

**Culture-dependency of fungal-endophyte isolation and implications for natural
products research**

by

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ABSTRACT

The assemblage of fungi isolated from plant tissues is dependent on the isolation conditions and cannot be assumed to represent the entire endophytic community. A comparison of the two media most used for endophyte isolation [2.0% malt extract (MEA), and 2.4% potato dextrose (PDA)] using three plants (*Pyrola elliptica*, *Pinus strobus*, and *Sarracenia purpurea*) consistently showed that PDA had a higher isolation frequency of endophytic fungi, and a greater number of morphologically distinct fungi recovered per sampling unit. Estimations of the total number of distinct fungi isolable using PDA also exceeded those for MEA. Culture-independent methods of detecting endophytic fungi were assessed for *S. purpurea* and revealed an additional fungal species, *Penicillium spinulosum*, that had not been isolated on PDA or MEA. Extracts of the isolated fungi were evaluated in antimicrobial assays where there was no difference in the proportion of active extracts observed between the two isolation media used.

DEDICATION

To the memory of my grandparents, William Sanford Rice and Hazel Jemima Davis

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List of Abbreviations

ATCC	American type culture collection
BHI	Brain-heart infusion
BLAST	Basic local alignment search tool
bp	Base-pairs
CAMH	Cation-adjusted Mueller Hinton
CDA	Czapek-dox agar
CFU	Colony-forming unit(s)
CMA	Cornmeal agar
COL	Catalogue of Life
D	Simpson's diversity index value
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EtOAc	Ethyl acetate
EtOH	Ethanol
GPS	Global Positioning System
ICE	Incidence coverage estimator
ITS	Internal transcribed spacer
MEA	Malt extract agar
MEB	Malt extract broth
MeOH	Methanol
NBM	New Brunswick Museum
NCBI	National Center for Biotechnology Information
NMR	Nuclear magnetic resonance
NPRG	Natural Products Research Group
OD	Optical density
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
RFU	Relative fluorescence unit(s)
S	Species richness
SDB	Sabaroud dextrose broth
SDI	Simpson's Diversity Index
UNBSJ	University of New Brunswick Saint John
UNITE	User-friendly Nordic ITS ectomycorrhizal database
YM	Yeast mold
YPer	Yeast protein extraction reagent

1. Introduction

1.1 Endophytic fungi

Endophytic fungi, or endophytes, are fungi that colonize the internal tissues of plants for all or part of their life cycle without causing signs or symptoms of disease at the time of their isolation.¹⁻³ In the broadest sense, endophytic fungi may participate in a mutualistic or symbiotic relationship with their host, be neutral to the plant, or even be latent pathogens that have colonized the plant and later cause an infection under certain conditions.¹⁻³ Endophytes are common in plants, and the vast majority of plants studied have been found to host at least one endophyte.^{1,4,5} Endophytes are defined by habitat not taxonomy, and any fungus living asymptotically within plant tissue, regardless of species, is an endophyte.¹⁻³

Endophytes have a variety of roles in plants. Some endophytes help their host plant tolerate unfavourable conditions such as high-salinity environments, with increased salt-tolerance being conferred on plants by introducing halotolerant endophytes, such as the introduction of *Piriformospora indica* to barley plants.⁶⁻⁹ In some cases, endophytes benefit their host by altering the expression of host genes that affect the plant's response to unfavourable conditions.¹⁰ Increased drought tolerance has been observed in wheat, maize, cabbage, and cacao plants inoculated with endophytes that alter host plant gene responses, delaying metabolic processes that would hasten the effects of dehydration.¹⁰⁻¹⁴ Endophytes also provide plants protection from herbivores. Endophytes of grasses are known to produce alkaloid chemicals that can render plants unpalatable or even toxic to herbivorous insects and mammals.¹⁵⁻¹⁸ Endophytes inhabiting conifers produce chemicals

toxic to spruce budworm larvae, a herbivorous species known to damage or kill balsam fir trees.¹⁹⁻²² Chemicals produced by endophytes can also protect the host plant from microbial pathogens. A study comparing endophytes to soil fungi found that endophytes more commonly produced chemicals toxic to microbial plant pathogens than did fungi living in the surrounding soil.²³

Endophytes can colonize their host in one of two ways. Vertical transmission, or seed transmission, occurs when endophyte spores or hyphae, contained in seeds, are passed down from parent plant to offspring.^{1,15,24} Some host plants benefit from vertical transmission of symbiotic endophytes, a phenomenon well documented in grasses.^{15,24} Alternatively, endophytes can colonize plant tissue horizontally, meaning from the environment; these associations begin via airborne spores, insect transmission, or colonization by hyphae from the rhizosphere (soil surrounding the roots).^{4,15,24} Endophytes that colonize plants by horizontal transmission depend on which fungi the plants is exposed to that are able to colonize the plant.^{4,15}

With the definition of the term endophyte being based on habitat, endophytic fungi are not limited to any taxonomic group within the kingdom fungi, and endophyte assemblages can act as a source of undiscovered fungal species.²⁵⁻²⁷ A wide range of estimates have been proposed for the number of fungal species that exist. The earliest published estimate of the kingdom fungi from the early 1800s did not provide a numerical value, but simply stated that fungal species could be as numerous and diverse as insect species.²⁸ Throughout the 1900s, the number of fungal species was estimated based on the ratio of terrestrial plants to unique fungal species, with various estimates ranging from 75 000 to 100 000 species.²⁹ In 1990, the first widely accepted numerical

estimate, also based on the ratio of terrestrial plants to unique fungal species, was estimated at 1.5 million.^{26,30} With the ability to detect fungal strains from environmental samples using molecular techniques, newer estimates have placed the fungal kingdom in excess of 6.2 million species.^{31–33} Even the most conservative of these estimates dwarfs all estimates of the number of fungal species that have actually been isolated, described, and named, and an indeterminate number of these undiscovered fungi could be living as endophytes.^{25,34}

In 2019, the Catalogue of Life (COL), a curated checklist of all named species, reported they had indexed 135 110 species of fungi.³⁵ The number of fungal species listed by the National Centre for Biotechnology Information (NCBI), a division of the U.S. Library of Medicine, is 152 926 species as of 2020.^{36,37} Considering the number of species estimated to exist, it is likely that less than 10% of the fungal species on earth have been isolated and described.

The disparity between the number of fungi that are believed to exist and the number of fungi that have been discovered is referred to as the isolation gap.^{25,38,39} The magnitude of this isolation gap means undiscovered microbial species must occupy many environmental niches, including many that live as endophytic fungi.^{25,27} The isolation gap is partly due to culture-dependency in microbial isolation.^{25,38,39} Culture-dependency means that the microbial strains isolated from environmental sources, including endophytic fungi, are dependent on the isolation conditions used.^{27,38–42} Isolated species of endophytic fungi, therefore, cannot be assumed to represent all species present in an assemblage.^{27,38–42}

Endophyte isolation is a culture-dependent process where some fungi may remain unisolated from plant tissue even after exhaustive attempts to culture them using multiple and varied isolation media and isolation techniques.^{27,34,38-41} The magnitude of microbial uncultivability was first noted in 1985 when Staley and Konopka observed that less than 1% of the bacterial cells they could see in environmental samples using microscopy could be grown in pure culture.⁴³ This disparity was dubbed “the great plate count anomaly” and, since then, attempts at bringing the other 99% into culture have been a recurring theme in microbiology.^{27,38,41,43-45} Recently, genetic research on endophyte assemblages has shown that the great plate count anomaly also applies to fungal communities.^{27,46,47}

Endophyte assemblages are of interest to researchers studying a variety of topics: fungal taxonomists as potential reservoirs of undiscovered species, ecologists as an important part of ecosystems and sometimes a predictor of plant fitness, and to natural products researchers for the natural products endophytes produce.^{11,46,48-54}

1.2 Endophytic fungi as a source of natural products

Natural products, chemicals produced by living organisms, play many roles in daily human life, including as medicines, fertilizers, food, and cosmetics.^{50,55} Natural products are also important to ecological processes, plant life, and microbial life as micronutrients, and growth factors.^{42,45,55}

Small molecule natural products have a wide variety of uses and chemical discovery efforts continue today.^{50,55} The need to continue natural products discovery is particularly evident in the antimicrobial field. Although synthetic chemistry has provided new avenues for drug development, the search for new natural products continues to be

one of the driving forces behind antimicrobial development.⁵⁵⁻⁵⁷ To fully explore the chemical diversity available from nature, researchers turn toward sources with unexplored biodiversity; one such source being endophytic fungi.^{46,49,51}

In many cases it is not the plant that produces the natural products of interest, but microbes, such as endophytes, that live within the plant. One such case of a natural product being produced by an endophytic fungus is that of the anticancer drug paclitaxel (Taxol®), which was found in an extract of a *Taxomyces andreanae* fungus isolated from a *Taxus brevifolia* tree.⁵⁸ Paclitaxel was a known natural product produced by the tree, but this discovery demonstrated that endophytic microorganisms living within a plant could be producing natural products of interest as well. Investigations of endophytic fungi for natural products discovery have been on the rise ever since.^{49,51,52,54,59} Part of the appeal of endophytic fungi to natural product researchers, is the vast amount of undiscovered diversity that remains within the kingdom.^{27,59}

The Natural Products Research Group (NPRG) at the University of New Brunswick – Saint John (UNBSJ) investigates plants and endophytic fungi for natural product production, particularly production of natural products with antimicrobial activity.⁶⁰⁻⁶⁵ Three of the plants the NPRG has investigated for natural products production include: *Pyrola elliptica*, *Pinus strobus*, and *Sarracenia purpurea*.⁶⁵⁻⁶⁷ While extracts from the plants themselves have been studied, the endophytes within these three plants have not been isolated and investigated for natural products biosynthesis by the NPRG, and therefore they were chosen for this study.

The working hypothesis among natural products researchers bioprospecting for new chemicals is that biological diversity and chemical diversity are linked: having a

greater number of potential biological sources to investigate will potentially lead to a greater number of new chemicals.^{27,49,68,69} To maximise the potential for chemical discovery the number of organisms studied should also be maximised.^{27,49,68,69}

1.3 Isolating endophytic fungi

Endophytic fungi are typically isolated by surface sterilizing plant tissue and isolating on solid media.^{70,71} Using this procedure ensures the fungi isolated are endophytic by using sterilants to destroy any fungi on the exterior surface of the plant.^{5,71,72} Plant tissue is immersed in one or more sterilants such as 5.25% sodium hypochlorite, 70% ethanol, 5% hydrogen peroxide, or 0.01% mercuric chloride, for a determined period of time to remove microbes from the outer surface of the plant.^{5,71,72} Effective surface sterilization, meaning killing or removing any fungi on the outside surface of the plant, is verified by streaking the intact plant tissue across the surface of a solid growth medium and monitoring for fungal growth.⁷¹ Plant samples are then cut into segments and fungi that grow from the cut edge of a surface sterilized plant segment in conjunction with the absence of fungal growth on the associated verification medium are considered endophytes.⁷¹ The surface sterilization procedure must be carefully developed for each plant as there is potential damage to the endophytes and the plant itself if the method and chemicals are too harsh.⁷¹ An effective surface sterilization procedure is one that minimizes contact time with sterilants, while killing all external fungi.⁷¹ A suitable surface sterilization procedure must be experimentally determined for each plant separately because of differences in plant structure and the fungi living there.⁷¹

Endophytic fungi are isolated from microbial assemblages of unknown taxonomic composition.^{25,27,40,71-73} Endophytes may remain unisolated or be damaged or killed by the surface sterilization process.^{5,71-73} Studies on cultivability of bacteria and fungi have shown it is unlikely that all microbial species present in a sample can be isolated using a single isolation procedure.^{39-41,43} This presents a problem for researchers planning to investigate endophytes for chemical discovery, because fungi that remain unisolated also remain uninvestigated for natural product production.^{27,34,40}

