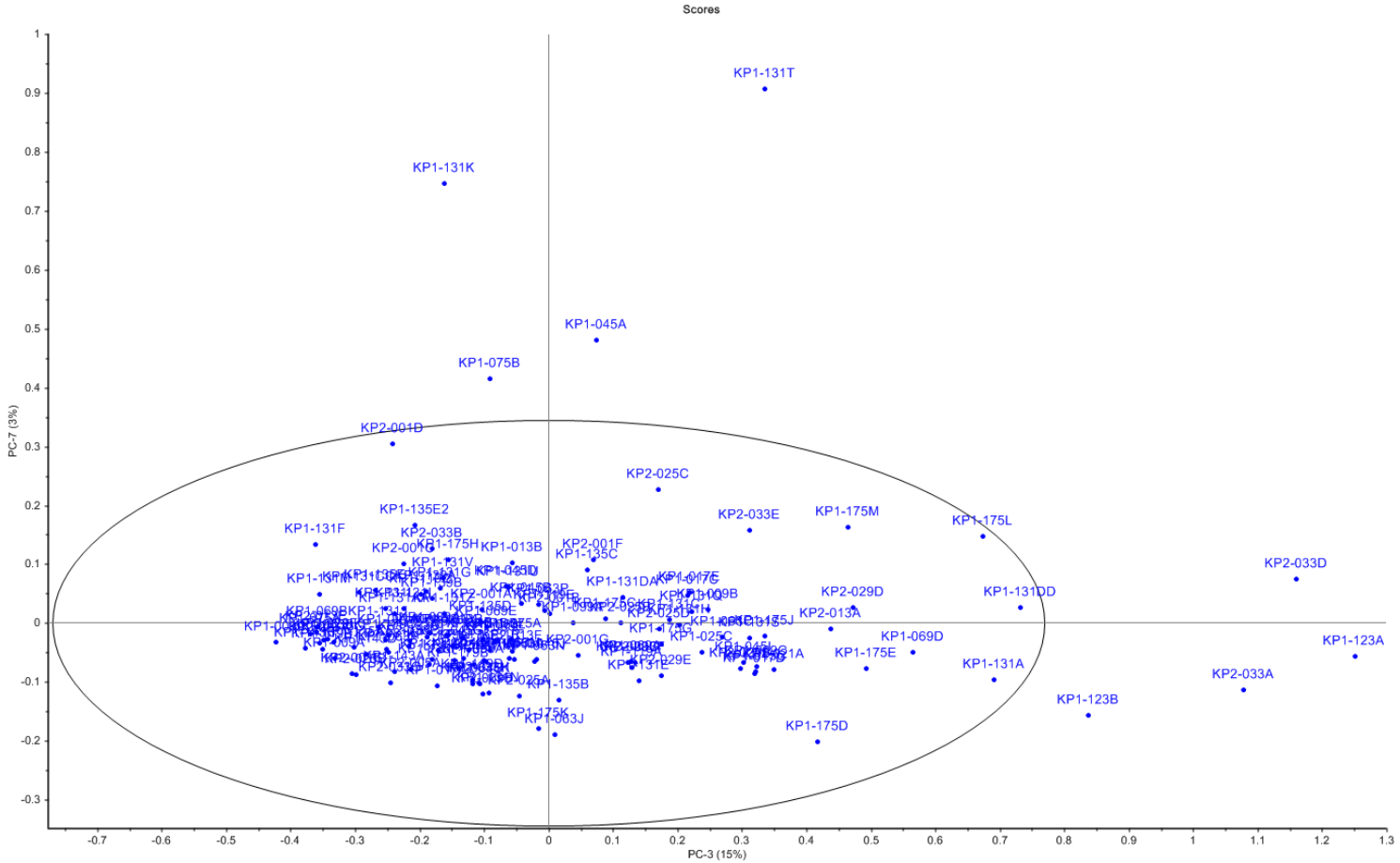


Figure A9.22. Principal component 3 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

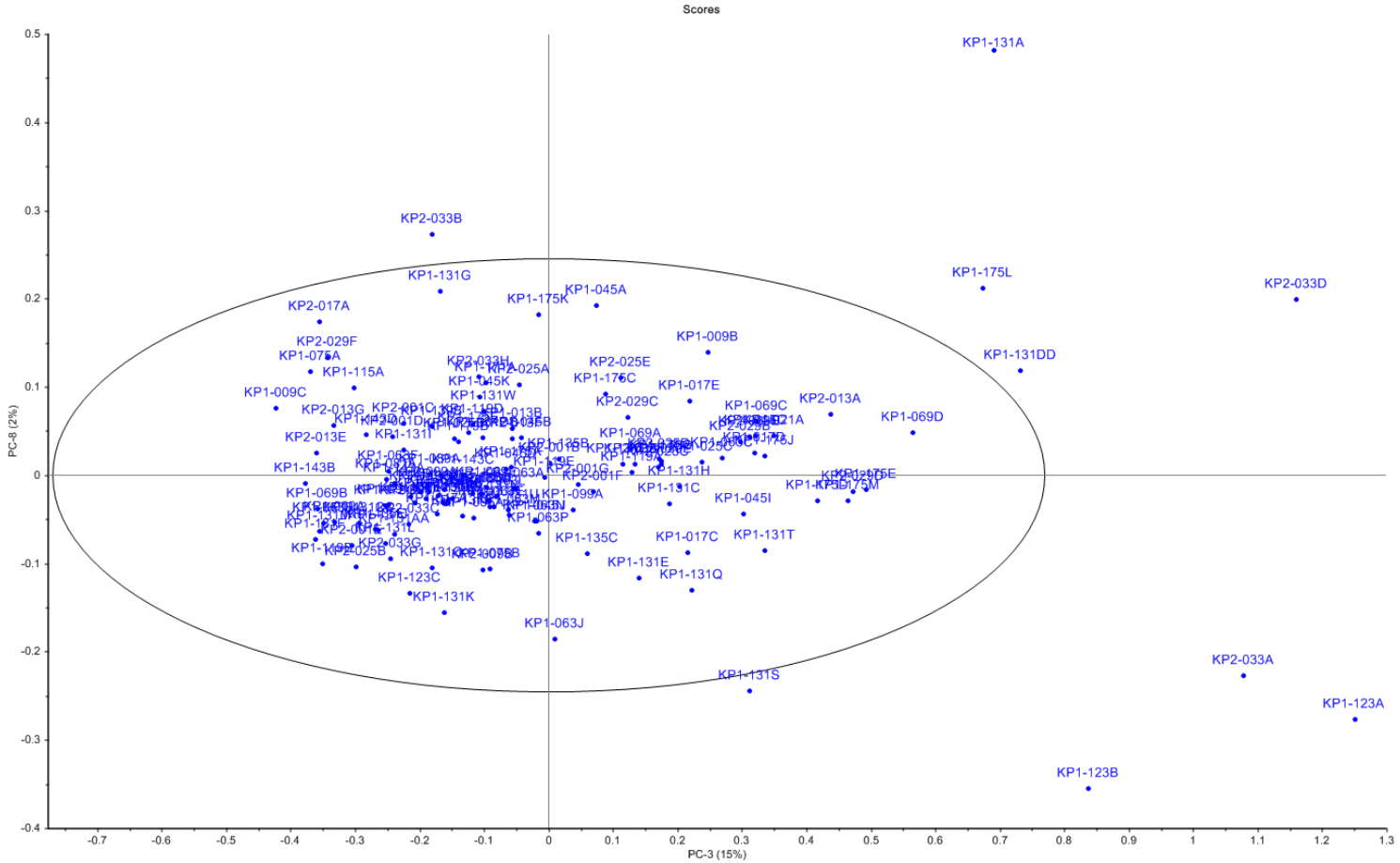


Figure A9.23. Principal component 3 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

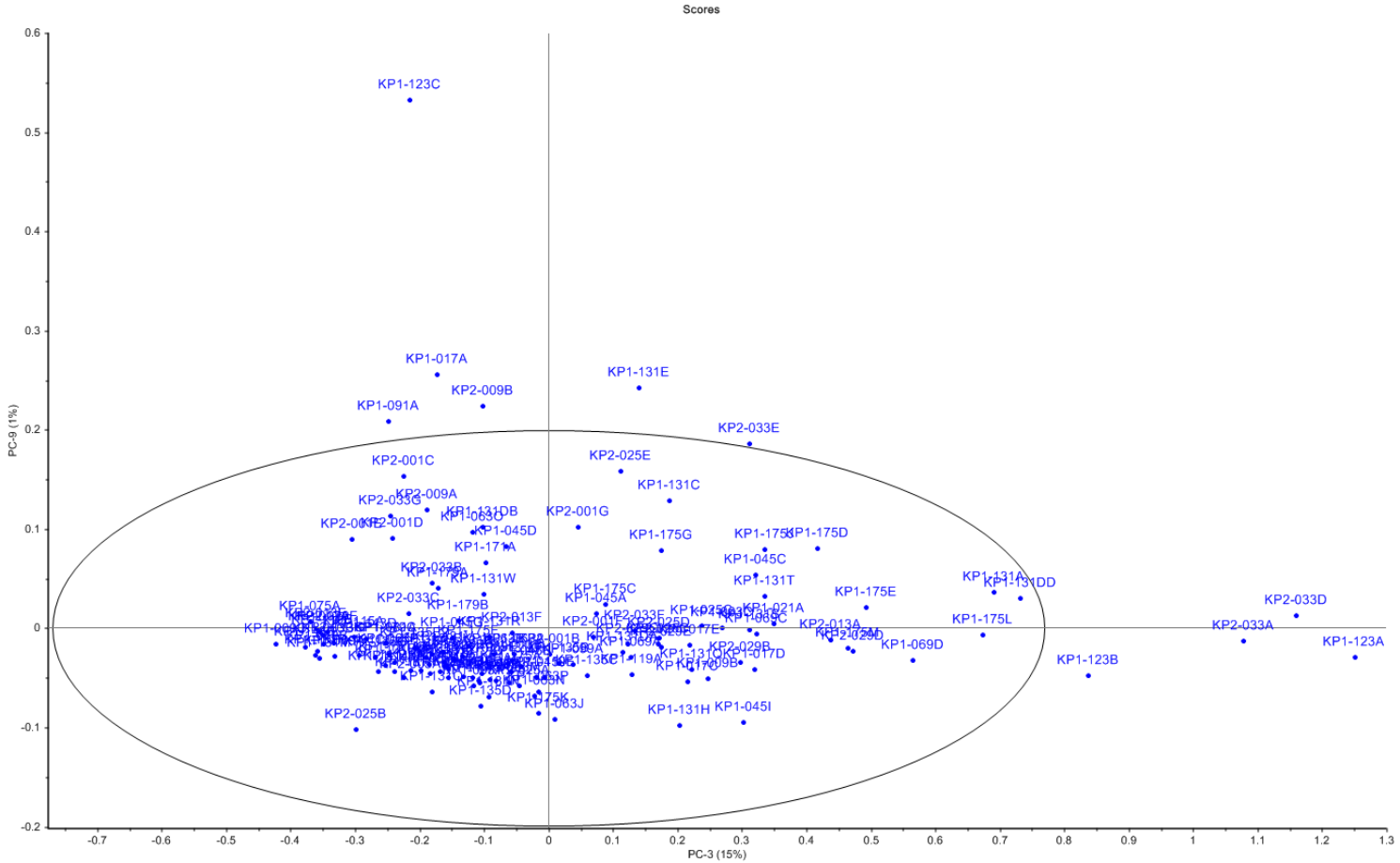


Figure A9.24. Principal component 3 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