Fungi may remain unisolated from an environmental sample for a variety of reasons.^{25,38,44,45} There are many factors in a surface sterilization procedure that can affect cultivability.^{5,71,73-76} The choice of sterilant(s), choice of isolation medium, preparation of isolation medium, and even laboratory conditions such as temperature and humidity can all affect whether or not a specific fungus is able to grow.^{5,71,73,76,77} In the case of the lichen *Flavoparnelia caperata*, *Nemania* spp. could be isolated when the surface sterilization chemical used was 70% EtOH, but not when 0.4 % sodium hypochlorite was used.^{73,78} Slow growing fungi can also remain unisolated, as they become obscured by faster growing fungi which they compete with for both space and nutrients on the medium during isolation.^{71,79} There are also certain nutritional requirements that must be met for fungi to grow.^{1,25,38,44,80} Not every fungus has the same nutritional requirements but there are some commonalities. Fungi require a carbohydrate source and a nitrogen source to grow, and most strains studied reportedly grow best at a pH between five and six, and between 15 °C and 37 °C.⁷⁷

The simple fact that fungi are being cultured in an artificial environment, rather than *in situ*, puts some fungi at a chemical disadvantage. In addition to the carbohydrates

provided by the isolation medium, fungi can require additional chemicals in order to grow.³⁹ These additional chemicals, called growth factors, include things like siderophores which help with iron uptake.^{34,39,45} Supplementing growth media with siderophores has led to the discovery of new fungi, but growth factors vary between fungi, making it difficult to formulate a single growth medium for all fungi.^{39,77,81,82} Growth factors for endophytes often come from other microorganisms living in the same community, or even the host plant itself.^{39,77,81,82} Fungi that rely on these growth factors may remain unisolated if these chemical interactions are missing in a laboratory setting.³⁹

1.4 Isolation media for fungi

Media composition is an important factor in fungal cultivability. Procedural steps such as autoclaving media play a role by affecting the pH of solidifying agents of some media.⁷⁶ Many fungal collections are isolated using only one of a few general-purpose media for fungi, namely 2.0% malt-extract agar (MEA), 2.4% potato-dextrose agar (PDA), or, for single-celled fungi, Sabaroud-dextrose agar.^{74,77} Some media are selective, specifically designed to target the isolation of certain types of fungi, often those of clinical relevance.^{77,81,82} Others are more general, making them the preferred medium of researchers looking to isolate endophytes from an assemblage of unknown taxonomic composition.⁷⁷ The most commonly used medium for endophyte isolation is 2.0% MEA.^{83,84}

The prominence of 2.0% MEA in endophyte isolation is due to its long history of use, not the result of any comprehensive study on the effectiveness of a wide variety of isolation media.^{77,83} Had another medium been used in early endophyte research, that

medium may still be in heavy use today. While 2.4% PDA has managed to break through as a second general purpose isolation medium for fungi, it has not been determined if either medium is superior to the other in terms of number of distinct fungi isolated. The rationale behind the choice of isolation medium is rarely, if ever, mentioned in endophyte isolation research, and may simply be a matter of researcher preference in many cases. The most popular media for fungal isolation have attained this status due to circumstance, and historical precedent, not experimental inquiry.

Malt extract is considered a general-purpose isolation medium for fungi.^{77,83} Malt extract is a high-carbohydrate medium containing the water soluble components of malted barley.⁸⁴ The low pH (5.4 – 5.8) of malt extract suppresses most bacteria while allowing for fungal growth.⁷⁷ Bacto™ brand malt extract is advertised as having been “used successfully in microbial fermentation applications” and has been recommended for isolating fungi from marine environments.^{60,63,70,84} In some reviews and protocol papers 2.0% MEA is the recommended isolation medium for fungi for researchers in a variety of fields from ecology to natural products research.^{5,70,72,83} The recommendations to use 2.0% MEA over the various other media available are not based off experimental comparison, but simply that 2.0% MEA has been successfully used by mycologists to isolate fungi for decades.^{70–72,83}

Potato dextrose medium is a general-purpose medium for fungi made from an infusion of potatoes.^{74,75} It is a popular medium for promoting pigment development and spore formation to aid in identification of fungal species.⁷⁷ Like malt extract, it is slightly acidic and nutrient rich with dextrose and potato starch being the carbohydrate sources.^{75,85} Potato dextrose has a long history of use as a fungal growth medium with

references to its use dating back to 1908.⁸⁶ Becton-Dickinson describes their Difco™ brand of potato dextrose medium as being, “(for) isolation of yeasts and moulds”.⁸⁵

Both malt extract and potato dextrose can be combined with a solidifying agent such as agar to create a semi-solid surface for isolating endophytic fungi.^{76,77} Isolating endophytes from surface sterilized plant segments on a semi-solid surface allows for visual confirmation that the fungus is growing from a cut edge of the plant, and makes it easier to separate fungi into individual cultures when multiple fungi grow from a single plant piece. Growing fungi on a solid medium also allows for the observation of morphological characteristics used to differentiate fungi such as colony shape, texture, and growth pattern, that are not seen in liquid culture or *in situ*. While 2.0% MEA and 2.4% PDA are effective isolation media for a broad range of fungi, no experimental work has been done to determine if one can be used to isolate distinct fungi more efficiently than the other.

1.5 Identification and classification of fungi

Accurate taxonomic identification is an important step in studying endophytic fungi.⁸⁷ A taxonomic name can link an organism to existing knowledge about its ecological relevance, related species, and its chemical producing potential.⁸⁸ Despite the importance of taxonomic identifications there are several unanswered questions in the field of endophyte taxonomy. Species concepts in fungi are still a hotly debated topic, and numerous phylogenetic analyses have been conducted to resolve confusing families and genera, such as *Colletotrichum*, *Teratosphaeria*, and *Cladosporium*, genera that have all been found living as endophytes.⁸⁹⁻⁹² Before molecular techniques came to

prominence in the early 1990s the delineation of many common endophyte genera and species was accomplished by observing morphological characteristics, and, as a result, errors persist.^{25,88,88,93–96} One of these errors are that separate taxonomic names have frequently been given to the teleomorph and anamorph (sexual and asexual stages) of the same fungal species.⁹⁷ Single species names have frequently been applied to groupings of fungi that should be identified as multiple species, because of a naming error these species remain hidden from taxonomic records and are called cryptic species.^{89,91,92,97–100} Even among the well-studied genus *Lophodermium*, an endophyte of conifer trees, cryptic species have been revealed through genetic analysis.⁹⁹

Even with an accurate taxonomic identification the relationship between taxonomy and natural products production is not well understood, and while taxonomic identifications are a valuable communication tool, cultures should not be prioritized for natural products research based off a taxonomic identification alone.^{25,27,46,49,51,52}

As stated, fungi were initially classified taxonomically solely based on morphological characteristics.^{25,93,101} Fungi (in culture) are most easily identified by examining asexual spores (conidia) and spore bearing structures (conidiophores), but many endophytes do not sporulate *in situ* or in culture.^{93,101} When these reproductive structures are present, many fungi are still not easy to differentiate due to the similarity of these structures across closely related species.^{93,101} Hyphae have relatively few distinguishing characteristics comparatively, and therefore provide little information about the identity of the fungus making identification nearly impossible without reproductive structures or DNA sequences.^{93,101}

Today, fungal identifications are aided by DNA barcoding. DNA barcoding works by comparing short (400 – 800 base pair) characteristic sequences of a sample organism against sequences of known origin to identify the sample.^{94,95,102,103} The internal transcribed spacer (ITS) genetic region has been used as the barcode marker for fungi since the first primers for this region were developed in 1990 as it is highly variable between species, making identifications to the species level possible in many cases.^{94,95,104,105} The ITS region was formally chosen as the universal barcode marker for fungi by a consortium of mycologists in 2012.⁹⁵

In 2010 the User-friendly Nordic ITS Ectomycorrhizal (UNITE) database was launched in an attempt to reevaluate fungal taxonomy and fit species within a phylogenetic framework based on ITS sequences.^{106,107} UNITE places all fungal ITS sequences from GenBank, the world's most comprehensive collection of environmentally-sourced DNA sequences, into a massive phylogenetic framework.^{106,107} Highly related sequences are grouped into operational taxonomic units (OTUs) at a 97% similarity threshold and given a species hypothesis in an attempt to more accurately identify the fungal sequences uploaded to GenBank, which otherwise rely on the identification provided by the researcher uploading the sequence.^{37,87,106,107} While identifying fungal sequences by placing them in a phylogenetic framework may conflict with morphology-based identifications of fungi, the widespread adoption of tools like UNITE will ultimately lead to a more rigidly defined and systematic process for differentiating fungi and confirming taxonomic identifications.^{88,94,106} While UNITE has the ambitious goal of defining the ITS sequences of each species of fungi, the boundaries of many genera and families have

already been redrawn based on the information from smaller scale phylogenetic analyses.^{89,90,92,105,108,109}

Due to ambiguity at the species level, the presence of cryptic species, and the ever-changing understanding of the fungal tree-of-life, a taxonomic identification may not be the best way to prioritize fungi for natural products discovery research.^{110,111} Instead, a dereplication strategy for fungi isolated for natural products research should be designed to separate fungi into individual strains with different growth patterns and metabolic potentials.^{111,112} Fungi can be differentiated by comparing colony morphology on multiple media such as cornmeal or Czapek-Dox agar and other culture media made for the differentiation of specific groups of fungi.^{74,75,112,113}

1.6 Culture-independent methods of detecting fungi

A culture-independent analysis, the detection and identification of fungi without the need to isolate them, is possible using high-throughput molecular techniques.^{114,115} Culture-independent identification works similarly to the DNA barcoding procedure. In a culture-independent analysis DNA of the entire endophyte community, meta-DNA, is extracted directly from plant tissue and sequenced using a next-generation platform capable of sequencing multiple barcodes from a single sample, a process called meta-barcoding.¹¹⁶⁻¹¹⁹ Each of the individual barcodes are then matched to sequences in a database to identify the fungi.¹¹⁶⁻¹¹⁹ The ITS region, being the universal barcode marker for fungi, is the target gene region sequenced in most culture-independent analyses of fungi.^{94,95,105,116,120,121}

A significant amount of the natural products yet to be discovered from microbial sources are likely produced by strains that have never been cultured or identified.²⁷ By identifying the unisolated species in a community, efforts can be focused on isolating specific fungi using methods such as single cell sequencing, selective isolation media, diluted media, and specially developed isolation chambers such as the iChip.^{44,68,122–125} Sequencing of unisolated fungi also has the added benefit of helping to close the isolation gap, as many fungi identified in culture-independent analyses do not represent known species of fungi.^{27,38,40,42,115}

1.7 Research objectives

Natural product discovery efforts are reliant on biodiversity; having more biological sources to extract chemicals from increases the chances of discovering new chemistry.⁴² Researchers isolating fungi for natural products discovery must understand the factors that affect fungal isolation and should aim to maximize the efficiency of the isolation process to isolate as many unique fungi as possible for their sampling effort.¹²²

The objective of this thesis was to isolate and identify distinct endophytic fungi using two common isolation media, 2.0% malt-extract agar (MEA) and 2.4% potato dextrose agar (PDA) and determine which medium should be used for future endophyte isolation projects with the goal of efficiently isolating morphologically distinct endophytic fungi. Recommendations about which medium to use were based on the number of fungi isolated using each medium, measures of biodiversity, accumulation of distinct fungi, and estimations of the total number of cultivable fungi when using each medium.

Extracts from the fungi isolated on each medium were compared to determine if extracts from fungi isolated on one of the media were more likely to exhibit antimicrobial activity.

Methods for conducting a culture-independent analysis were also tested and an estimation of the endophyte community in *Sarracenia purpurea* was compared to the fungal species isolated from the plant.

2. Methods

2.1 Media preparation

Agar plates for endophyte isolation were prepared by mixing either Bacto™ malt extract medium (20 g/L), or Difco™ potato dextrose broth (24 g/L), and agar [14 g/L (Fisher Scientific Ltd., Ottawa, ON)] in distilled water. The fungal isolation media were autoclaved for 30 minutes at 121 °C before use. Autoclaved media were poured into sterile plastic petri dishes (100 × 15 mm, Fisher Scientific Ltd., Ottawa, ON) and allowed to solidify. Media plates were stored at 4 °C until use.