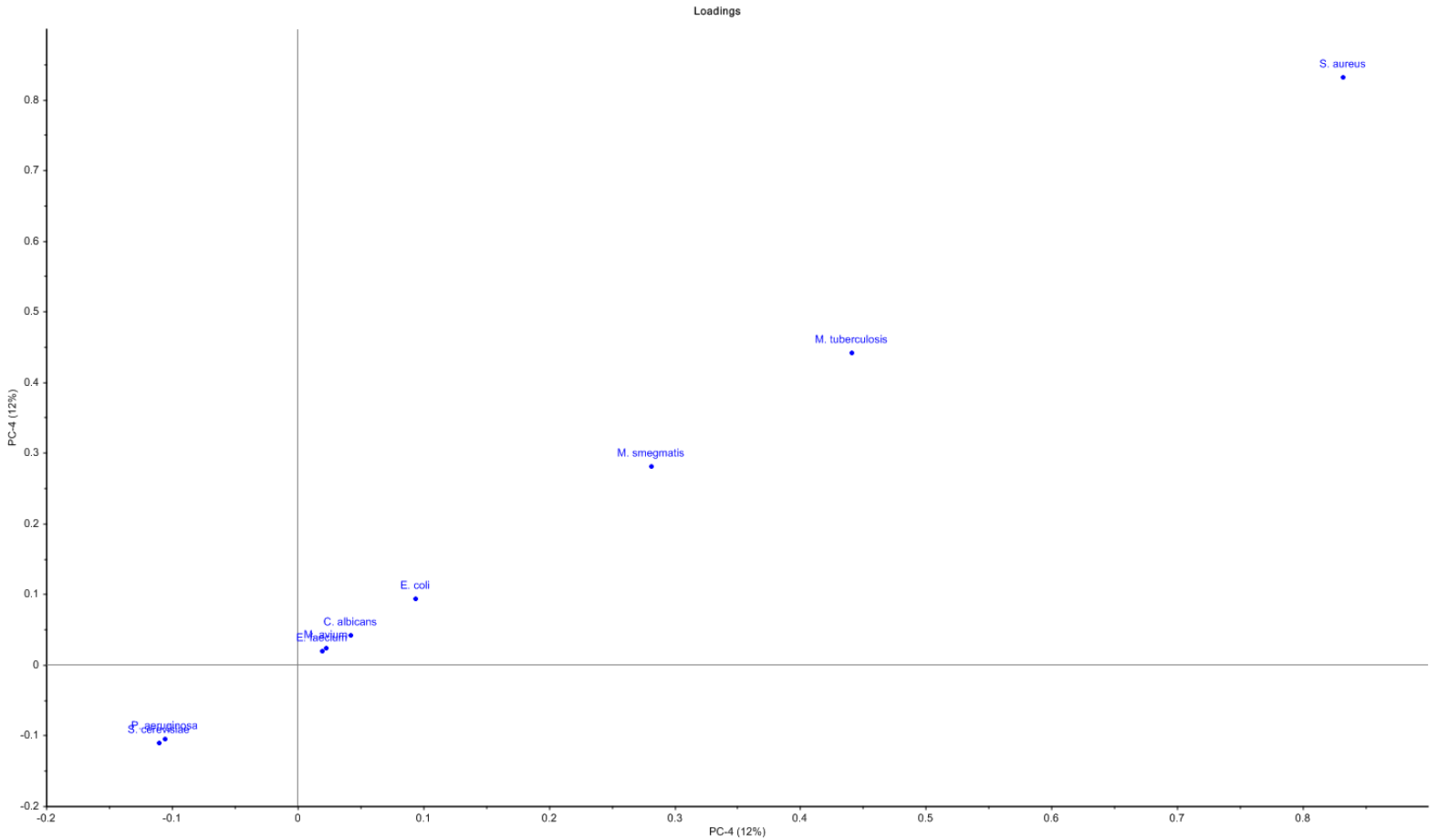


Figure A9.25. Loadings plot for principal component 4 plotted against principal component 4 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

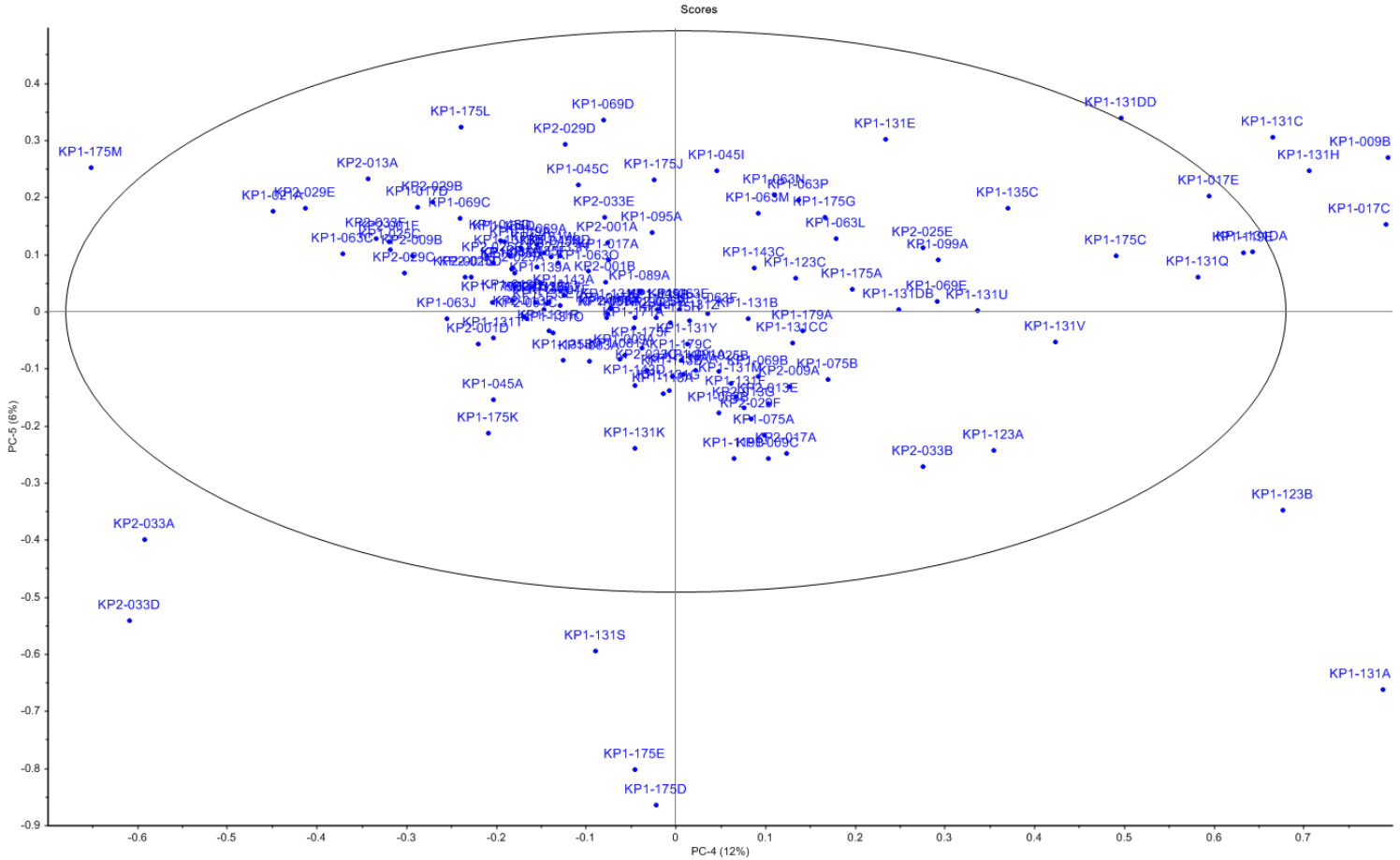


Figure A9.26. Principal component 4 plotted against principal component 5 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

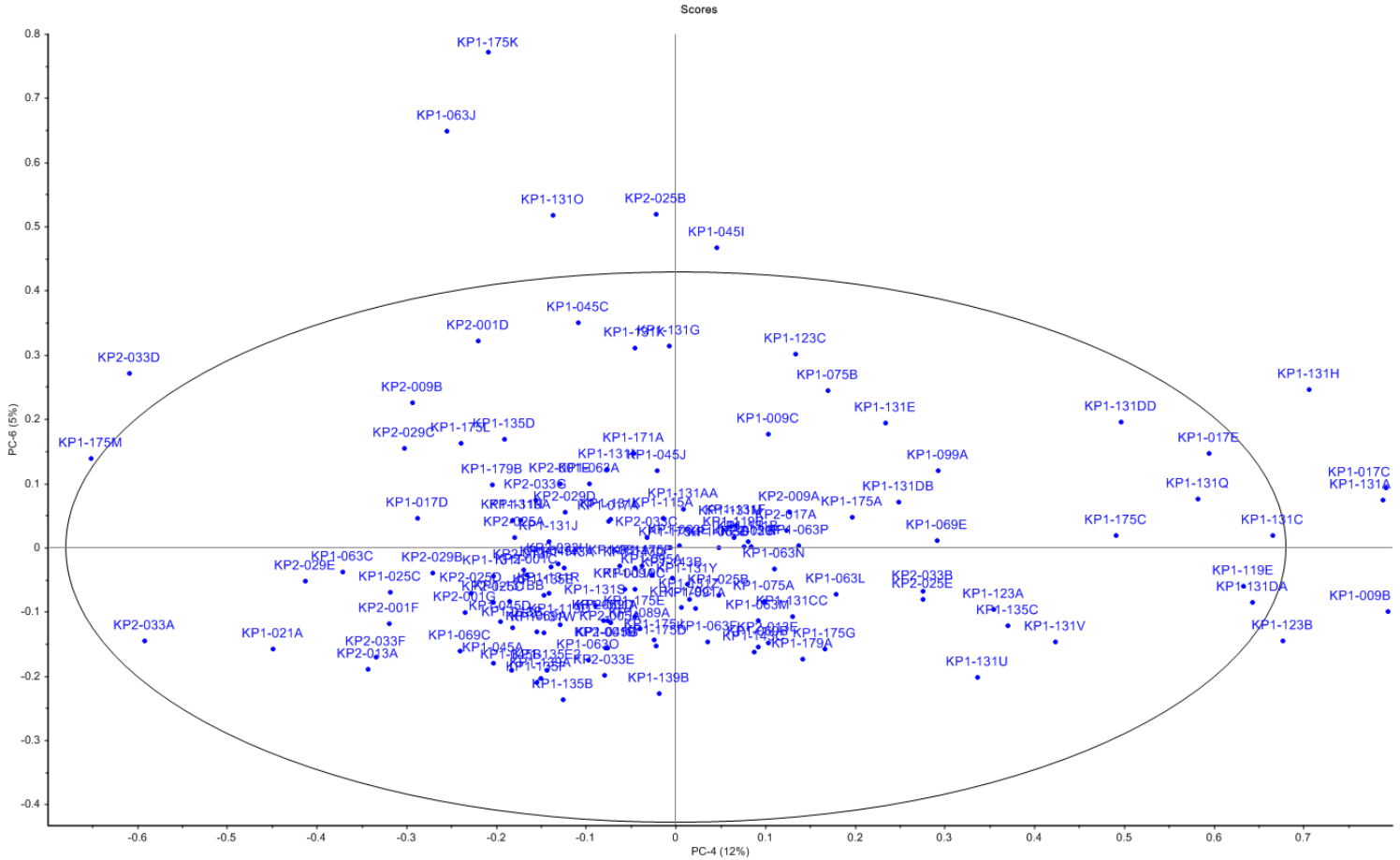


Figure A9.27. Principal component 4 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

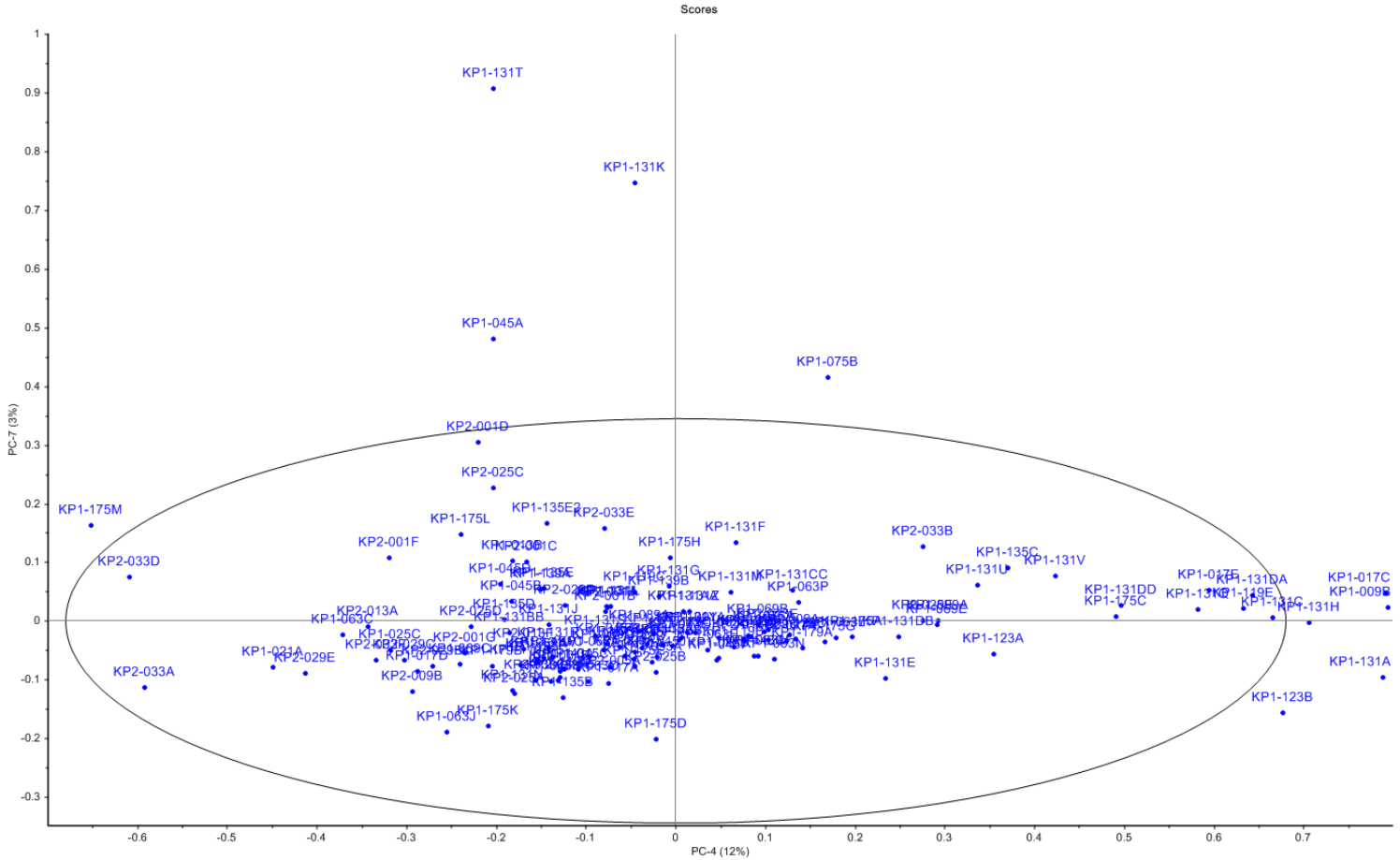


Figure A9.28. Principal component 4 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

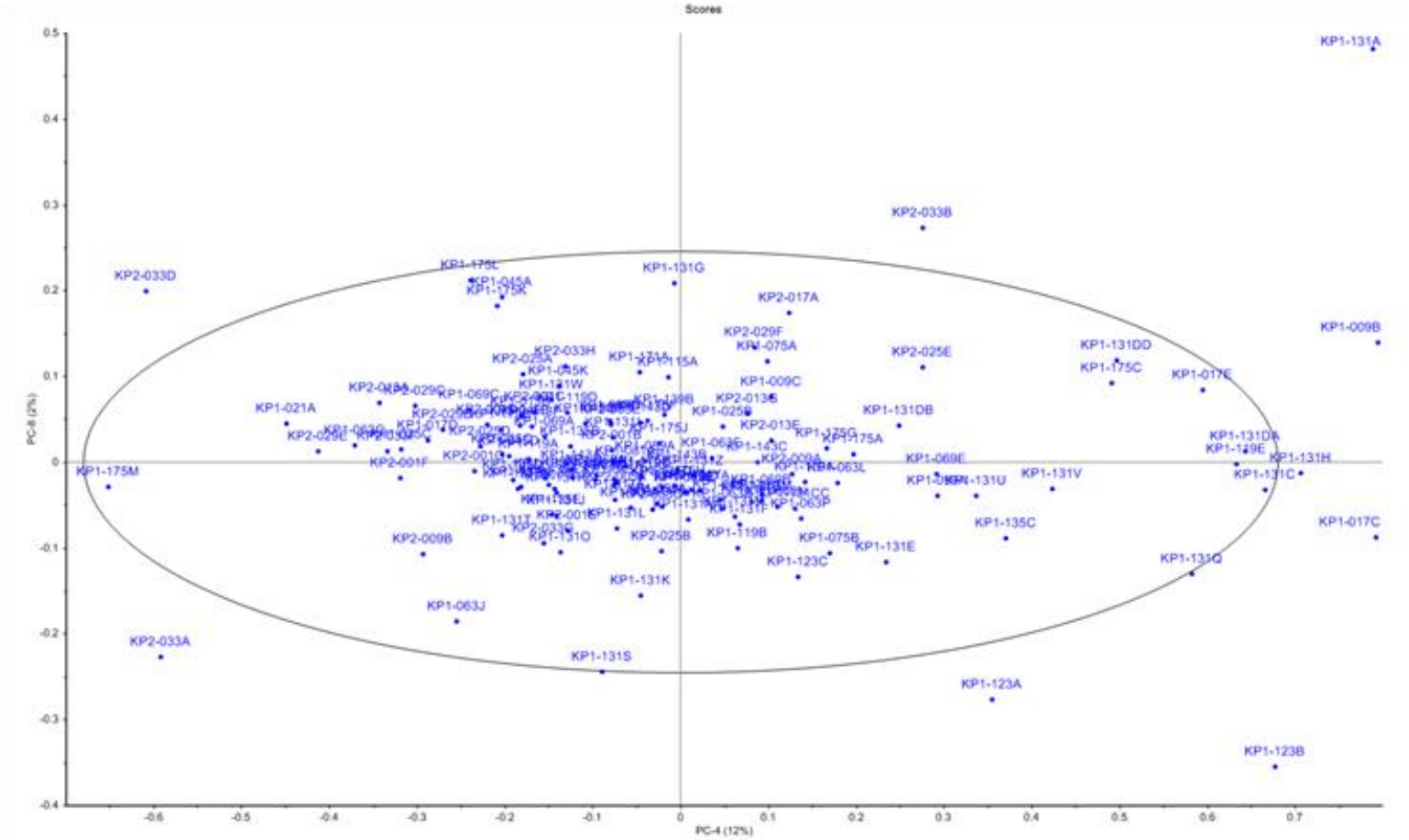


Figure A9.29. Principal component 4 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

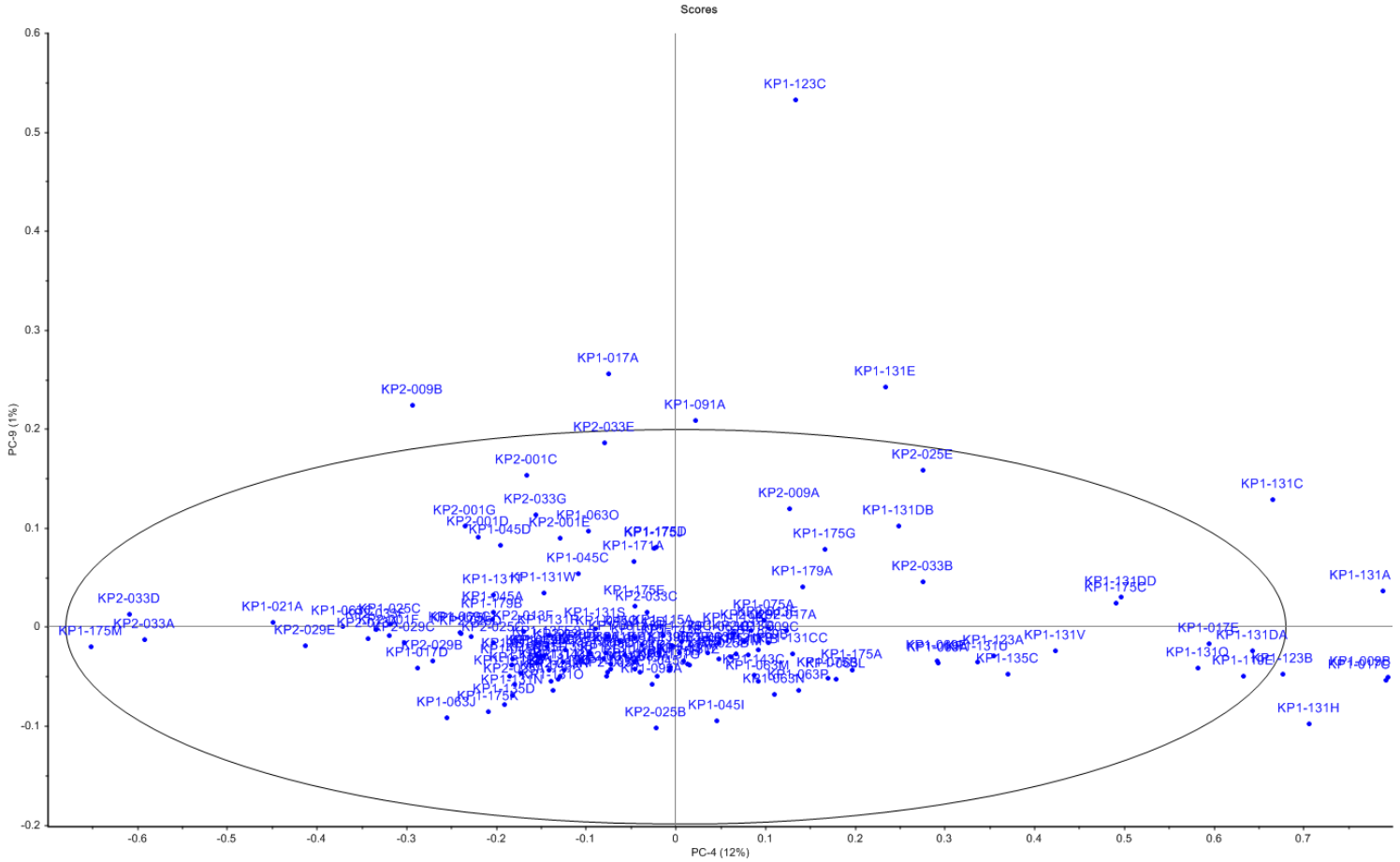


Figure A9.30. Principal component 4 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

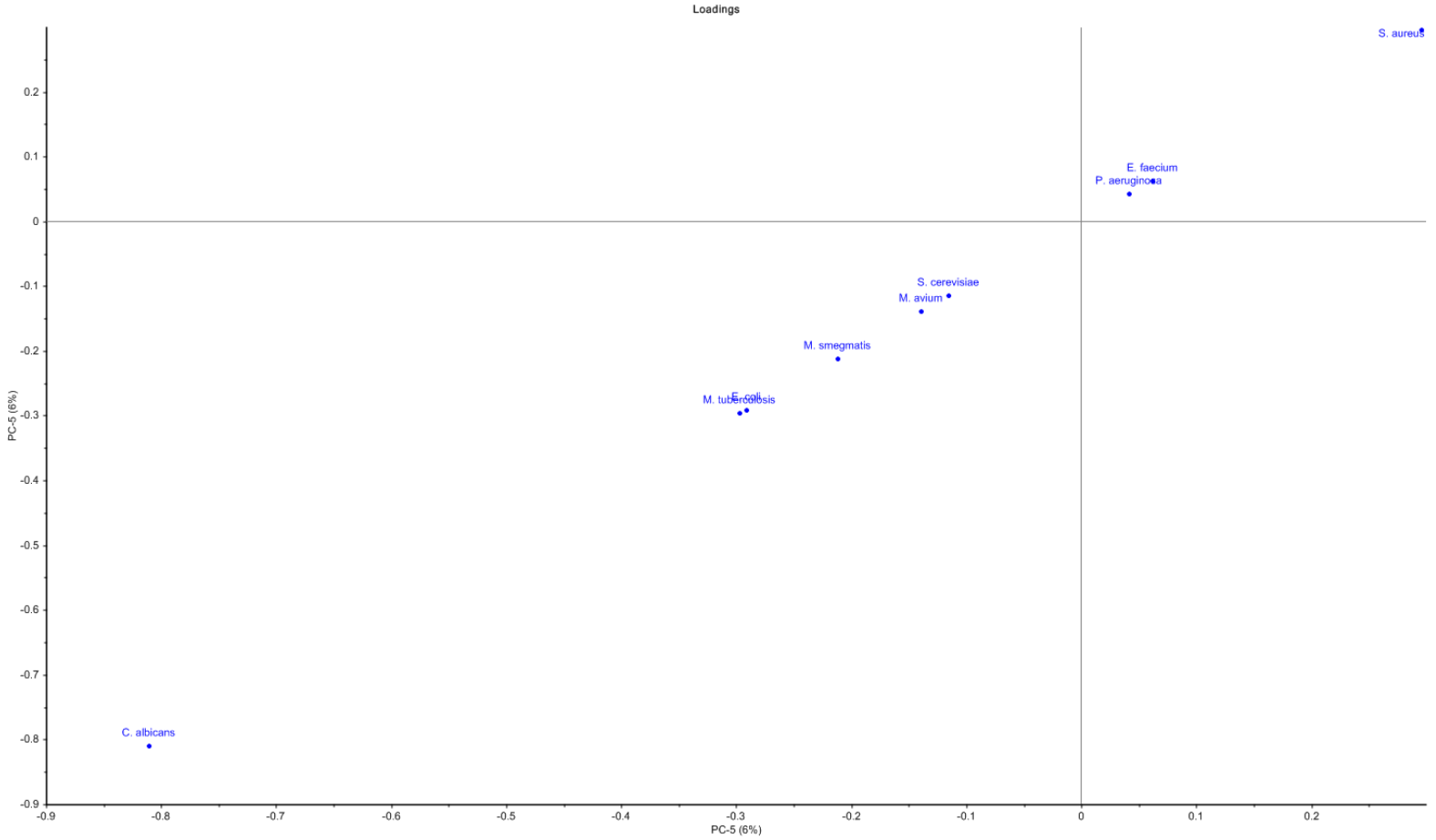


Figure A9.31. Loadings plot for principal component 5 plotted against principal component 5 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