Each fungus isolated on 2.0% MEA and 2.4% PDA was subcultured and grown on Czapek-Dox agar (CDA), and cornmeal agar (CMA). Czapek-Dox agar was prepared by mixing Difco™ Czapek-Dox broth (35 g/L) (Becton Dickinson, Mississauga, ON) and agar [14 g/L (Fisher Scientific Ltd., Ottawa, ON)] in distilled water. Cornmeal agar was prepared by boiling 50 g yellow cornmeal (Purity, Markham, ON) in 1 L of distilled water for 1 hour. The solution was then left for 24 hours after which the cornmeal infusion was decanted, and the supernatant filtered through cheesecloth and diluted two-fold with distilled water before agar (14 g/L) was added. Czapek-Dox and cornmeal agar solutions were autoclaved for 30 minutes at 121 °C before being poured into sterile petri dishes (100 × 15 mm, Fisher Scientific Ltd., Ottawa, ON) and allowed to solidify. Media plates were stored at 4 °C until use.

Fungal cryopreservation medium (MexA agar) was prepared as described by Kjer et al. (2010) using Bacto™ malt extract medium (20 g/L), glycerol (62 g/L), agar (13 g/L), and yeast extract (0.1 g/L) in distilled water.⁷⁰ Cryopreservation medium was brought to

a boil and 2.5 mL was then transferred into 5 mL screw-cap mailing tubes (VWR International, Mississauga, ON), loosely capped, and autoclaved (121 °C, 30 minutes). Tubes were then tightly sealed and placed on their side until the medium solidified. Tubes of cryopreservation medium were stored at 4 °C until used.

2.2 Plant selection and collection

Plants previously found to produce chemicals with antimycobacterial activity by the Natural Products Research Group (NPRG, UNBSJ) were chosen for endophyte isolation.⁶⁵⁻⁶⁷ Plants were collected from sites in southern New Brunswick between August 2017 and January 2018 (Table 1). Voucher specimens were deposited at the New Brunswick Museum (NBM) in Saint John, New Brunswick (Table 1).

Table 1. Locations and voucher specimen numbers of host plants used for isolation of endophytic fungi

Plant species	Location	GPS coordinates	Voucher number
<i>Pinus strobus</i>	Mechanic Settlement, NB	45°43'58.1"N 65°15'58.9"W	NBM# VP-40988
<i>Pyrola elliptica</i>	Grand Bay-Westfield, NB	45°23'11.9"N 66°16'49.8"W	NBM# VP-40987
<i>Sarracenia purpurea</i>	Prince of Wales, NB	45°11'59.3"N 66°13'48.8"W	NBM# VP-40989

Pyrola elliptica was selected for endophyte isolation based on the antimycobacterial activity exhibited by fractions of a methanolic extract from the plant.⁶⁶ *Pinus strobus* was selected for endophyte isolation based on the isolation of the antimycobacterial diterpenes copalic acid and isopimaric acid from a methanolic extract of the plant.⁶⁷ *Sarracenia purpurea* was selected for endophyte isolation based on reports of antimycobacterial triterpenes having been isolated from a methanolic extract of the

plant.⁶⁵ In addition to the reports of antimycobacterial activity of extracts and their fractions, or compounds isolated from extracts, all three plants have a documented history of medicinal use among North-American First Nations communities.¹²⁶

Needles from *P. strobus* used for endophyte isolation were collected near Mechanic Settlement, New Brunswick. Leaves of *P. elliptica* were collected from a trail near Grand Bay-Westfield, New Brunswick. *S. purpurea* was collected for endophyte isolation from Prince of Wales, New Brunswick. Leaves from *P. strobus* and *S. purpurea* used for endophyte isolation were each collected from a single plant. Due to its smaller size and limited number of leaves, at two per plant, a total of 10 *P. elliptica* plants were used for endophyte isolation.

2.3 Surface sterilization method determination

A suitable surface sterilization method was determined for each plant separately. Leaves from each species were first rinsed under distilled water. Leaves were then placed into a 5.25% sodium hypochlorite solution for between 1 and 10 seconds, followed by immersion in sterile water for 10 seconds (Table 2). Leaves were then placed in 70% ethanol (ACS, Fisher Scientific Ltd., Ottawa, ON) for between 10 and 20 seconds, followed by a final rinse in sterile water for 10 seconds (Table 2). Surface sterilized leaves were then blotted dry on autoclaved paper towel. When surface sterilizing *S. purpurea*, one drop of Tween 80 was added to the sodium hypochlorite solution to reduce surface tension and improve sterilant surface contact through hairs on the surface of the leaf. Surface sterilization verification plates were prepared by rubbing a treated leaf across the surface of a 2.0% MEA plate which was then sealed with Parafilm™ (Bemis

Company Inc., Neenah, WI, USA) and monitored for growth of epiphytic fungi for six weeks. Leaves were cut into segments (approximately 0.5 cm × 0.5 cm, or 0.5 cm in length for needles of *P. strobus*) and placed on fresh 2.0% MEA (five segments per plate) and sealed with Parafilm™. This process was then repeated using 2.4% PDA for both the verification and isolation plates. Up to six different surface sterilization conditions were tested for each plant on both media. Fungi were considered endophytic when observed growing from the cut edge of a surface sterilized plant in conjunction with a clean verification plate. The potential effects of the surface sterilization process on the endophytes within each plant were minimized by choosing the surface sterilization method which minimized exposure times to sterilants while also preventing the growth of epiphytes on surface sterilization verification plates.

Table 2. Surface sterilization methods tested to determine a suitable endophyte isolation procedure for leaves of three host plants

Sterilization method	Seconds in 5.25% NaOCl¹	Seconds in sterile H₂O	Seconds in 70% Ethanol	Seconds in sterile H₂O*
1 ²	1	10	10	10
2	5	10	10	10
3	5	10	15	10
4	5	10	20	10
5	10	10	10	10
6	10	10	15	10

*Following surface sterilization leaves were blotted dry on autoclaved paper towel

¹One drop of Tween 80 added when surface sterilizing *S. purpurea*

²Only tested on *P. strobus* after other sterilization methods failed to allow for growth of endophytes

2.4 Endophyte isolation

Leaves from each plant were surface sterilized using the appropriate method determined for that plant. Each surface sterilized leaf/needle was smudged across the

surface of an 2.0% MEA plate to verify effective surface sterilization by monitoring for growth of epiphytic fungi. Plant pieces were cut and placed on 2.0% MEA (five segments per plate) as previously described. The endophyte isolation procedure was then repeated using 2.4% PDA in place of MEA. A total of 200 segments of leaves from each plant (100 on MEA and 100 on PDA) were used for endophyte isolation. Endophyte isolation plates were sealed with Parafilm™ and incubated at ambient temperature and light for up to 4 weeks.

When fungal hyphae were observed growing from a cut edge of a plant segment associated with a clean surface sterilization verification plate, they were subcultured onto the surface of fresh media. Fungi were further subcultured, as necessary, to obtain pure cultures.

2.5 Dereplication of endophytes

Each pure fungal culture was subcultured onto 2.0% MEA, 2.4% PDA, cornmeal agar (CMA), and Czapek-Dox agar (CDA). Duplicate endophytes were identified by comparing their colony morphology on all four of these media (Figure 1). Groups of endophytes were dereplicated if they were isolated from the same plant, on the same medium, and were identical on each of the four media with respect to colour, form, elevation, margin pattern, and texture. A representative culture of each morphologically distinct endophyte was selected and used for all subsequent work and duplicate cultures were counted and removed.

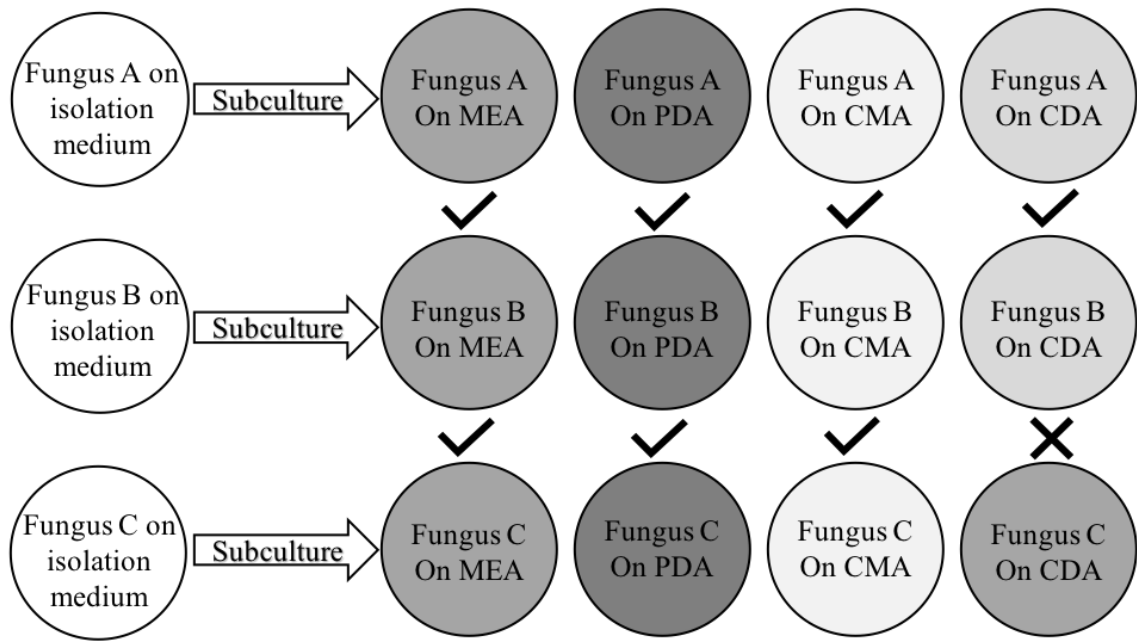


Figure 1. Example of morphological dereplication of three endophytic fungi (Fungus A, B, and C) using four media. Colony morphology is represented by shade for simplicity. In this example fungus A and B would be dereplicated into a single distinct endophyte. Due to morphological differences on CDA fungus C would also be kept as its own distinct endophyte.

2.6 Cryopreservation of fungal isolates

Distinct endophytes were cryopreserved according to the procedure described by Kjer et al.⁷⁰ Each distinct endophyte was subcultured onto 11 malt cryopreservation medium slants, 10 for long term storage and one to be used for fermentation and extraction (Section 2.11). After fungal cultures had grown to cover approximately 50% of the surface of the slants or had been growing on the slants for 4 weeks, they were cooled to 4 °C for two hours, then frozen at -20 °C for two hours, and finally, cryopreserved at -70 °C.

2.7 Endophyte identification

Endophytic fungi were identified through a combination of examination of morphological structures and DNA barcoding. Initially, a tentative identification was proposed based on colony and spore morphology followed by confirmation using DNA barcoding of the internal transcribed spacer (ITS) region.

Fungi were grown on 2.4% PDA and transferred by scraping hyphae (approximately 1 mg) into sterile 1.5 mL Eppendorf tubes to which Lysis buffer AP1 (400 μ L) from the DNeasy® plant mini kit (Qiagen, Toronto, ON) was added. Fungi were homogenized in the buffer using a VWR handheld homogenizer for 30 seconds. RNase A (4 μ L; Qiagen, Toronto, ON) was added and DNA was extracted by following steps eight to nineteen of the DNeasy® plant mini kit protocol.¹²⁷ The final elution of DNA was done using two 50 μ L aliquots of elution buffer AE (Qiagen, Toronto, ON). Extracted DNA was stored at -20 °C until needed.

The ITS region of the extracted fungal DNA was amplified by the polymerase chain reaction (PCR) using the ITS 1F and ITS 4 primers (5 μ mol) (Table 3) and a HotStar Taq master mix kit (Qiagen, Toronto, ON) in 68 μ L reactions.^{95,104} PCR was run in a MyCycler Thermal Cycler (Bio-Rad Laboratories Ltd., Mississauga, ON) using the following parameters: initial activation at 95 °C for 5 minutes, followed by 35 cycles of denaturation (95 °C, 1 minute), annealing (49 °C, 30 seconds), and extension (72 °C, 1 minute), followed by a final extension at 72 °C for 10 minutes. PCR products were stored at -20 °C until use.