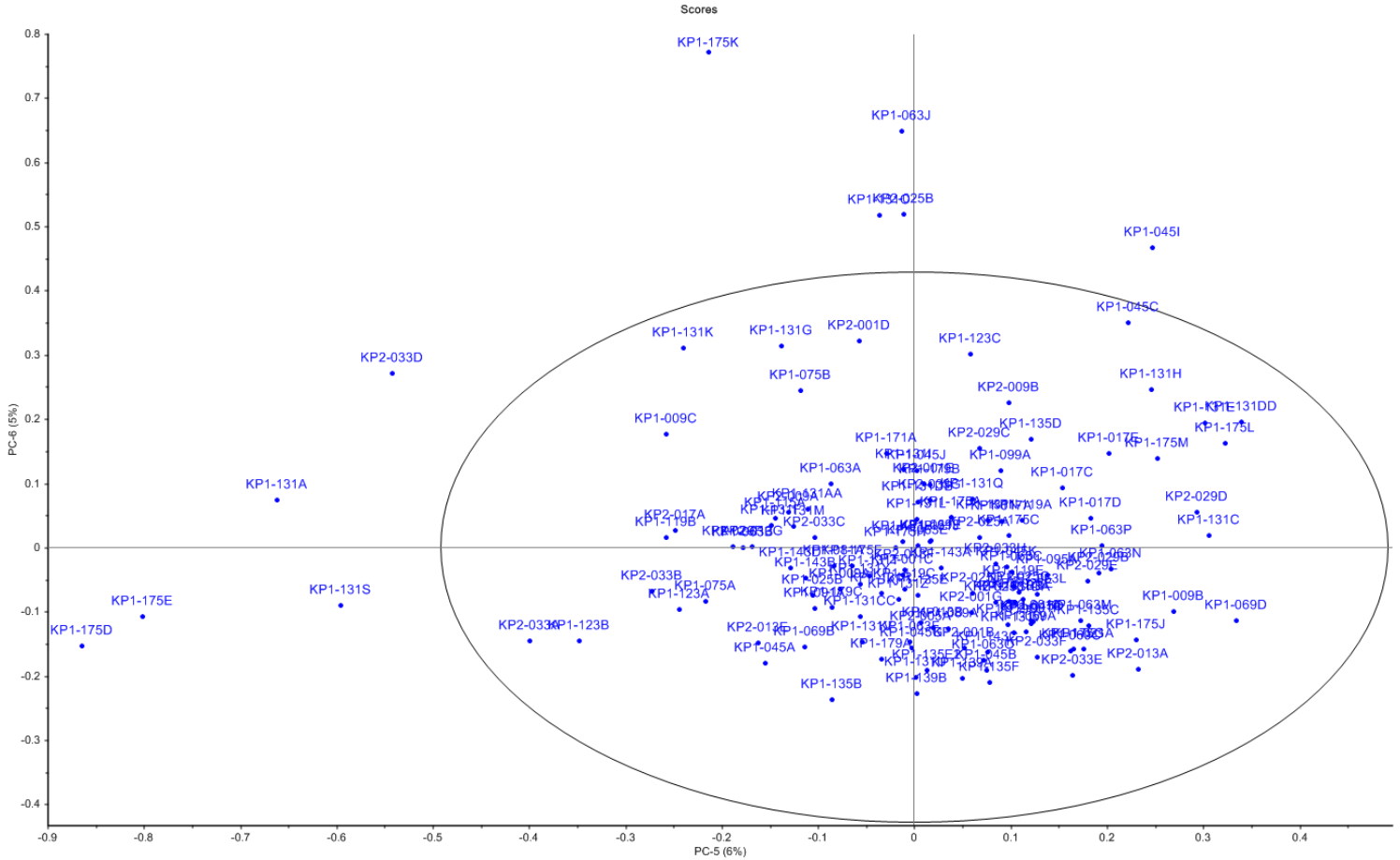


Figure A9.32. Principal component 5 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

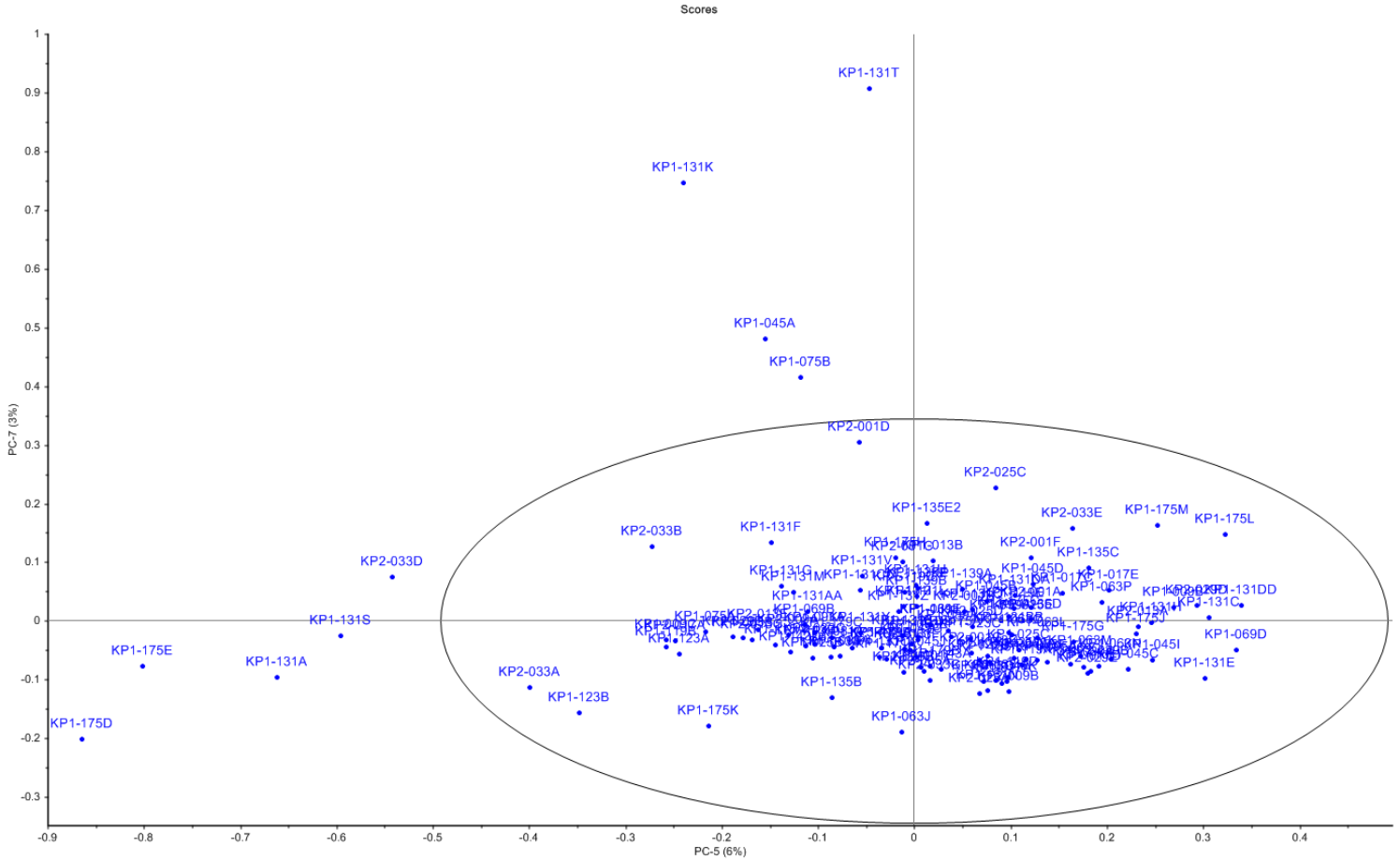


Figure A9.33. Principal component 5 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

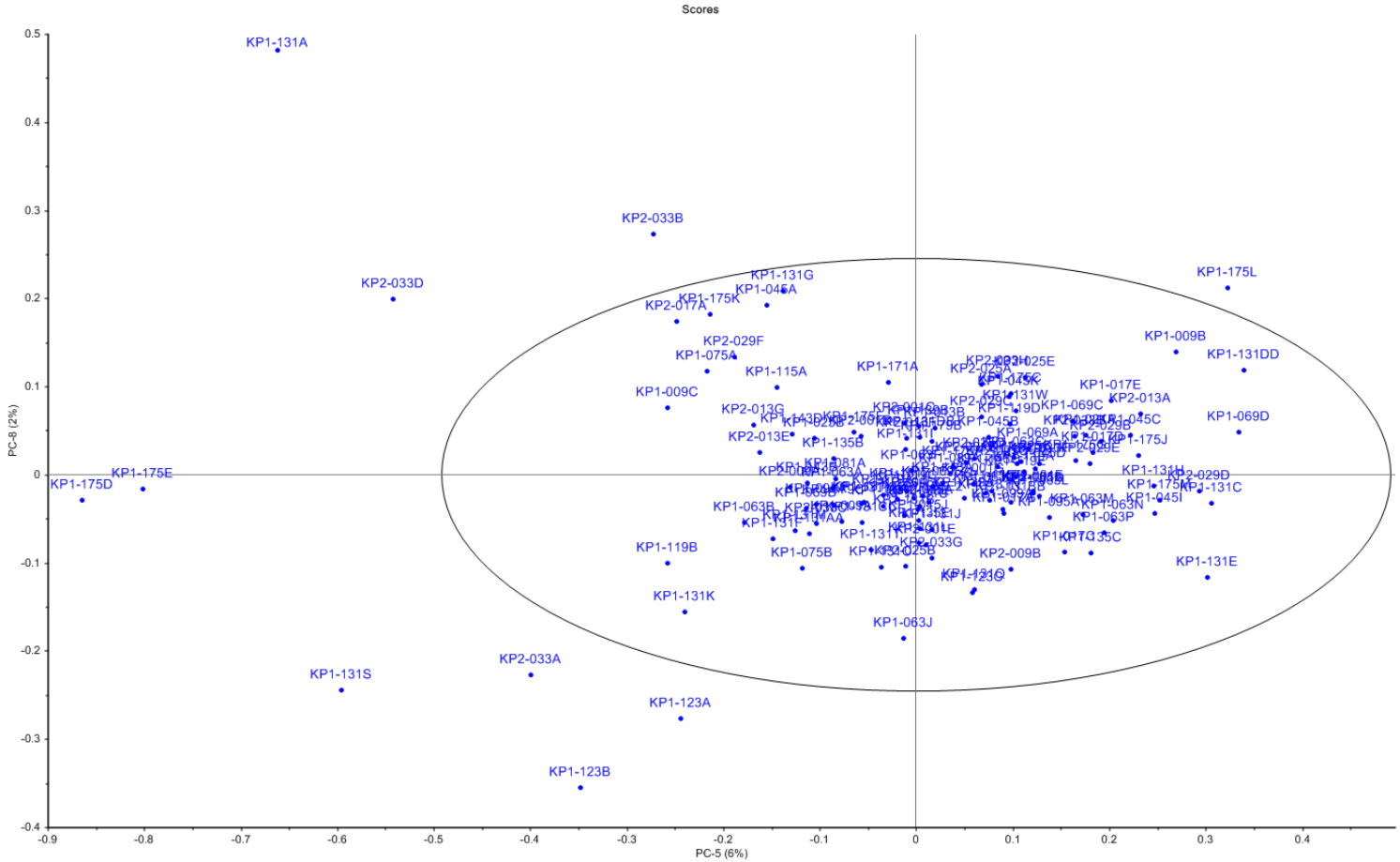


Figure A9.34. Principal component 5 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

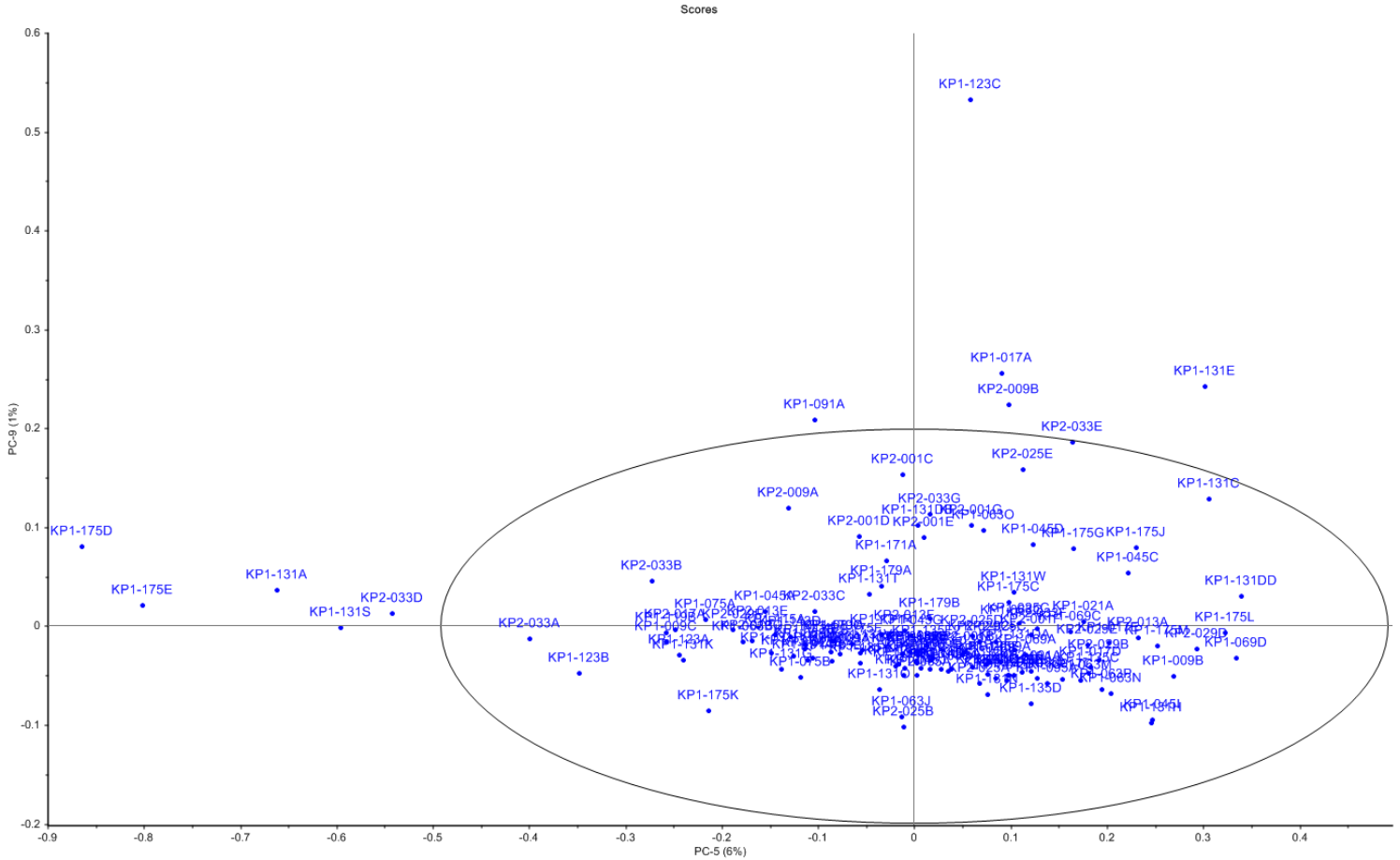


Figure A9.35. Principal component 5 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

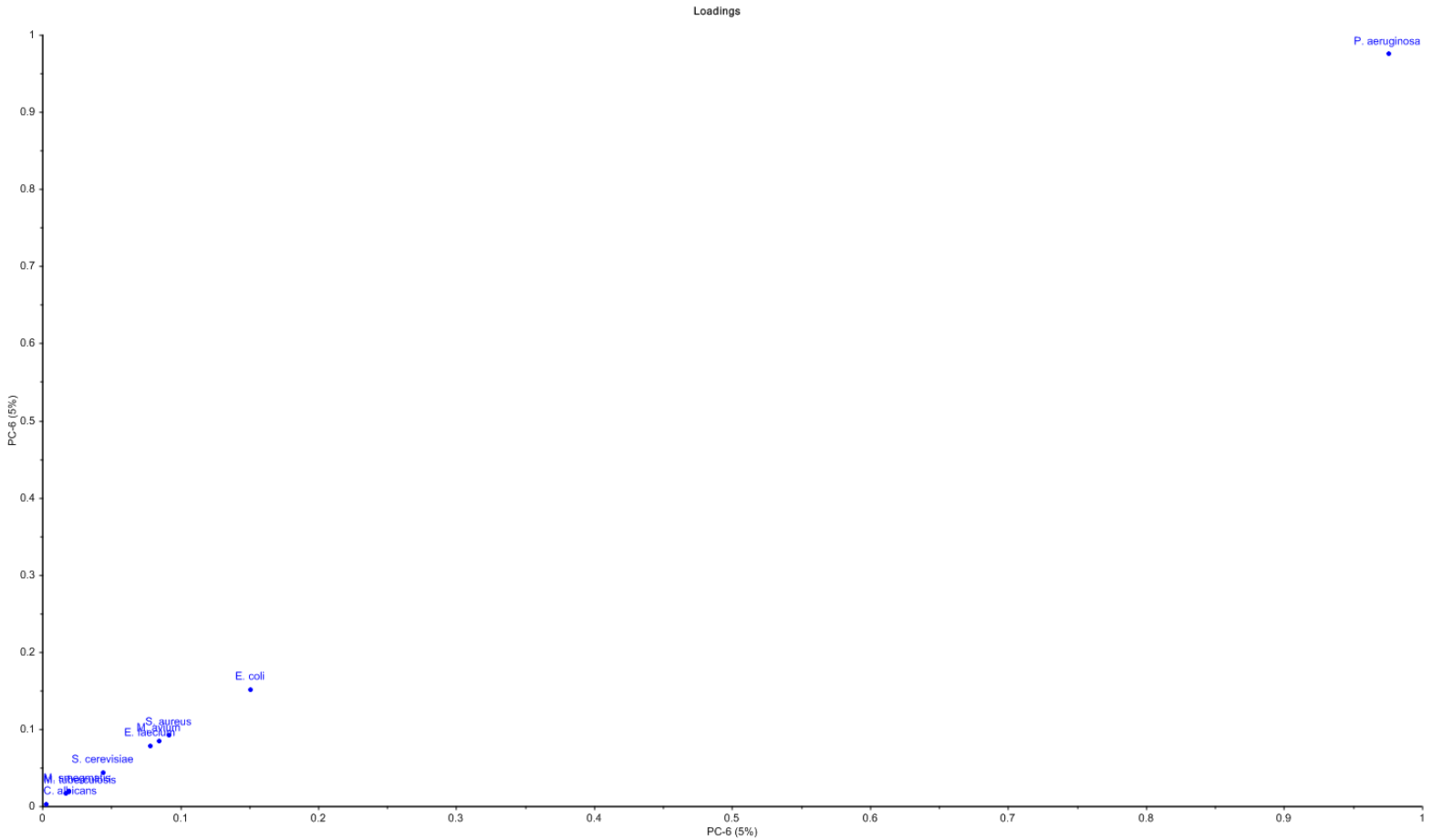


Figure A9.36. Loadings plot for principal component 6 plotted against principal component 6 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

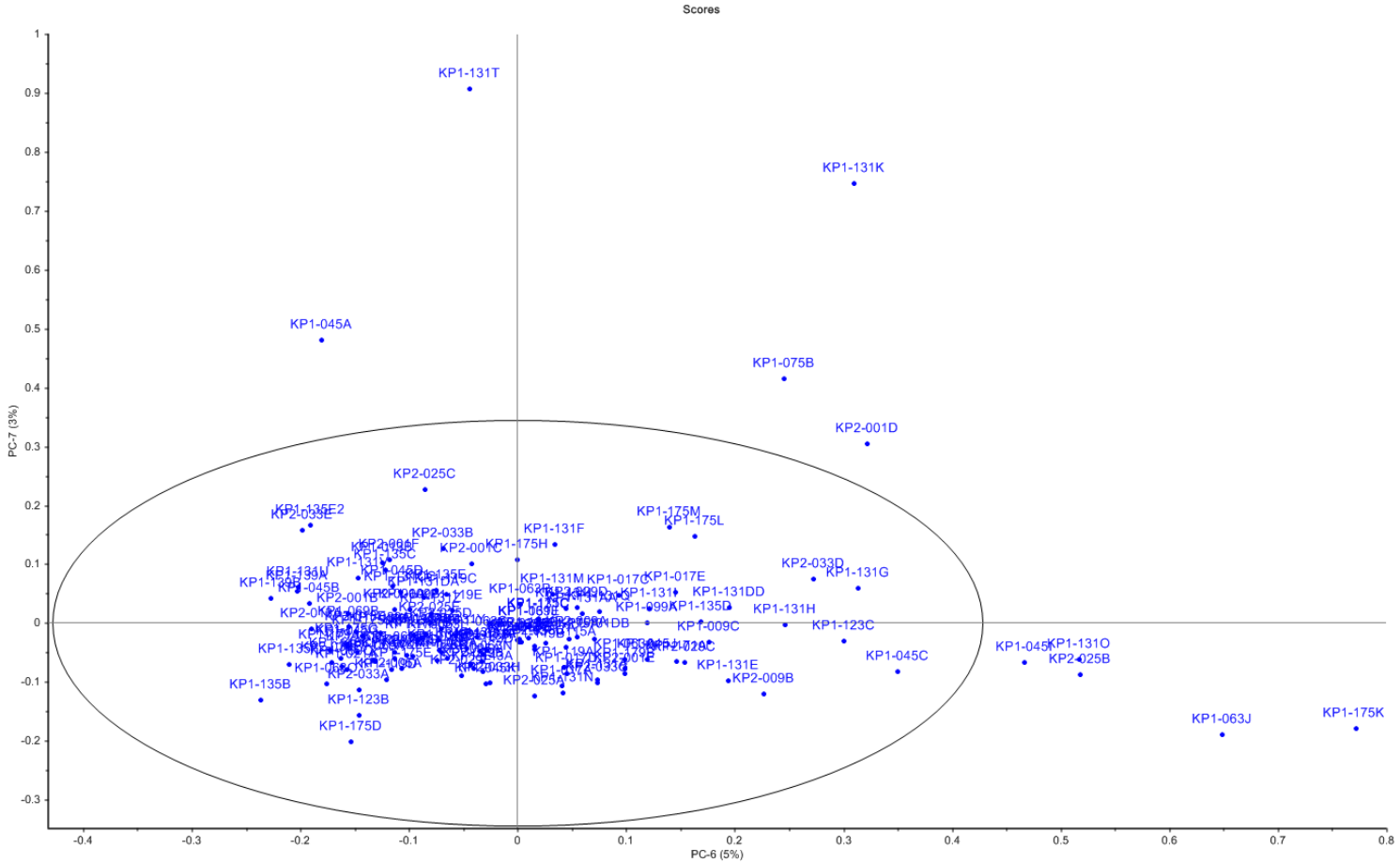


Figure A9.37. Principal component 6 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

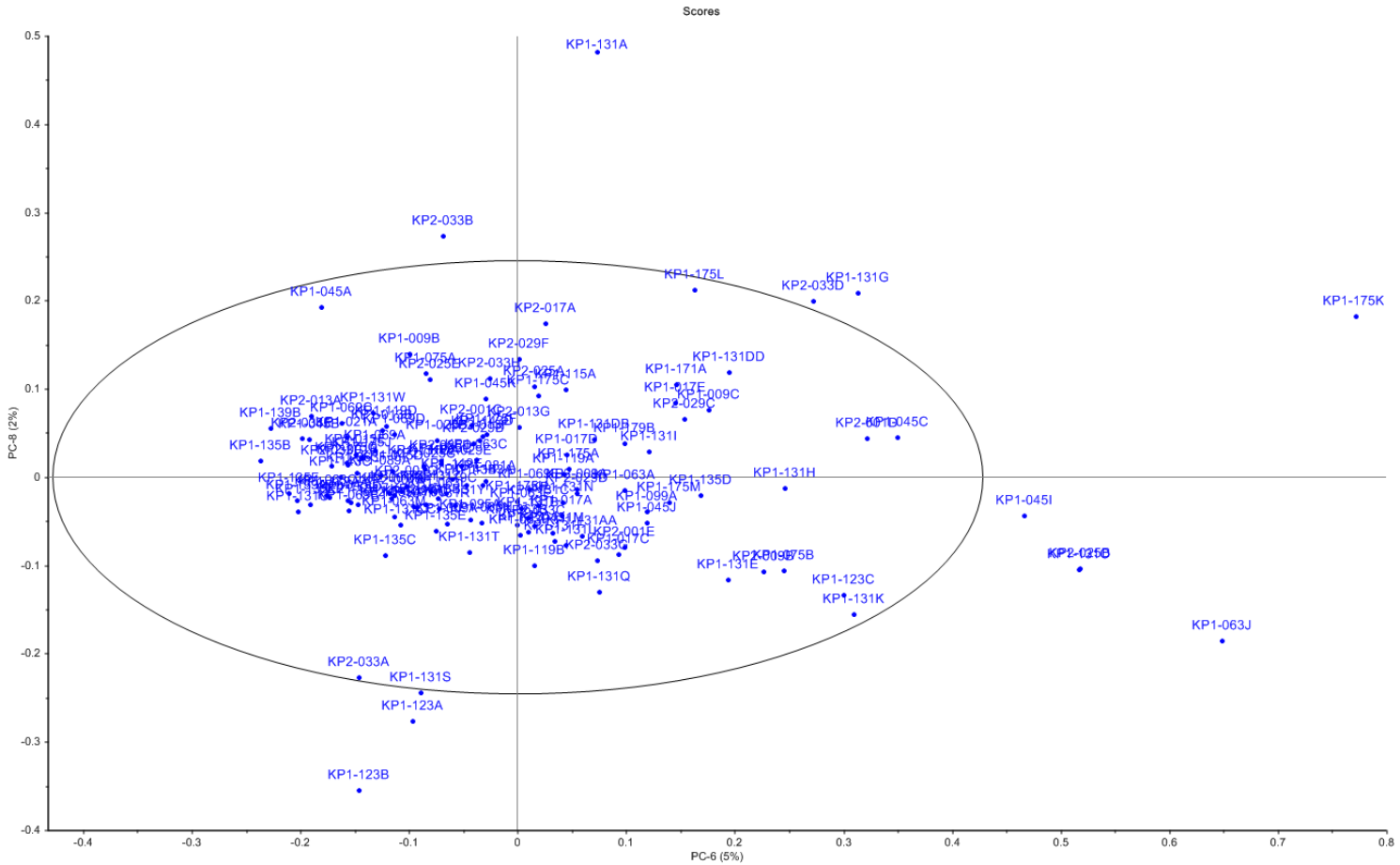


Figure A9.38. Principal component 6 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