Table 3. Primer sequences

Primer	Direction	Target regions	Primer sequence
ITS 1F	Forward	ITS1 and ITS2	5' CTTGGTCATTTAGAGGAAGTAA 3'
ITS 4	Reverse	ITS1 and ITS2	5' TCCTCCGCTTATTGATATGC 3'

PCR products were verified by gel electrophoresis. Agarose gels (0.5%) were prepared by adding 0.5 g agarose to 50 mL tris-borate-EDTA (TBE) buffer (Fisher Scientific, Ottawa, ON) and heating until all agarose melted. After the agarose solution had cooled for 5 minutes SYBR[®] Safe gel stain [5 µL (Invitrogen, Burlington, ON)] was added and the gel solidified in a gel casting tray with well combs added. Samples of amplified DNA (10 µL) and a 100-2000 bp DNA ladder [5 µL (Invitrogen, Burlington, ON)] were each added to separate wells and electrophoresis run for 25 minutes at 120 V in TBE buffer. Images of the gels were taken using a ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories, Mississauga, ON) and viewed using Bio-Rad Image Lab software.

Successfully amplified fungal DNA, as evidenced by the presence of a band in gel electrophoresis corresponding to an approximate 550 bp strand, was submitted for Sanger Sequencing (Génome Québec, Montreal, QC) along with the ITS 1F and ITS 4 primers. When both were obtained, forward and reverse sequences were aligned in a pairwise alignment using BioEdit Sequence Alignment Editor 7.0.5.3 creating a single consensus sequence of the ITS region.

In cases where DNA could not be successfully extracted, amplified, and sequenced using the DNeasy[®] plant mini kit after two attempts, a Yeast DNA Extraction Kit was used (Thermo Scientific[™], Ottawa, ON). Fungal material was prepared and homogenized as above except yeast protein extraction reagent (YPer) was used in place

of AP1 buffer. The DNA extraction was then performed according to the manufacturer's instructions. At the end of the extraction DNA was dissolved in 100 μ L AE buffer (Qiagen, Toronto, ON) and stored at -20 $^{\circ}$ C until needed for PCR.

When DNA could not be successfully extracted and amplified after two attempts with the DNEasy plant mini kit or the Yeast DNA Extraction Kit, a cetrimonium bromide (CTAB) based extraction method modified from Healey et al. was used.¹²⁸ CTAB extraction buffer was prepared as described by Healey et al. Fungal material was prepared and homogenized as above except CTAB extraction buffer (500 μ L) was used in place of AP1 buffer. The homogenate was incubated at 65 $^{\circ}$ C for 30 minutes and centrifuged at 5000 \times g for 5 minutes. The supernatant was recovered and extracted with 500 μ L chloroform: isopropyl alcohol (24:1). The mixture was centrifuged at 5000 \times g for 10 minutes and the aqueous layer transferred to a new 1.5 mL Eppendorf tube. RNase A (5 μ L) was added and mixed by vortex (30 seconds). The mixture was incubated at 37 $^{\circ}$ C for 15 minutes. The mixture was then extracted a second time with 500 μ L chloroform: isopropyl alcohol (24:1). The mixture was centrifuged at 5000 \times g for 10 minutes and the aqueous layer transferred to a new 1.5 mL Eppendorf tube. Sodium chloride solution (5 M, 250 μ L) was added and mixed by vortex (30 seconds). Cold ethanol (95%, -20 $^{\circ}$ C, 750 μ L) was added and mixed by vortex (30 seconds). The mixture was incubated at -20 $^{\circ}$ C for one hour after which DNA was precipitated by centrifuge (5000 \times g for 10 minutes) and the supernatant decanted away. The DNA pellet was washed with ethanol (70%, 300 μ L), air-dried, and resuspended in 100 μ L AE buffer (Qiagen, Toronto, ON). Extracted DNA was stored at -20 $^{\circ}$ C until needed for PCR.

Sequences obtained from each endophyte were aligned to sequences in GenBank (National Center for Biotechnology Information (NCBI), US National Library of Medicine, Bethesda, MD, USA) using the basic local alignment search tool (BLAST; NCBI; U.S. National Library of Medicine). Sequences were also aligned to curated reference sequences for fungal species from the User-friendly Nordic ITS Ectomycorrhizal Database (UNITE) using BLAST+.¹⁰⁷ Sequences were also matched with a UNITE species hypothesis (a grouping of highly similar, publicly available ITS sequences) using the UNITE online search tool.^{106,107} Decisions on fungal identification were made to the lowest possible taxonomic level with information from examination of culture and spore morphology as well as annotations of matched sequences from DNA barcoding. An analysis of sequences from high-throughput sequencing studies on fungi determined sequences as short as 100 bp can be used effectively for DNA barcoding, because of this a 100 bp query length was chosen as the threshold for a valid DNA barcoding comparison.¹²⁹ Species level identifications were made when 97% of base pairs or more matched a reference sequence.^{106,130} In cases where fungi were matched to multiple species over the 97% threshold fungi were identified to the level of genus. Thresholds for higher level identifications were set at 94% for genus, 89% for family, and 81% for order.¹³¹ Where possible reference sequences curated by UNITE were used for DNA barcoding, however the assignment of reference sequences for known fungal species is incomplete. In cases where results from a BLAST search of the GenBank database returned a genus or species for which there was no curated reference sequence available DNA barcoding results from sequences available on GenBank had to be used.

After a taxonomic identification was confirmed the ITS sequence of each fungus was uploaded to GenBank and assigned an accession number (Appendix IV).

2.8 Estimating distinct culturable fungi

Accumulation curves were produced for the distinct endophytes isolated from each of the three host plants using both media types by random resampling of the 100 plant segments used for each isolation condition using the R package Vegan©. Normally referred to as species accumulation curves, the curves were instead calculated to show the accumulation of morphologically distinct endophytes, which would include cultures of the same species that grow differently.

The estimated number of culturable distinct endophytes from each plant, using each medium was calculated using three non-parametric species richness estimators: the Chao 2 estimate (bias-corrected), incidence coverage estimator (ICE), and the second-order Jackknife estimator. As with the accumulation curves, the number of culturable distinct fungi, rather than number of culturable species, was estimated. All richness estimates were calculated using the program EstimateS© as well as Simpson's diversity index (SDI) values. The SDI is a value between 0 and 1 representing the biodiversity based on both number of distinct strains in a dataset and the evenness of their isolation frequencies; a higher value indicates a more biodiverse assemblage.

2.9 Reagents, Cultures, and growth media for fermentation and bioassays

Ethyl acetate (EtOAc) and methanol (MeOH) were ACS reagent grade purchased from Fisher Scientific (Ottawa, ON, Canada). Methanol- d_4 was purchased from

MilliporeSigma (Oakville, ON, Canada). All microbial cultures used were ATCC[®] certified from Cedarlane[®] (Burlington, ON, Canada).

Malt extract broth (2.0%, MEB) was prepared using Bacto[™] malt extract medium (20 g/L) in distilled water. Malt broth (100 mL) was then poured into 250 mL Erlenmeyer flasks, stoppered with foam plugs, and autoclaved for 30 minutes at 121 °C. Autoclaved broth was stored at room temperature until use within 24 hrs.

Cation-adjusted Mueller Hinton II (2.2%, CAMH) broth was prepared by mixing 2.2 g CAMH medium (BBL[™], Difco, Fisher Scientific, Ottawa, ON, Canada) in 100 mL of double distilled water. Bacto[™] brain-heart infusion (3.7%, BHI) broth was prepared by mixing 3.7 g BHI medium (BBL[™], Difco, Fisher Scientific, Ottawa, ON, Canada) in 100 mL of double distilled water. Sabaroud dextrose broth (3.0%, SDB) was prepared by mixing 3.0 g Sabaroud dextrose medium (BBL[™], Difco, Fisher Scientific, Ottawa, ON, Canada) in 100 mL of double distilled water. Yeast-mold broth (2.1%, YM) was prepared by mixing 2.1 g YM medium (BBL[™], Difco, Fisher Scientific, Ottawa, ON, Canada) in 100 mL of double distilled water. All media were made in 100 mL media bottles, autoclaved at 121 °C for 30 minutes and sterile media stored at 4 °C until use.

2.10 Fermentation and extraction of fungi

One cryopreserved specimen of each endophyte was removed from cryopreservation by thawing the culture at -20 °C for two hours, then at 4 °C for two hours, then at room temperature for two hours, and finally subculturing onto 2.0% MEA.¹⁰⁵ Fungi were subcultured from MEA into 100 mL 2.0% MEB in 250 mL Erlenmeyer flasks for fermentation. The flasks were stoppered with foam plugs and

shaken at 150 rpm in the dark at 20-23 °C for fourteen days. Between three and fifteen 100 mL cultures of each fungus were fermented and extracted to produce a minimum of 2.5 mg of refined extract. On day 14, liquid cultures of fungi were sonicated for 30 seconds, filtered through cotton wool, and extracted three times with EtOAc (30 mL per 100 mL of liquid culture). Each extract was concentrated *in vacuo* before being transferred to a 3-dram vial using reagent grade methanol (MeOH) then concentrated *in vacuo*. Each freeze-dried extract was dissolved in 2.0 mL of pure water and loaded onto a 2 g C18 column (Sep-Pak[®], Waters, Mississauga, ON) pre-conditioned with 14 mL MeOH and equilibrated with 14 mL pure water. The samples were then washed with 14 mL pure water. Material that could not be transferred to columns using water was then transferred with 2 mL MeOH. Refined extracts were then eluted from the columns using 14 mL MeOH and concentrated *in vacuo*. Extracts were stored at -20 °C until used for antimicrobial assays.

2.11 Antifungal assay

All antimicrobial assays were performed in a Labconco Class II biological safety cabinet (Labconco, Kansas City, MS, USA).

Antifungal activity of each refined extract (100 µg/mL) was tested against *Candida albicans* (ATCC 14053) and *Saccharomyces cerevisiae* (ATCC 9763) in microbroth dilution assays. Stock solutions of each extract in dimethyl sulfoxide (DMSO; 5 mg/mL) were diluted to 4% DMSO in 3.0% SDB for *C. albicans* or 2.1% YM broth for *S. cerevisiae*. Extract test solutions (100 µL) were transferred to non-peripheral wells of clear, non-tissue cultured, 96-well microtitre plates in triplicate. Each plate also contained

three blank wells (200 μ L 2% DMSO in broth), three negative control wells (100 μ L 4% DMSO in broth), and three positive control wells. Positive controls were tested in triplicate (*C. albicans*: 2.5 μ g/mL Nystatin, *S. cerevisiae*: 2.5 μ g/mL Amphotericin B). The negative control, positive control, and sample wells were inoculated with 100 μ L of a 1.0×10^6 CFU/mL suspension of the test organism in the appropriate broth (SDB for *C. albicans*, YM broth for *S. cerevisiae*) resulting in a final cell concentration of 5.0×10^5 CFU/mL in each inoculated well. To reduce evaporation sterile water (200 μ L) was added to the perimeter wells of each plate.

Immediately after cells were added the optical density (OD) at 600 nm of each well was recorded on a molecular devices Emax microplate reader and subtracted from a second measurement after 24 hours of incubation in the dark at 37 $^{\circ}$ C to calculate Δ OD. The average Δ OD reading of the blank wells was subtracted from all other wells to account for absorbance of media. Percentage inhibition of fungal growth in each well was defined as:

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

2.12 Antibacterial assay

Antibacterial activity of each extract was tested at the same concentration as noted above against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), and *Enterococcus faecium* (ATCC 35667) using the same procedure as the antifungal assay using either 2.2% CAMH broth (*E. coli*, *S. aureus*, and *P. aeruginosa*) or 3.7% BHI broth (*E. faecium*). Final positive control concentrations for antimicrobial assays were as follows: 1.25 μ g/mL gentamicin (*E. coli*),

0.625 µg/mL erythromycin (*S. aureus*), 10 µg/mL gentamicin (*P. aeruginosa*), and 0.625 µg/mL tetracycline (*E. faecium*).