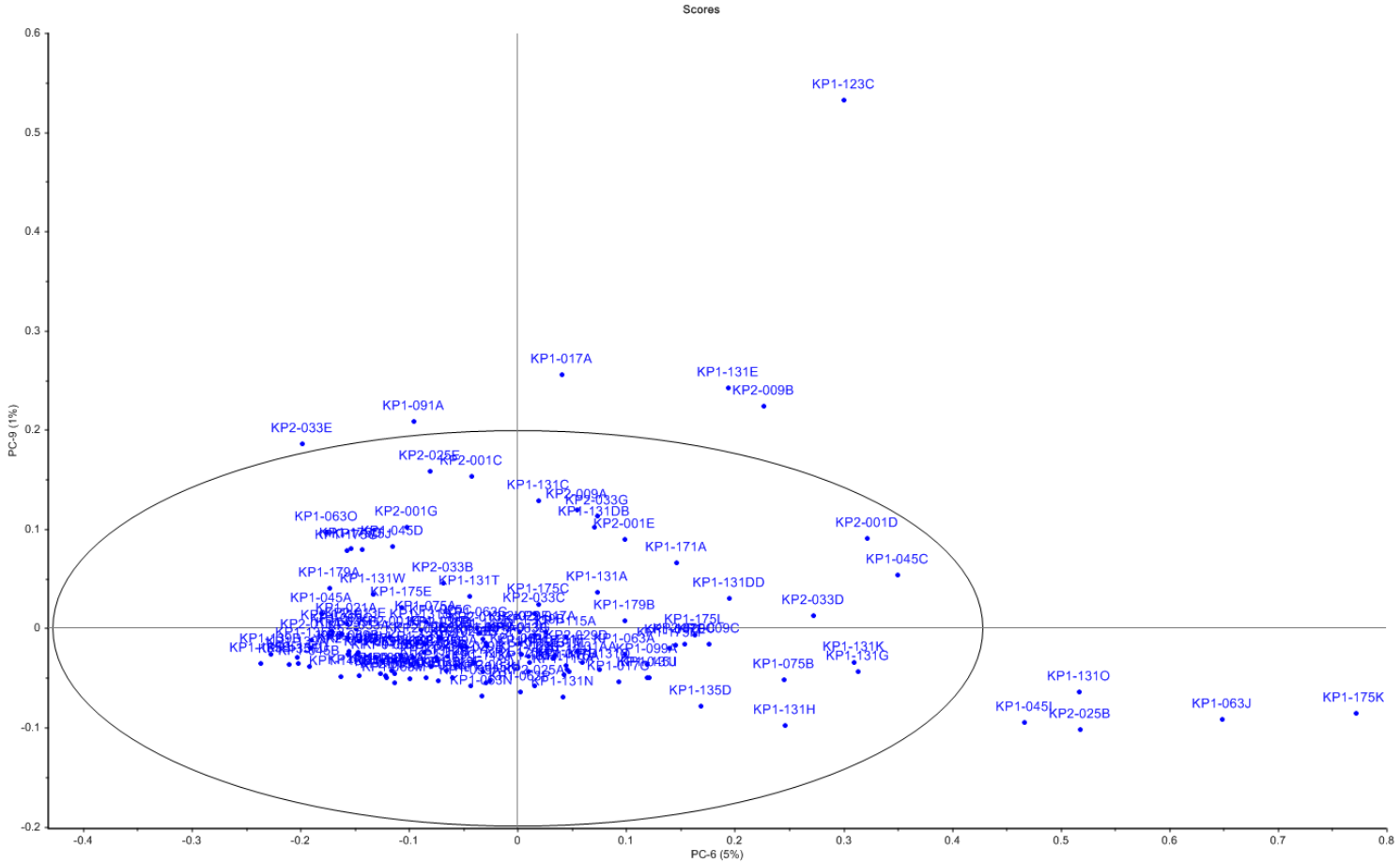


Figure A9.39. Principal component 6 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

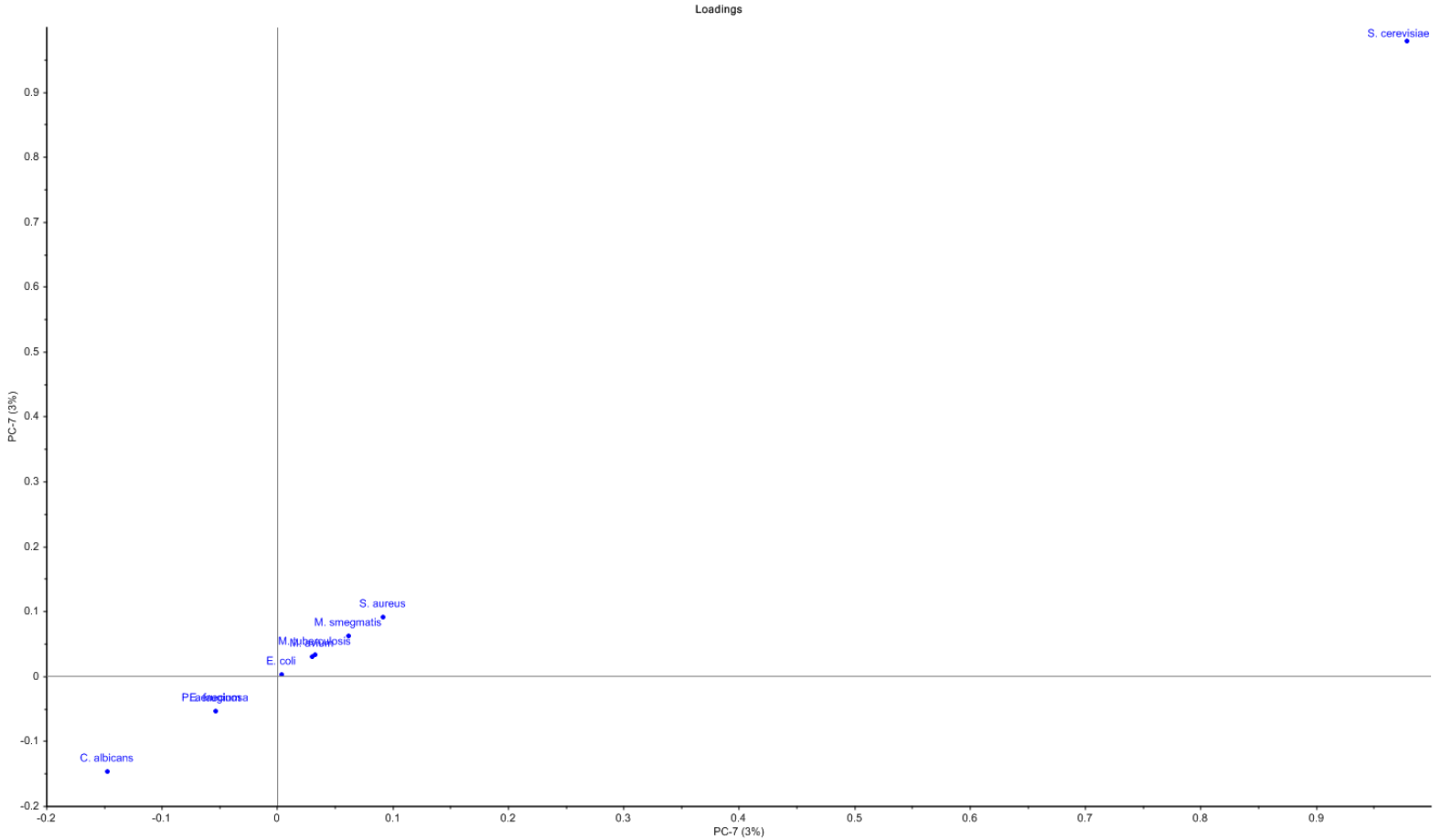


Figure A9.40. Loadings plot for principal component 7 plotted against principal component 7 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

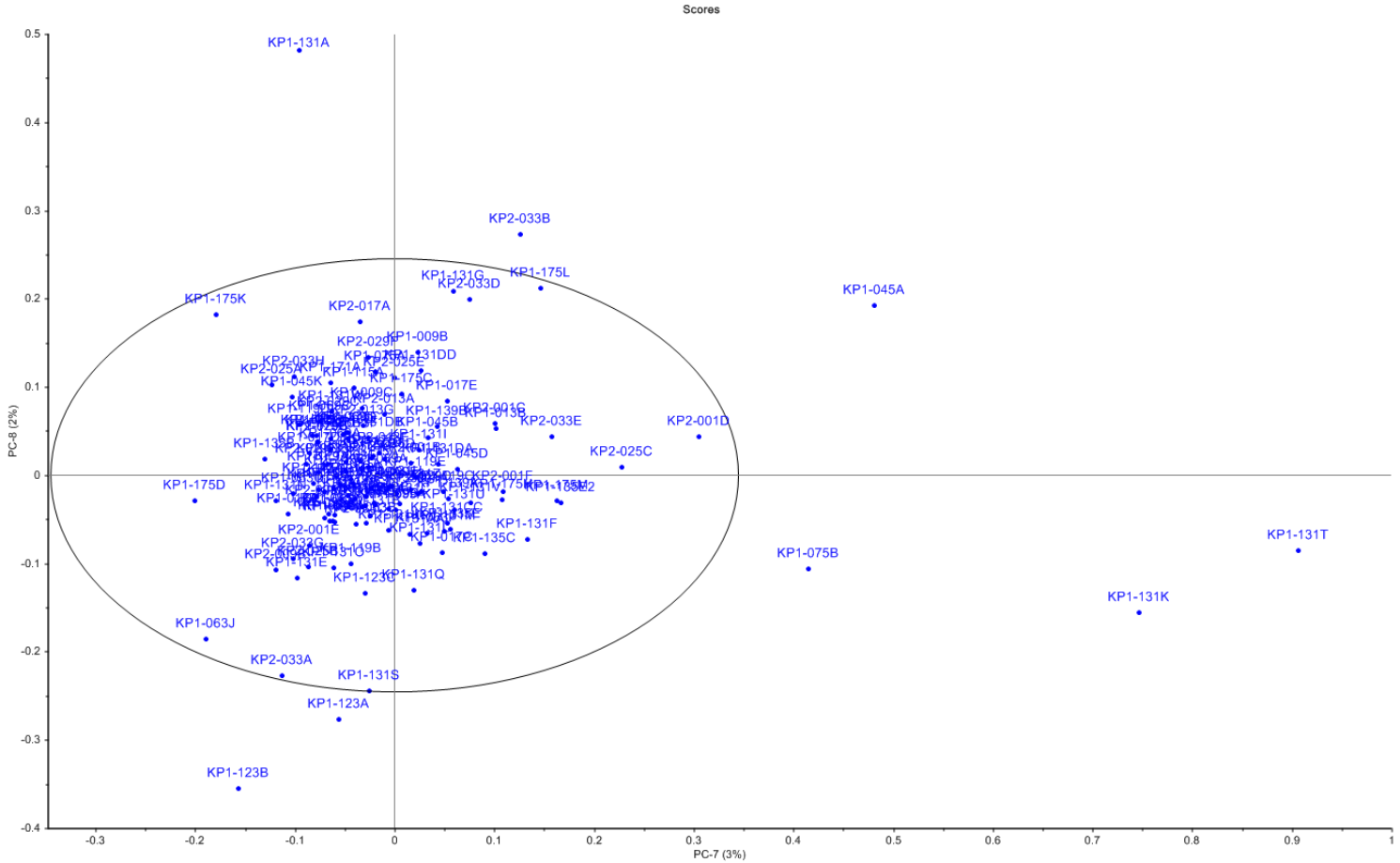


Figure A9.41. Principal component 7 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