2.13 Antimycobacterial assay

Antimycobacterial activity of each extract was tested against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *Mycobacterium smegmatis* (ATCC 700048) in microplate resazurin assays as previously described.¹³² Stock solutions of each extract in DMSO (5 mg/mL) were diluted to 4% DMSO in Middlebrook 7H9 broth (BBL™, MGIT™, Becton Dickinson, Mississauga, ON) and tested against each organism in black, non-tissue cultured 96 well plates in triplicate at 100 µg/mL along with a negative well to control for autofluorescence of the sample. Positive control concentrations were 0.1 µg/mL rifampin for *M. tuberculosis* H37Ra and 1.25 µg/mL ciprofloxacin for *M. smegmatis*. All antimycobacterial assay plates were incubated at 37 °C in the dark. *M. tuberculosis* H37Ra plates were incubated in 5% CO₂. After two days (*M. smegmatis*) or four days (*M. tuberculosis* H37Ra) 50 µL resazurin (1.25 µg/mL; 5% Tween 80) was added to each well. After an additional 24-hour incubation period each plate was measured fluorometrically in relative fluorescence units (RFU) on an EM microplate spectrofluorometer (SpectraMax®, Gemini™, Molecular Devices, San Jose, CA, USA) at 37 °C. Percentage inhibition of mycobacterial growth in each well was defined as:

$$[1 - (\text{mean control and test well RFU} / \text{mean negative control well RFU})] \times 100$$

2.14 Data analysis of bioactivity data

All statistical analyses were run in GraphPad Prism version 8.2.1 (GraphPad, San Diego, CA, USA). Each 96-well assay plate was tested in an ordinary one-way ANOVA ($\alpha = 0.05$) to test for significant differences between samples and the negative control. Unpaired t-tests ($\alpha = 0.05$) were run to check each percentage inhibition value against the negative control without correcting for multiple comparisons. A chi-square test ($df = 1$, $\alpha = 0.05$) was used to test for associations between presence or absence of significant antimicrobial activity of extracts, and the isolation medium of the source fungus.

2.15 Culture-independent analysis

A culture-independent analysis of the endophyte assemblage in each plant was attempted. During endophyte isolation (Section 2.4) additional leaf segments from each plant were cut and placed in 1.5 mL microcentrifuge tubes (approximately 100 mg tissue per tube).

Two types of DNA extraction of the leaf material were attempted. Leaf segments were homogenized by shaking with a sterile glass bead at 100 Hz for two minutes in 500 μ L either AP1 buffer (DNeasy plant mini kit) or CTAB extraction buffer (CTAB protocol). DNA was extracted from leaf segments using the DNeasy Plant Mini Kit as previously described. Leaf segments were also extracted using the CTAB-based extraction buffer and protocol modified from Healey et al. described in section 2.7.¹²⁸

Extraction products were verified using a NanoDrop™ 2000 spectrometer. A single sample of DNA extracted from *S. purpurea* using the modified CTAB protocol was sent to GeneWiz (South Plainfield, NJ, USA) for sequencing using an Illumina®

MiSeq platform in a specialized fungal diversity assay using a proprietary set of ITS primers as a pilot study.

Results from GeneWiz were returned fully processed with forward and reverse reads paired using the Quantitative Insights Into Molecular Ecology (QIIME) data analysis package, chimeric sequences removed using the UCHIME algorithm, and effective sequences grouped into operational taxonomic units (OTUs), groupings of highly similar sequences representing distinct taxa, using the clustering program VSEARCH (1.9.6).^{133–135}

Sequences representing each of the OTUs detected by GeneWiz were aligned to sequences in the UNITE database and GenBank using BLAST. Sequences representing each OTU were also aligned to the ITS sequences of fungi isolated from *S. purpurea* in section 2.4 using BLAST+ 2.9.0.

3. Results

3.1 Surface sterilization of plants

The surface sterilization method used for each plant was that which minimized the exposure time to sterilants and allowed for the growth of endophytes while preventing growth of epiphytic fungi on surface sterilization verification plates (Table 4). When surface sterilizing *S. purpurea*, two drops of Tween 80 were added to the sodium hypochlorite solution as a surfactant to promote surface contact through trichome hairs.

Table 4. Surface sterilization methods used for isolating endophytic fungi from medicinal plants

Plant species	Tissue	Surface sterilization method*
<i>P. strobus</i>	Needles	1 s sodium hypochlorite, 10 s 70% EtOH
<i>P. elliptica</i>	Leaves	10 s sodium hypochlorite, 10 s 70% EtOH
<i>S. purpurea</i>	Leaves	10 s sodium hypochlorite (w/Tween 80), 15 s 70% EtOH

*Leaves were rinsed in sterile water for 10 seconds after each sterilant, surface sterilized leaves were then blotted dry on autoclaved paper towel

3.2 Fungi isolated from medicinal plants

In total, 286 endophytes were isolated from 600 plant segments from all three host plant species for an overall isolation frequency of 47.7%. The isolation frequencies by growth medium were 42.7% from plant segments on 2.0% MEA and 52.7% from plant segments on 2.4% PDA (Table 55). *Pinus strobus* had the lowest isolation frequency in comparison to the other two plants with a total of 6.5% when compared to 61.5% for *P. elliptica* and 75.0% for *S. purpurea*.

Each endophyte was grown on four different media and compared morphologically to remove duplicates. This morphological dereplication step was used to prevent repeated attempts at extracting natural products from the same fungus and was

also necessary for the estimation of the number of distinct endophytic fungi isolable on each medium. After removing duplicates from the collection, a total of 71 distinct endophytes were found: nine from *P. strobus*, 17 from *P. elliptica*, and 45 from *S. purpurea* (Table 5). Fungi isolated on 2.0% MEA were not dereplicated with fungi isolated on 2.4% PDA.

Table 5. Number of endophytic fungi isolated³ from three host plants.

Plant name	2.0% MEA ¹		2.4% PDA ²		Total	
	Isolated ³	Distinct ⁴	Isolated ³	Distinct ⁴	Isolated ³	Distinct ⁴
<i>P. elliptica</i>	57	7	66	10	123	17
<i>P. strobus</i>	3	2	10	7	13	9
<i>S. purpurea</i>	68	20	82	25	150	45
Total	128	29	158	42	286	71

¹Malt extract agar

²Potato dextrose agar

³From 200 pieces of each plant (100 on MEA, 100 on PDA)

⁴Endophytes from the same plant, isolated on the same medium that were morphologically identical when compared on four media (CDA, CMA, MEA, and PDA) were dereplicated and one distinct representative copy was kept

3.3 Identification of distinct endophytic fungi

Distinct endophytic fungi were identified using a combination of molecular barcoding and morphological structures. In total, 63 of the 71 distinct endophytic fungi were assigned a taxonomic identification with 42 being identified to the species level, 16 to the genus level, and two to the family level, while two endophytes could only be identified as a member of Ascomycota, the phylum of fungi that includes most endophytic fungi (Tables 6-11).^{136,137}

Only 13 of the 71 distinct endophytes isolated produced spores on one or more of the four media they were grown on. Without spores, spore-bearing structures, or fruiting

bodies fungi have few identifiable characteristics. Of these 13 fungi, six produced dark-pigmented, segmented ascospores consistent with the genus *Alternaria*; the *Alternaria* assignments were further confirmed by DNA barcoding results which also led to species-level identifications. One fungus produced dark, ovoid spores consistent with either the genus *Chaetomium* or *Cladosporium*, using DNA barcoding the identity was confirmed to be *Cladosporium cladosporioides*. Three fungi had long, ellipsoid ascospores, each containing eight distinct conidia in rows of two, these spores were consistent with a *Physalospora* fungus that was confirmed by DNA barcoding to be *Physalospora vaccinii*, with 99% of base pairs matching the reference sequence assigned by UNITE (Accession number: FJ603608) and a query length of between 476 and 480 base pairs in each case. Two sporulating fungi, *Leptospora rubella*, and *Ramularia* sp. had round spores consistent with many taxa, these identifications were therefore assigned based on DNA barcoding results. The remaining 58 sterile or unicellular fungi were assigned taxonomic identifications solely based on the results of DNA barcoding.

Sequences of the fungal ITS region available from GenBank were used for DNA barcoding. An independent service, UNITE, aims to use the data available in GenBank to fit new sequences into phylogenetic framework and help researchers identify their samples without relying on the identifications proposed by other mycologists for a sequence.^{107,138} Identifications based on a curated reference sequence from the UNITE database were preferred when assigning identifications.

Several fungal species were identified multiple times from the collection of distinct fungi isolated on the same medium from the same plant, such as *Alternaria*

alternata, which was isolated five times on 2.4% PDA from *S. purpurea*, but due to unique culture morphology, multiple copies of this species were kept in the collection.

In cases where two or more distinct fungi were assigned the same taxonomic identification the identifier was appended with a roman numeral. In cases where no identification could be determined due to a lack of DNA sequence or morphological features, endophytes were identified using a brief morphological description.

Table 6. DNA barcoding results and taxonomic identification of endophytes isolated from *P. elliptica* on MEA

Endophyte code	Sequence length (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-105A	601	JQ948292	<i>Colletotrichum fioriniae</i>	506	100	<i>Colletotrichum fioriniae</i> I
BRD3-105B	596	JQ948292	<i>Colletotrichum fioriniae</i>	506	100	<i>Colletotrichum fioriniae</i> II
BRD3-105M	663	JN692543	<i>Phyllosticta pyrolae</i>	569	99	<i>Phyllosticta pyrolae</i> I
BRD3-113C	No sequence	-	-	-	-	White-filamentous
BRD3-113P	575	KU516491	Ascomycota sp.	491	100	Sordariomycetes sp.
BRD3-113Y	No sequence	-	-	-	-	White yeast II
BRD3-124A	595	JQ948402	<i>Colletotrichum godetiae</i>	504	100	<i>Colletotrichum godetiae</i>

Table 7. DNA barcoding results and taxonomic identification of endophytes isolated from *P. elliptica* on PDA

Endophyte code	Sequence (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-107A	601	JX658441	<i>Daldinia decipiens</i>	526	97	<i>Daldinia</i> sp.
BRD3-111A	No sequence	-	-	-	-	Beige yeast
BRD3-111B	587	GU227829	<i>Colletotrichum lineola</i>	515	100	<i>Colletotrichum lineola</i>
BRD3-111E	666	JN692543	<i>Phyllosticta pyrolae</i>	569	99	<i>Phyllosticta pyrolae</i> II
BRD3-111J	577	MT560377	<i>Gibberella acuminata</i>	577	100	<i>Gibberella acuminata</i>
BRD3-111K	595	JQ948292	<i>Colletotrichum fioriniae</i>	506	100	<i>Colletotrichum fioriniae</i> III
BRD3-111N	610	JN979424	<i>Hypoxyylon fuscum</i>	537	100	<i>Hypoxyylon fuscum</i>
BRD3-111T	588	KT214565	<i>Chaetomium globosum</i>	490	100	<i>Chaetomium globosum</i>
BRD3-112E	No sequence	-	-	-	-	White yeast I
BRD3-125A	548	KC867794	<i>Ramularia</i> sp.	459	100	<i>Ramularia</i> sp.

Table 8. DNA barcoding results and taxonomic identification of endophytes isolated from *P. strobus* on MEA

Endophyte code	Sequence length (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-155A	738	KY576897	<i>Lophodermium nitens</i>	432	96	<i>Lophodermium</i> sp. III
BRD3-155C	741	MH457132	<i>Lophodermium nitens</i>	726	98	<i>Lophodermium nitens</i> II

Table 9. DNA barcoding results and taxonomic identification of endophytes isolated from *P. strobus* on PDA

Endophyte code	Sequence length (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-154A	No sequence	-	-	-	-	White yeast III
BRD3-154C	746	FJ861987	<i>Lophodermium pini-excelsae</i>	431	98	<i>Lophodermium pini-excelsae</i>
BRD3-154E	749	KY576897	<i>Lophodermium nitens</i>	433	95	<i>Lophodermium</i> sp. I
BRD3-154F	738	KY576897	<i>Lophodermium nitens</i>	433	96	<i>Lophodermium</i> sp. II
BRD3-154H	No sequence	-	-	-	-	Brown filamentous
BRD3-154J	645	MG722803	<i>Naganishia liquefaciens</i>	532	100	<i>Naganishia liquefaciens</i>
BRD3-154L	690	MH457132	<i>Lophodermium nitens</i>	681	97	<i>Lophodermium nitens</i> I

Table 10. DNA barcoding results and taxonomic identification of endophytes isolated from *S. purpurea* on MEA

Endophyte code	Sequence length (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-079A	552	FJ603608	<i>Physalospora vaccinii</i>	476	100	<i>Physalospora vaccinii</i> III
BRD3-079B	585	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> V
BRD3-079E	564	MH790419	<i>Cladosporium cladosporioides</i>	564	100	<i>Cladosporium cladosporioides</i>
BRD3-079G	593	FJ603608	<i>Physalospora vaccinii</i>	476	100	<i>Physalospora vaccinii</i> IV
BRD3-082B	538	MH855281	<i>Godronia cassandrae</i>	522	100	<i>Godronia</i> sp. II
BRD3-082C	561	MF943022	<i>Hypoxylon</i> sp.	555	95	<i>Hypoxylon</i> sp. III
BRD3-082M	588	JX010230	<i>Colletotrichum kahawae</i>	485	100	<i>Colletotrichum kahawae</i> II
BRD3-082O	565	FJ603608	<i>Physalospora vaccinii</i>	476	100	<i>Physalospora vaccinii</i> V
BRD3-083A	551	FJ603608	<i>Physalospora vaccinii</i>	476	100	<i>Physalospora vaccinii</i> VI
BRD3-083D	559	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	516	94	<i>Pseudoteratosphaeria</i> sp. I
BRD3-083K	570	KM186831	<i>Bryochiton</i> sp.	479	95	<i>Bryochiton</i> sp.
BRD3-083P	560	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	516	94	<i>Pseudoteratosphaeria</i> sp. II
BRD3-083W	719	HQ211705	Ascomycota sp.	429	95	Ascomycota sp. I
BRD3-083Y	571	KX928830	Pleosporales sp.	454	93	Pleosporales sp.
BRD3-084B	584	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> VI
BRD3-084F	494	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	472	94	<i>Pseudoteratosphaeria</i> sp. III
BRD3-084I	543	MH855281	<i>Godronia cassandrae</i>	523	100	<i>Godronia</i> sp. III
BRD3-115A	566	NR_155967	<i>Zasmidium fructigenum</i>	554	100	<i>Zasmidium fructigenum</i> I
BRD3-126B	No sequence	-	-	-	-	Black filamentous
BRD3-126C	566	NR_155967	<i>Zasmidium fructigenum</i>	554	100	<i>Zasmidium fructigenum</i> II

Table 11. DNA barcoding results and taxonomic identification of endophytes isolated from *S. purpurea* on PDA

Endophyte code	Sequence length (bp)	Reference sequence	Identity of reference sequence	Query length (bp)	Match (%)	Taxonomic identification assigned to endophyte
BRD3-078A	532	JX010230	<i>Colletotrichum kahawae</i>	485	100	<i>Colletotrichum kahawae</i> I
BRD3-078B	584	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> I
BRD3-078C	572	FJ603608	<i>Physalospora vaccinii</i>	480	100	<i>Physalospora vaccinii</i> I
BRD3-078E	585	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> II
BRD3-078F	583	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> III
BRD3-078I	549	FJ603608	<i>Physalospora vaccinii</i>	476	100	<i>Physalospora vaccinii</i> II
BRD3-078J	562	HM148081	<i>Cladosporium delicatulum</i>	498	100	<i>Cladosporium</i> sp.
BRD3-078K	538	MH855281	<i>Godronia cassandrae</i>	521	99	<i>Godronia</i> sp. I
BRD3-078L	577	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> IV
BRD3-078M	609	MZ571367	<i>Dothiora</i> sp.	609	100	<i>Dothiora</i> sp. II
BRD3-081C	536	MH859479	<i>Encoeliopsis rhododendri</i>	524	99	<i>Encoeliopsis rhododendri</i>
BRD3-081D	597	AY969550	Helotiales sp.	508	98	Helotiales sp.
BRD3-081G	632	HQ652066	<i>Candida pseudoglaebosa</i>	534	100	<i>Candida pseudoglaebosa</i>
BRD3-081H	615	KX496897	<i>Diaporthe</i> sp.	541	96	<i>Diaporthe</i> sp. I
BRD3-081I	643	MF943022	<i>Hypoxylon</i> sp.	555	95	<i>Hypoxylon</i> sp. I
BRD3-081J	642	MF943022	<i>Hypoxylon</i> sp.	555	95	<i>Hypoxylon</i> sp. II
BRD3-085E	593	KF465761	<i>Alternaria alternata</i>	481	100	<i>Alternaria alternata</i> VII
BRD3-085G	555	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	516	94	<i>Pseudoteratosphaeria</i> sp. IV
BRD3-085P	No sequence	-	-	-	-	Yellow yeast
BRD3-085R	549	MH178719	<i>Hypoxylon</i> sp.	513	96	<i>Hypoxylon</i> sp. IV
BRD3-085T	625	MZ493116	<i>Leptospora rubella</i>		97	<i>Leptospora rubella</i>
BRD3-090A	563	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	516	94	<i>Pseudoteratosphaeria</i> sp. V
BRD3-121B	539	MH855281	<i>Godronia cassandrae</i>	520	100	<i>Godronia</i> sp. IV
BRD3-121F	727	HQ211705	Ascomycota sp.	434	94	Ascomycota sp. II
BRD3-121J	559	AF309604	<i>Pseudoteratosphaeria ohnowa</i>	516	94	<i>Pseudoteratosphaeria</i> sp. VI

The relative abundance of distinct endophytes isolated from *P. elliptica* is shown in Figure 2. On 2.0% MEA, 46 of the 57 endophytes isolated were *Phyllosticta pyrolae*. The dominance of a single strain is shown by the Simpson's Diversity Index (SDI) value for endophytes isolated from *P. elliptica* on 2.0% MEA ($D = 0.347$). *Phyllosticta pyrolae* was also frequently isolated on 2.4% PDA along with *Colletotrichum lineola*, a species of fungus that was not isolated on 2.0% MEA. The presence of a second dominant strain in the endophytes isolated on 2.4% PDA, as well as the presence of more rare endophytes contributes to a higher SDI value ($D = 0.605$). The higher SDI value for endophytes isolated on 2.4% PDA shows that this collection is more biodiverse.



Figure 2. Relative abundance and isolation frequency of distinct endophytic fungi isolated from *Pyrola elliptica* leaves by isolation medium

As noted with endophytes from *Pyrola elliptica*, endophytes isolated on 2.4% PDA from *Pinus strobus* were more biodiverse ($D = 0.945$) than those isolated on 2.0% MEA ($D = 0.667$) (Figure 3). These measures of diversity are based on a small sample size as the isolation frequency from *P. strobus* was low (3% on MEA, 10% on PDA). No single distinct endophyte was dominant in this collection, but endophytes of the genus *Lophodermium* were the most abundant in the community making up nine of the 13 endophytes isolated from the plant.



Figure 3. Relative abundance and isolation frequency of distinct endophytic fungi isolated from *Pinus strobus* by isolation medium

The Simpson's Diversity index values for fungi isolated from *S. purpurea* were calculated to be 0.788 on 2.4% PDA and 0.880 on 2.0% MEA. There were fewer distinct endophytes isolated on MEA, but because the Simpson's index takes evenness of the populations into account fungi isolated on MEA have a higher value on the Simpson's index, due to the dominance of a single *Pseudoteratosphaeria* sp. endophyte repeatedly isolated on 2.4% PDA (Figure 4).

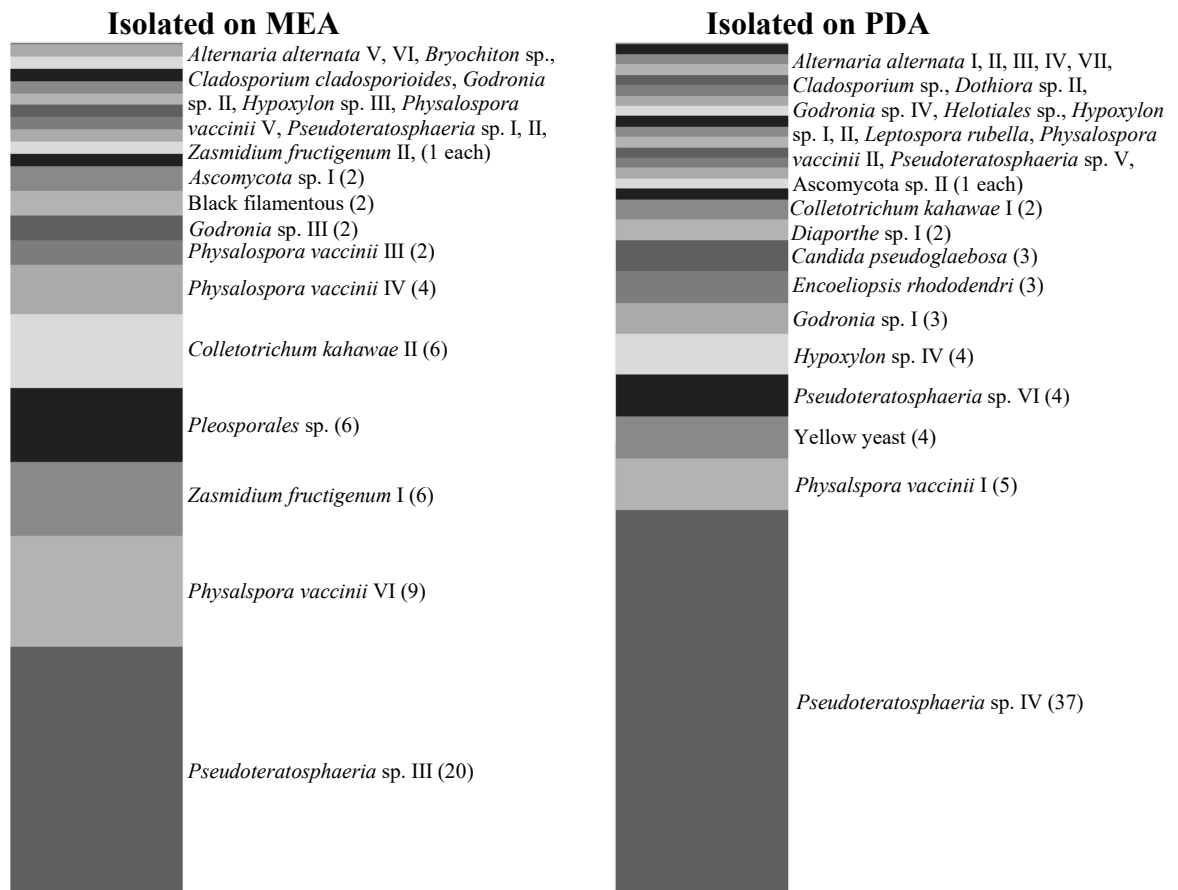


Figure 4. Relative abundance and isolation frequency of distinct endophytic fungi isolated from *Sarracenia purpurea* by isolation medium.

3.4 Accumulation of distinct endophytic fungi

The goal when isolating biological resources for natural products discovery research is to isolate as many distinct fungi as possible while minimizing sampling effort, maximizing the efficiency of the isolation process. When isolating fungi from cut plant pieces on solid media, sampling effort can be measured in number of plant segments. When sampling a microbial community, the number of species or distinct fungi isolated increases with sampling effort until all isolable fungi have been discovered. This relationship can be shown visually using a species accumulation curve; in this case, it is not the accumulation of species being measured however, but the accumulation of morphologically distinct endophytic fungi.

In total 17 distinct fungi were isolated from *P. elliptica*, seven using 2.0% MEA and 10 using 2.4% PDA. The accumulation of distinct fungi from *P. elliptica* is seen in Figure 5 with more distinct fungi expected per plant segment from 2.4% PDA up to 100 plant segments. Neither accumulation curve reaching an asymptote at 100 plant segments suggests that more distinct fungi could be isolated by continuing isolation attempts using the same methods.