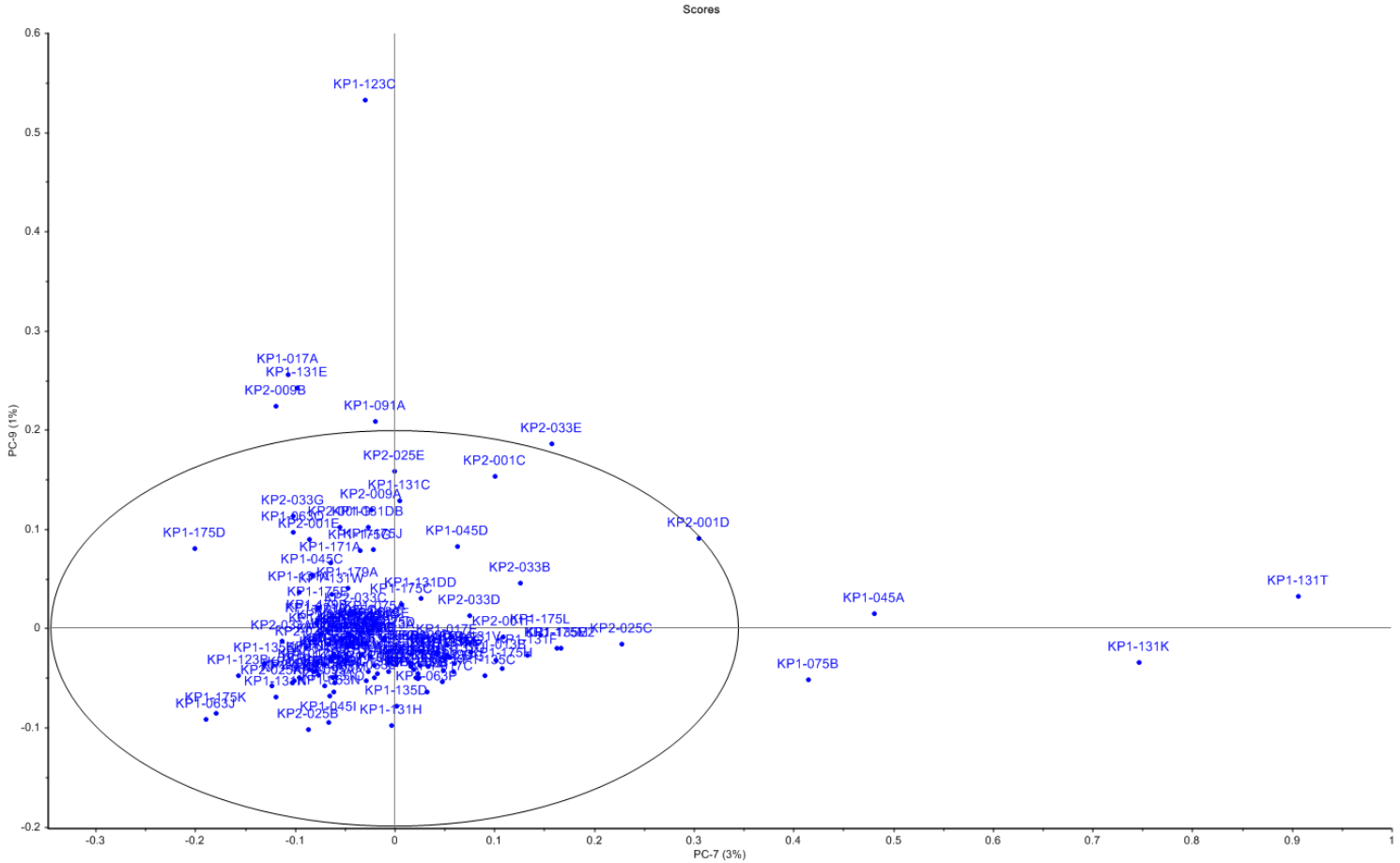


Figure A9.42. Principal component 7 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

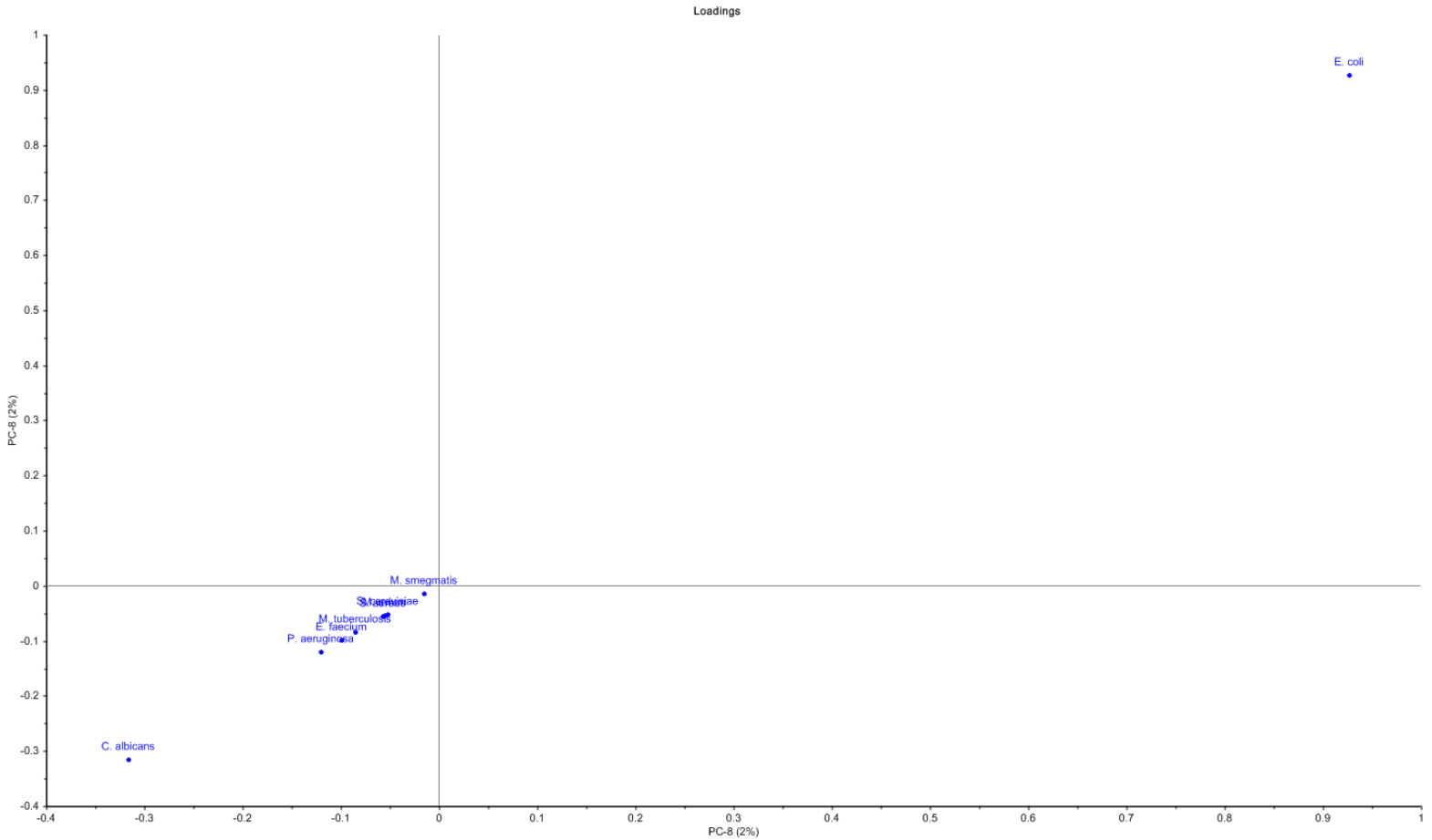


Figure A9.43. Loadings plot for principal component 8 plotted against principal component 8 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

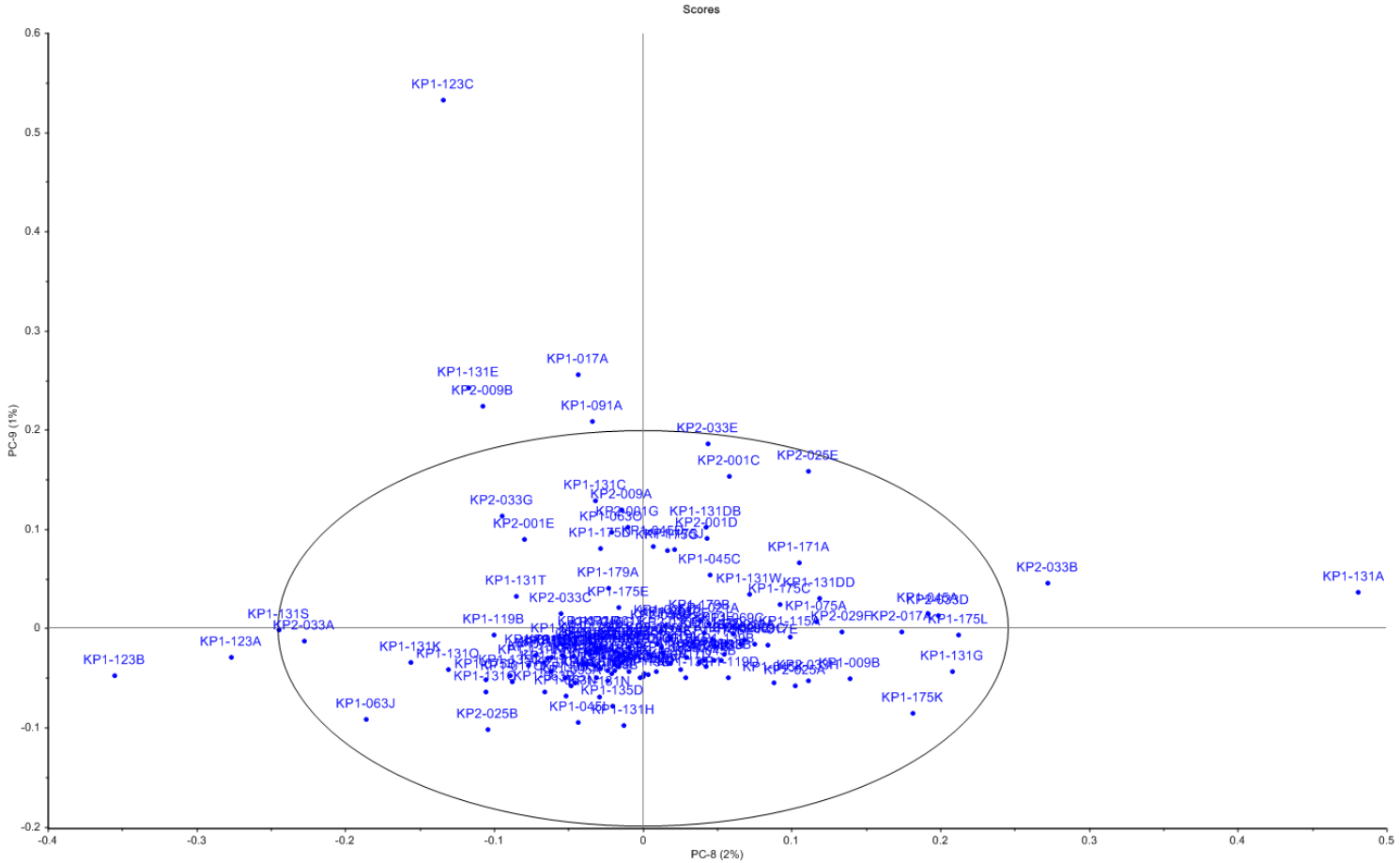


Figure A9.44. Principal component 8 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada. Bioactivity profiles that were outliers were identified as those found outside of the 95% confidence circle for each PCA plot.

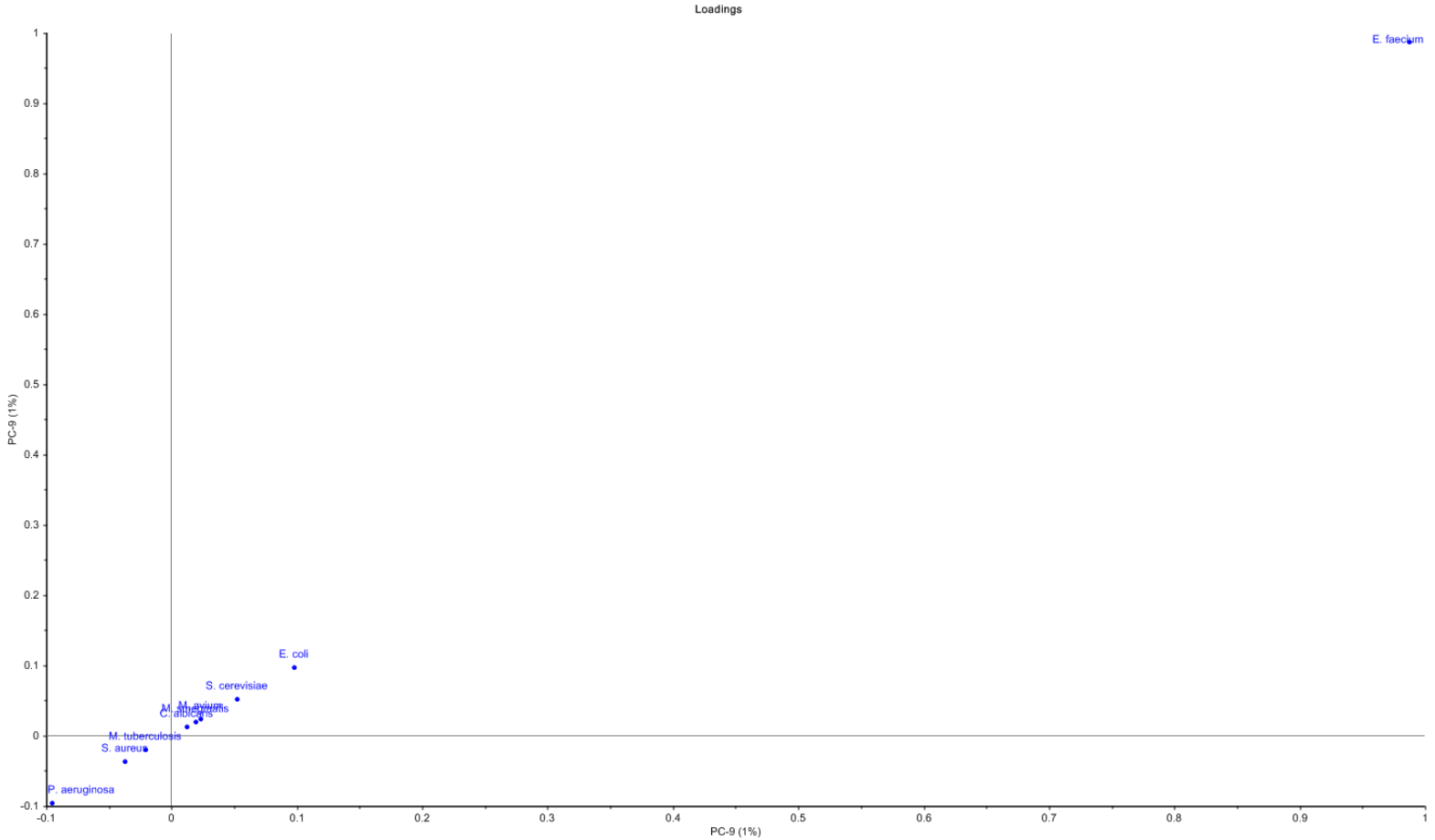


Figure A9.45. Loadings plot for principal component 9 plotted against principal component 9 obtained from the principal component analysis of bioactivity profiles obtained from extracts of endophytes obtained from marine macroalgae of the Bay of Fundy, New Brunswick, Canada.

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Universities Attended

University of New Brunswick, Saint John
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Publications

Flewelling, A.J., Johnson, J.A., Gray, C.A. 2016. Application of a simple bioactivity profiling strategy to natural product discovery from endophytes of marine macroalgae. Planta Medica. 82(S 01): S1-S381. (Conference abstract)

Flewelling, A.J., Currie, J., Gray, C.A., Johnson, J.A. 2015. Endophytes from marine macroalgae: promising sources of novel natural products. Current Science. 109(1): 88-111.

Flewelling, A.J., Bishop, A.I., Johnson, J.A., Gray, C.A. 2015. Polyketides from an endophytic *Aspergillus fumigatus* isolate inhibit the growth of *Mycobacterium tuberculosis* and MRSA. Natural Product Communications. 10(10): 1661-1662.

Flewelling, A.J., Johnson, J.A., Gray, C.A. 2013. Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Botanica Marina. 53(3): 287-297.

Flewelling, A.J., Johnson, J.A., Gray, C.A. 2013. Antimicrobials from the Marine Algal Endophyte *Penicillium* sp. Natural Product Communications. 8(3): 373-374.

Flewelling, A.J., Ellsworth, K.T., Sanford, J., Forward, E., Johnson, J.A., Gray, C.A. 2013. Macroalgal endophytes from the Atlantic coast of Canada: A potential source of antibiotic natural products? Microorganisms. 1: 175-187.

Flewelling, A.J., Johnson, J.A., Gray, C.A. 2012. Bioactive natural products from North Atlantic algal endophytes. Planta Medica. 78(11): 1155. (Conference abstract)

Hebert, M.J.G., **Flewelling, A.J.**, Clark, T.N., Levesque, N.A., Jean-Francois, J., Surette, M.E., Gray, C.A., Vogels, C.M., Touaibia, M., Westcott, S.A. 2015. Synthesis and biological activity of arylspiroborate salts derived from caffeic acid phenethyl ester. International Journal of Medicinal Chemistry. 9 pages

Li, H., Doucet, B., **Flewelling, A.J.**, Jean, S., Webster, D., Robichaud, G.A., Johnson, J.A., Gray, C.A. 2015. Antimycobacterial natural products from endophytes of the medicinal plant *Aralia nudicaulis*. Natural Product Communications. 10(10): 1641-1642.

Patterson, A.E., **Flewelling, A.J.**, Clark, T.N., Geier, S.J., Vogels, C.M., Masuda, J.D., Gray, C.A., Westcott, S.A. 2015. Antimicrobial and antimycobacterial activities of aliphatic amines derived from vanillin. Canadian Journal of Chemistry. 93: 1305-1311.

Salsbury, L.E., Robertson, K.N., **Flewelling, A.J.**, Li, H., Geier, S.J., Vogels, C.M., Gray, C.A., Westcott, S.A. 2014. Anti-mycobacterial activities of copper (II) complexes. Part II. Lipophillic hydroxypyridinones derived from maltol. Canadian Journal of Chemistry. 93: 334-340.

Webb, M.I., O'Neill, T., Li, H., **Flewelling, A.**, Halcovitch, N.R., Bowes, H.R., Lee, G.M., Swetnam, M.E., Vogels, C.M., Decken, A., Baerlocher, F.J., Gray, C.A., Westcott, S.A. 2014. Arylspiroborates derived from 4-tert-Butylcatechol and 3,5-di-tert-Butylcatechol and their Antimicrobial Activities. Journal of Heterocyclic Chemistry. 51: 157-161.

Geier, M.J., Bowes, E.G., Lee, G.M., O'Neill, T., Li, H., **Flewelling, A.**, Vogels, C.M., Decken, A., Gray, C.A, Westcott, S.A. 2013. Synthesis and biological activities of arylspiroborates derived from 2,3-dihydroxynaphthalene. Heteroatom Chemistry. 24: 116-123.

Patterson, A.E., Bowes, E.G., Bos, A., O'Neill, T., Li, H., **Flewelling, A.**, Vogels, C.M., Decken, A., Gray, C.A., Westcott, S.A. 2013. Anti-mycobacterial activities of copper (II) salicylaldimine complexes derived from long chain aliphatic amines. Canadian Journal of Chemistry. 91(11): 1093-1097.

Stewart, E.L., Patterson, A.E., O'Neill, T., Li, H., **Flewelling, A.**, Vogels, C., Decken, A., Lloyd, V.K., Gray, C.A., Westcott, S.A. 2013. Synthesis, characterization and bioactivities of platinum(II) complexes bearing pyridinecarboxaldimines containing aliphatic groups. Canadian Journal of Chemistry. 91: 131-136.

Conference Presentations

Maritime Natural Products Conference, University of Prince Edward Island, Charlottetown, PEI (August 2017)
Oral Presentation
Title: "Application of a simple bioactivity profiling strategy to natural product discovery from endophytes of marine macroalgae"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, University of Prince Edward Island, Charlottetown, PEI (August 2017)
Poster Presentation
Title: "Isolation and identification of 3,5-dihydroxyhexanoic acid oligomers from an unidentified sterile endophyte of the brown alga *Scytosiphon lomentaria*"
Authors: Nicholas J. Morehouse, **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, University of Prince Edward Island, Charlottetown, PEI (August 2017)
Poster Presentation
Title: "The NPRG fungal endophyte library"
Authors: Julie Therrien, Allyson Bos, Matthew L. Clinton, Kathleen Complak, Samantha Cox, Trevor N. Clark, Katelyn Ellsworth, **Andrew J. Flewelling**, Lauren Forgrave, Nicholas Morehouse, Artabaz Nazari, Kelsey Pendleton, Marija Valjanovska, John A. Johnson and Christopher A. Gray.

Maritime Natural Products Conference, Dalhousie University, Halifax, Nova Scotia (August 2016)
Poster Presentation
Title: "Application of a simple bioactivity profiling strategy to natural product discovery from macroalgal endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

9th Joint Natural Products Conference 2016, Copenhagen, Denmark (July 2016)
Poster presentation
Title: "Application of a simple bioactivity profiling strategy to natural product discovery from macroalgal endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

99th Canadian Chemistry Conference and Exhibition, Halifax, NS (June 2016)
Poster Presentation
Title: "Application of a simple bioactivity profiling strategy to natural product discovery from macroalgal endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2016)
Poster Presentation

Title: "Application of a simple bioactivity profiling strategy to natural product discovery from macroalgal endophytes"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, UNBSJ Campus, Saint John, New Brunswick (August 2015)
Oral Presentation

Title: "Bioactivity profiling: a tool to prioritise novel antibiotic discovery"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Science Atlantic - CIC Chemistry Conference (ChemCon), UNB Fredericton (May 2015)
Oral Presentation

Title: "Can pictures avoid redundancy? The use of bioactivity profiling to prioritise novel antibiotic discovery"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2015)
Poster Presentation

Title: "Wanted: Less redundancy in natural products discovery"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

New Brunswick Health Research Foundation 6th Annual Health Research Conference, Moncton, New Brunswick (November 2014)
Poster Presentation

Title: "Wanted: Less redundancy in natural products discovery"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, University of Prince Edward Island, Charlottetown, PEI (August 2014)
Oral Presentation

Title: "Bioactivity profiling of marine macroalgal endophytes from the Bay of Fundy, Canada"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2014)
Poster Presentation

Title: "Discovery of natural product based efflux inhibitors"

Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

New Brunswick Health Research Foundation 5th Annual Health Research Conference, Saint John, New Brunswick (November 2013):
Poster Presentation
Title: "Discovery of natural product based efflux inhibitors"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, Saint Francis Xavier University, Antigonish, Nova Scotia (August 2013)
Oral Presentation
Title: "Antibiotic Natural Products of Endophytes from North Atlantic Marine Macroalgae"
Authors: **Andrew J. Flewelling**, Kelsey K. Pendleton, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2013)
Oral Presentation
Title: "Bioactive Natural Products from Marine Macroalgal Endophytes of the Shetland Islands, UK
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

International Congress on Natural Products Research, New York, NY, USA (July/August 2012)
Poster Presentation
Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, Dalhousie University, Halifax, Nova Scotia (August 2012)
Oral Presentation
Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2012)
Poster Presentation
Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

New Brunswick Health Research Foundation 3rd Annual Health Research Conference, Moncton, New Brunswick (November 2011)
Poster Presentation
Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, UNBSJ Campus, Saint John, New Brunswick (August 2011)
Oral & Poster Presentation

Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Interprofessional Health Research Day, Saint John Regional Hospital (March 2011)
Poster Presentation

Title: "Bioactive Natural Products from North Atlantic Algal Endophytes"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

New Brunswick Health Research Foundation 2rd Annual Health Research Conference, Moncton, New Brunswick (November 2010)
Poster Presentation

Title: "Isolation of culturable endophytes from green and brown algae as a source of new bioactive compounds"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray

Maritime Natural Products Conference, University of Prince Edward Island (September 2010)
Oral Presentation

Title: "NMR spectroscopic and bioassay screening of marine algae endophyte metabolites for new natural products."
Authors: Alexander D. Colquhoun; Justin D. Stewart; Katelyn T. Ellsworth; **Andrew J. Flewelling**; John A. Johnson; Christopher A. Gray

Title: Endophytes of marine algae from the Bay of Fundy, Canada.
Authors: Katelyn T. Ellsworth; **Andrew J. Flewelling**; John A. Johnson; Christopher A. Gray*

Interprofessional Health Research Day, Saint John Regional Hospital (March 2010)
Poster Presentation

Title: "Isolation of Endophytes from Green and Brown Algae"
Authors: **Andrew J. Flewelling**, John A. Johnson, and Christopher A. Gray