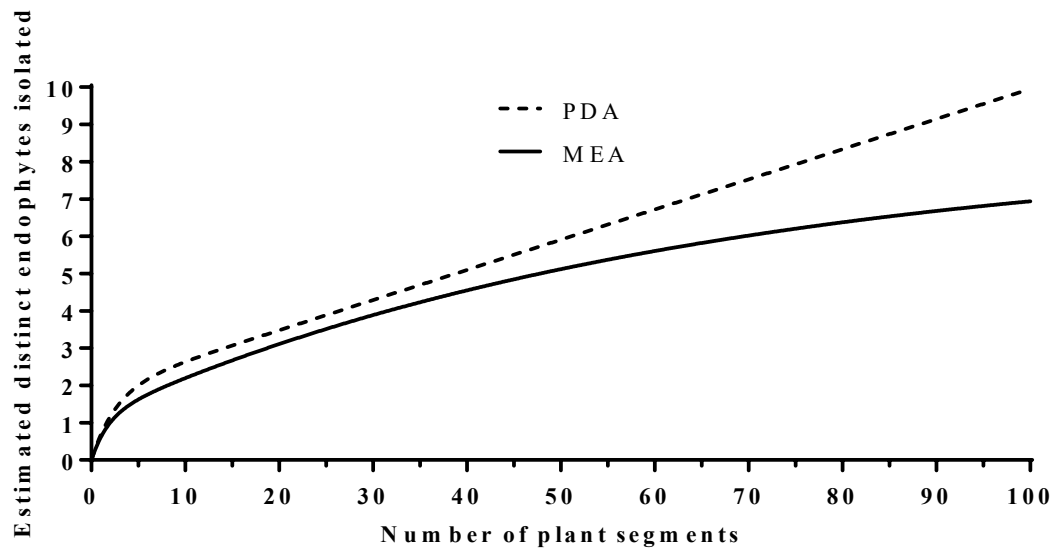


Figure 5. Estimated accumulation of distinct endophytic fungi from *P. elliptica* on 2.4% PDA and 2.0% MEA based on random resampling of plant segments used for endophyte isolation (100 permutations).

As seen in figure 6, as sampling effort of *P. strobus* increases few new distinct endophytic fungi are recovered, and visually the accumulation curve for endophytes isolated on 2.0% MEA does not look like it will rise past two or three distinct endophytes, an estimation which is reflected in Table 12. A horizontal or nearly horizontal accumulation curve suggests that an increase in sampling effort will lead to little or no increase in distinct endophyte cultures. The accumulation curve for distinct endophytes isolated from *P. strobus* on 2.4% PDA is consistently above the curve for MEA up to 100 segments, meaning that regardless of sample size more distinct fungi can be expected from isolation attempts using 2.4% PDA than 2.0% MEA.

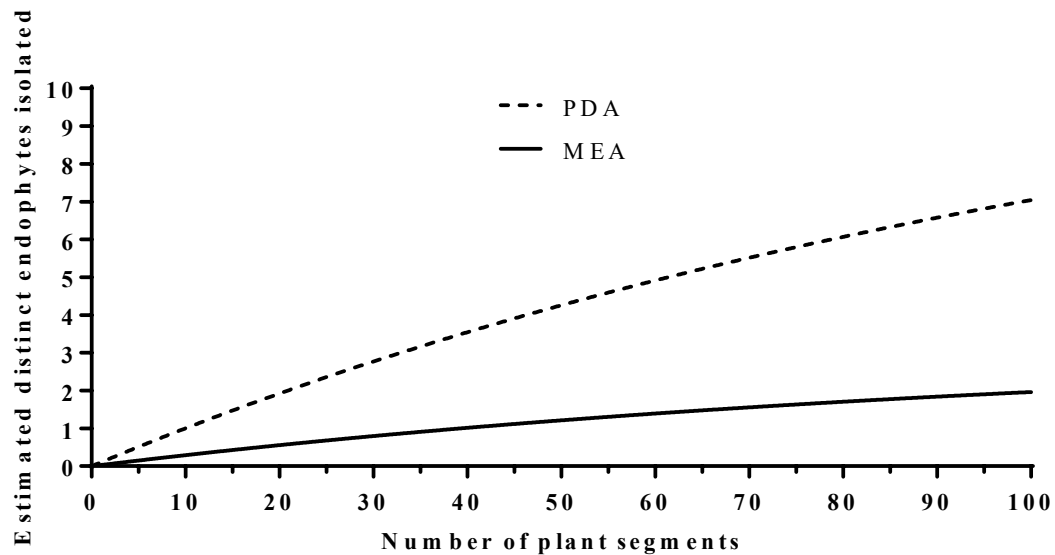


Figure 6. Estimated accumulation of distinct endophytic fungi from *P. strobus* on 2.4% PDA and 2.0% MEA based on random resampling of plant segments used for endophyte isolation (100 permutations).

The accumulation curves for distinct fungi from *S. purpurea* also show that regardless of sampling effort more distinct fungi can be expected when using 2.4% PDA as the isolation medium as opposed to 2.0% MEA up to 100 segments (Figure 7). Neither of the accumulation curves for distinct fungi isolated from *S. purpurea* reach a horizontal asymptote.

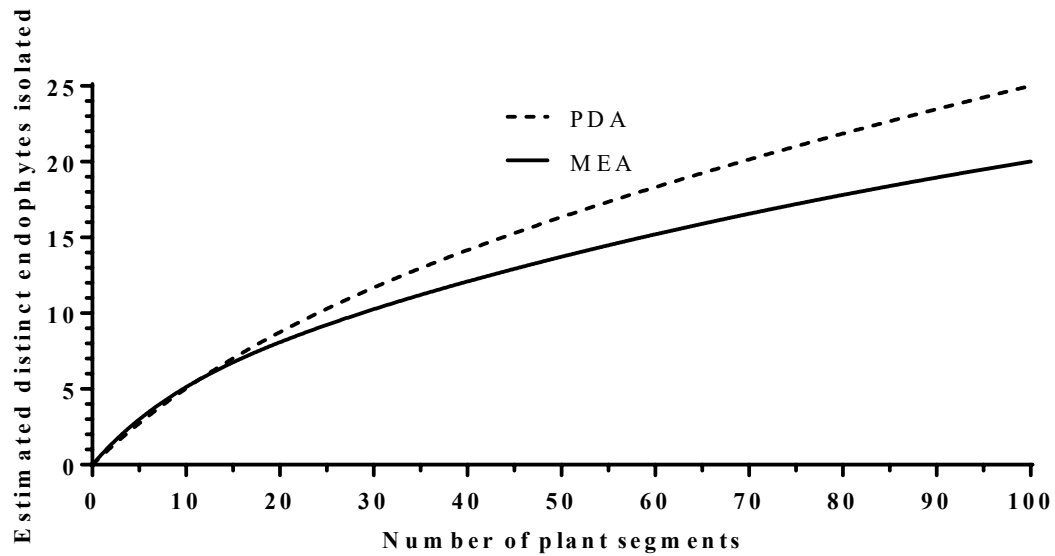


Figure 7. Estimated accumulation of distinct endophytic fungi from *S. purpurea* on 2.4% PDA and 2.0% MEA based on random resampling of plant segments used for endophyte isolation (100 permutations).

3.5 Richness estimates of distinct, culturable fungi

The number of distinct, culturable endophytic fungi in each plant was estimated using three non-parametric species richness estimators, the bias-corrected Chao 2 statistic, the incidence coverage estimator (ICE), and the second-order Jackknife estimator (Table 12). All three of these estimators base their results on the number of observations of a species rather than the number of individuals observed. An observation-based estimator is necessary because it is unknown if two identical fungi growing from separate cut plant edges should be counted as separate individuals or a single individual, cut in half during the isolation process. Because these estimates are based on cultured fungi, Table 12 shows an estimate of the number of distinct fungi, culturable using a particular isolation medium, with the particular surface sterilization method used, and is not an estimate of the actual number of distinct fungi living in the tissue.

Table 12. Estimated number of distinct culturable endophytes from tissues of *P. elliptica*, *P. strobus*, and *S. purpurea* when isolating on 2.0% MEA and 2.4% PDA using three non-parametric, observation-based species richness estimators.

Plant name	Chao 2		ICE		2nd-order Jackknife	
	MEA	PDA	MEA	PDA	MEA	PDA
<i>P. elliptica</i>	8.49	37.72	9.26	37.72	11.94	25.76
<i>P. strobus</i>	2.00	8.49	3.00	11.67	3.00	11.97
<i>S. purpurea</i>	28.91	59.65	35.43	59.57	35.82	52.61

Although the three richness estimators use different formulae leading to different values, the estimated number of isolable fungi on 2.4% PDA is greater than on 2.0% MEA within each plant regardless of which estimator is used. This means that more distinct fungi are culturable when using 2.4% PDA than when using 2.0% MEA.

3.6 Bioactivity of extracts from fungi isolated on PDA and MEA

The antimicrobial activity of an extract from each distinct fungus was tested in triplicate in a microplate assay against eight test organisms consisting of two Gram-positive bacteria (*E. faecium*, and *S. aureus*), two Gram-negative bacteria (*E. coli*, and *P. aeruginosa*), two mycobacteria (*M. smegmatis*, and *M. tuberculosis*) and two fungi (*C. albicans*, and *S. cerevisiae*) (Appendix VII). Extracts were deemed active at 100 µg/mL if they significantly inhibited the growth of the test pathogen when compared to an untreated control in a one-way unpaired t-test ($\alpha = 0.05$) (Table 13).

The number of extracts active against each test organism was compared between isolation medium of the source fungus in a chi-square test to determine if the probability an extract is active is dependent on the isolation medium of the source fungus.

A higher proportion of extracts from fungi isolated on 2.0% MEA were active against *E. coli*, and *P. aeruginosa* than extracts from fungi isolated on 2.4% PDA (Table 11). A higher proportion of extracts from fungi isolated using 2.4% PDA were active against the remaining test organisms (Table 13). When compared using chi-square analyses (d.f. = 1, N = 71, $\alpha = 0.05$) there is insufficient evidence to conclude that significant bioactivity of an extract is dependent on the isolation medium of the source fungus for any of the eight test organisms (Table 13).

Table 13. Number of extracts showing significant antimicrobial activity against eight test organisms by isolation medium, and results of Chi-Square tests (d.f. = 1, N = 71) comparing the proportion of active extracts from fungi isolated on 2.0% MEA to 2.4% PDA against eight test organisms

Test Organism	Extracts from fungi isolated on 2.0% MEA		Extracts from fungi isolated on 2.4% PDA		Chi square statistic (χ^2)	<i>p</i> -Value	Significant ($\alpha = 0.05$)
	Active	Inactive	Active	Inactive			
<i>E. faecium</i>	2	27	6	36	0.94	0.33	No
<i>S. aureus</i>	7	22	13	29	0.39	0.53	No
<i>M. smegmatis</i>	4	25	9	33	0.67	0.41	No
<i>M. tuberculosis</i>	16	13	29	13	1.42	0.23	No
<i>E. coli</i>	11	18	10	32	1.64	0.20	No
<i>P. aeruginosa</i>	11	18	15	27	0.04	0.85	No
<i>C. albicans</i>	1	28	7	35	3.00	0.083	No
<i>S. cerevisiae</i>	8	21	17	25	1.25	0.26	No
Combined	60	172	106	230	2.14	0.143	No

A Chi-square analysis was also used to compare the number of active extracts by medium for all test organisms together. A total of 29 extracts were obtained from fungi isolated on 2.0% MEA and tested against eight test organisms for a total of 232 tests, of which 60 (26%) were active. From the 42 fungi isolated on 2.4% PDA, there were 106 cases of significant antimicrobial activity from the 336 total tests of extracts (32%). Comparing these produces a non-significant result, χ^2 (d.f. = 1, $N = 568$) = 2.14, $p = 0.143$ (Table 13), meaning the effect of isolation medium of the source fungus on the proportion of extracts showing significant antimicrobial activity is insignificant at $\alpha = 0.05$.

3.7 Culture-independent analysis

GeneWiz obtained 70 432 effective sequences in the fungal diversity assay and clustered them into nine OTUs. Of these nine OTUs five, accounting for 70 220 sequences, matched the ITS region of the host plant, *Sarracenia purpurea* (Table 14). The remaining sequences ($n = 212$) were organized into four OTUs representing four fungal taxa (Table 14). Searches of the GenBank and UNITE databases matched these OTUs to *Colletotrichum* sp., *Pseudoteratosphaeria* sp., *Candida glabrosa*, and *Penicillium spinulosum*.

Table 14. Identities of operational taxonomic units detected in meta-gene analysis of *Sarracenia purpurea* and closest related isolated endophytes

OTU	Number of Sequences	Taxonomic Identity	Closest related isolated endophyte (% match)
OTU 1	69 959	<i>Sarracenia purpurea</i>	N/A
OTU 2	109	<i>Pseudoteratosphaeria</i> sp.	BRD3-090A (100%)
OTU 3	72	<i>Colletotrichum</i> sp.	BRD3-082M (100%)
OTU 4	65	<i>Sarracenia purpurea</i>	N/A
OTU 5	64	<i>Sarracenia purpurea</i>	N/A
OTU 6	49	<i>Penicillium spinulosum</i>	None
OTU 7	46	<i>Sarracenia purpurea</i>	N/A
OTU 8	39	<i>Sarracenia purpurea</i>	N/A
OTU 9	29	<i>Candida pseudoglaebosa</i>	BRD3-081G (100%)

Colletotrichum sp., *Pseudoteratosphaeria* sp., and *Candida glaebo*sa were isolated from *S. purpurea*. *Penicillium spinulosum* was the only fungal taxon detected in the culture-independent analysis that was not isolated from *S. purpurea*. Other species that had been isolated from *S. purpurea* in this study were not detected in the culture-independent analysis of meta-DNA from the plant (Figure 8).

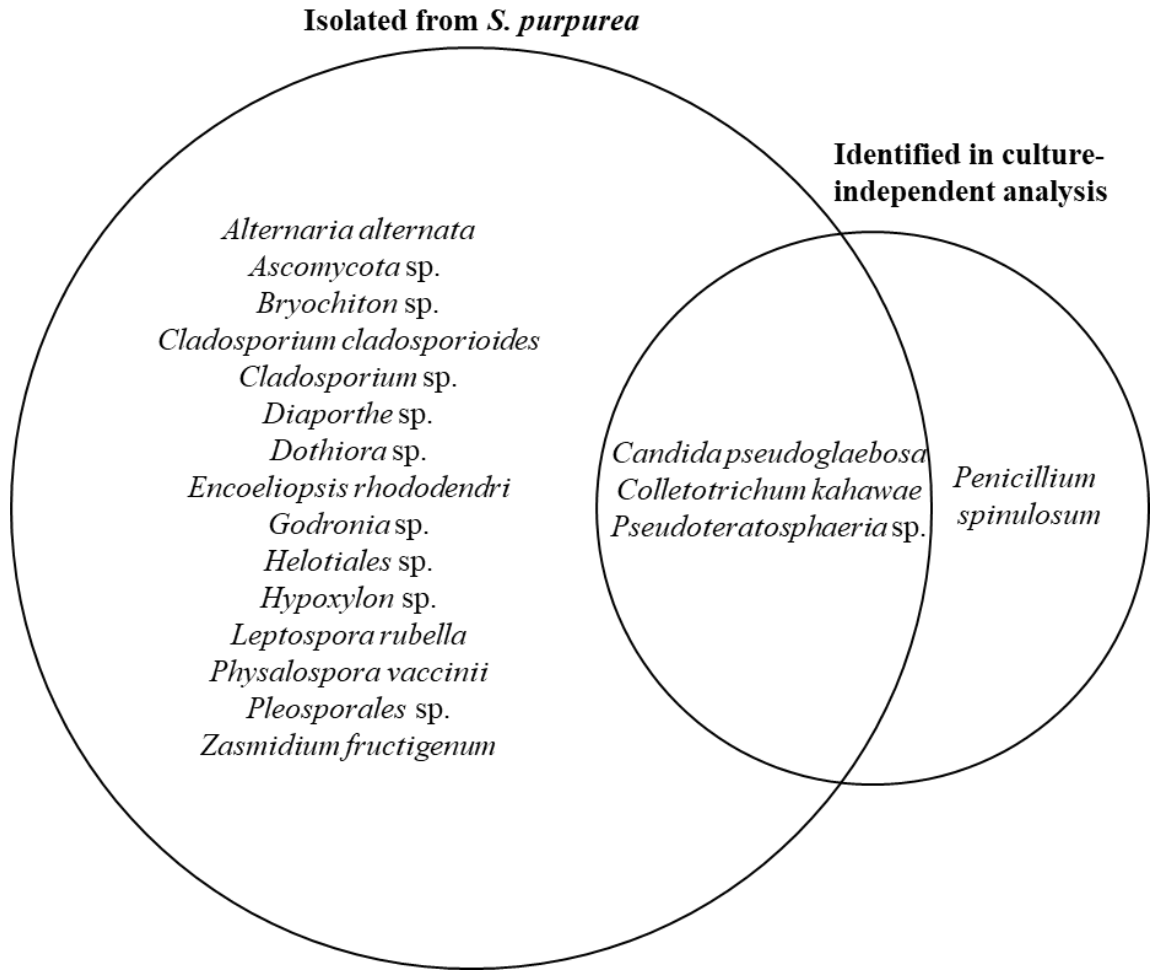


Figure 8. Endophytic fungal taxa isolated from *Sarracenia purpurea* and detected in a culture-independent analysis based on meta-barcoding of the ITS region.

4. Discussion

4.1 Endophytic fungi isolated from medicinal plants

Every plant has the potential to host its own unique microbial community, meaning the sampling effort required to isolate a distinct endophyte also varies by plant. The simplest measure of sampling effort in endophyte isolation is the isolation frequency, the percentage of sampling units (in this case leaf segments) that yield an endophyte.

Endophytes were isolated from 61.5% of *Pyrola elliptica* leaf segments. One distinct endophyte, identified as *Phyllosticta pyrolae*, dominated the collection making up 65.9% of the total endophytes isolated. Single, dominant endophytes, like this *Phyllosticta pyrolae* endophyte, have been observed in other collections, in some cases making up as many as 80% of the endophytes isolated from a plant.^{4,139,140}

This is the first report of endophytic fungi being isolated from *Pyrola elliptica*, more commonly known as the elliptic-leaved shinleaf. The only other report of endophytic fungi from a *Pyrola* species comes from Estonia where a total of 45 species, mainly basidiomycetes, were isolated from *Pyrola rotundifolia*.¹⁴¹ None of the endophytes isolated from *P. elliptica* in this study are known endophytes of *P. rotundifolia*.¹⁴¹

The endophyte community in *Pinus strobus* has been extensively investigated. *Lophodermium nitens* and other species from the *Lophodermium* genus, which were isolated in this study, are known endophytes of *P. strobus* and have been extensively investigated as producers of natural products.^{142–144} There are reports of other endophyte species isolated from *P. strobus* that were not found in this study including *Xylaria* spp.

and *Coccomyces strobiliferus*.^{145,146} The isolation of *Naganishia liquefaciens* reported in Table 9 is the first report of this species as an endophyte of *P. strobus*.

A study on *Pinus* endophytes found varying isolation frequencies of endophytes from segments of *P. strobus* needles, between 0.02% and 66.5%.¹⁴³ These varying isolation frequencies could be a result of the age of the needles sampled.¹⁴⁷ Endophyte infection rates of conifers are known to increase with needle age and decrease with height on the tree.¹⁴⁷⁻¹⁴⁹ In this study endophytes from *Pinus strobus* had the lowest isolation frequency of the three plants at 6.5%. The consequence of a low isolation frequency is that more sampling effort is required to isolate only a few distinct endophytes. No distinct endophyte was isolated from *P. strobus* more than twice, although the collection was dominated by endophytes from the genus *Lophodermium*.

An endophyte was isolated from 75% of the 200 total *S. purpurea* leaf segments sampled. *Sarracenia purpurea* was host to the greatest number of distinct fungi of the three plants, but the fungi are not evenly represented in the community.

Pseudoteratosphaeria sp. is dominant in the collection, making up 44.0% of the total fungi isolated from *S. purpurea*. Dominant species are common in endophyte isolation studies with a single species sometimes making up as many as 80% of the endophytes isolated.^{139,140,150}

There has been only one other report of endophytic fungi isolated from *S. purpurea*. *Paraconiothyrium* sp., *Phomopsis* sp., *Colletotrichum acutatum*, *Cryptosporiopsis actinidiae*, and two unidentified Basidiomycetes were isolated from leaves of *S. purpurea* collected in North Carolina.¹⁵¹ None of the same species were isolated from *S. purpurea* in this study. Additionally, despite reporting the isolation of

endophytes, that study did not investigate endophytes of *S. purpurea* for natural products production.

The three plants used in this study had a range of isolation frequencies between 6.5% and 75%. These differences show that isolation procedures, even when optimized for each plant individually, cannot change the fact that different plants host different fungi. Some may contain comparatively few distinct endophytes such as *P. strobus*, while others may host many fast-growing, easily isolable endophytes such as *S. purpurea*.

4.2 Endophyte identification

In this study each distinct fungus isolated from the three plants was identified using a combination of DNA barcoding and, where possible, examination of morphological structures. Even in cases where reproductive structures are present, identification of endophytes to species level without molecular analysis is sometimes impossible, as has been documented with species belonging to the genus *Lophodermium*.^{152,153} Of the 71 fungi isolated, eight could not be identified to any taxonomic level due to the absence of morphological structures or a sufficient ITS sequence. These eight fungi were given a description based on culture-morphology (Appendix II).

Taxonomic identification was not used as the determining factor in which endophytes were distinct, due to persistent errors in fungal taxonomic records. Several factors complicate the identification of fungi to the species level. Publications have been devoted to parsing out the species within a genus, such as with *Teratosphaeria* which through phylogenetic analysis has been split into multiple genera.^{92,108} In other cases,

such as with *Lophodermium nitens*, cryptic species or genetic variations within the species could provide more opportunities for chemical discovery.¹⁵⁴

4.3 Comparison of endophyte isolation media

Malt extract agar (2.0%, MEA) has been the isolation medium of choice for the isolation of endophytic and marine-derived fungi by the NPRG at UNBSJ.^{60–63,155} The goal of the NPRG when isolating fungi is to isolate as many distinct fungi as possible to use for natural products discovery efforts. The purpose of comparing the efficiency of 2.0% MEA and 2.4% PDA as isolation media was to determine if the NPRG should continue using 2.0% MEA for fungal isolation or if switching to 2.4% PDA would lead to the isolation of a greater number of distinct fungi without increasing sampling effort.

When isolating fungi for natural products research the same fungus is often isolated multiple times. These repeat isolations of the same fungus do not provide new opportunities for natural products discovery and amount to wasted sampling effort.^{25,27,122} Natural products researchers studying endophytic fungi should therefore not be concerned with biodiversity indices that account for evenness of the collection, but simply species richness, the raw count of how many unique fungi are isolated.^{27,156}

The isolation of distinct fungi can be visualized using a species accumulation curve. Researchers isolating endophytic fungi for natural products research can use accumulation curves to pilot their study and determine how many distinct endophytes they can expect to recover for their sampling effort.^{157–159} When an accumulation curve reaches a horizontal asymptote it can be inferred that all distinct fungi culturable using

that particular isolation method have been recovered, and continuing isolation attempts using the same method will not yield any new fungi.^{156,159,160}

Put simply, an accumulation curve measures the efficiency of an endophyte isolation process; how much sampling effort is required to continue to isolate new distinct fungi. In this study, the accumulation curves of distinct endophytes isolated on 2.4% PDA were consistently above the curves representing the accumulation of distinct endophytes isolated on 2.0% MEA for all three plants (Figures 5-7). This indicates that a greater number of distinct fungi can be expected when isolating fungi on 2.4% PDA, when sampling effort is equal, up to 100 sampling units (plant segments), for all three plants.

Interpolating culturable species richness from a species accumulation curve that has reached a horizontal asymptote is more accurate than extrapolating a rising curve.^{159,161,162} In this data set none of the accumulation curves for any plant/isolation medium combination, except *P. strobilus* on 2.0% MEA, reached a horizontal asymptote (Figures 5-7). Species richness estimators were then used to estimate the true number of distinct, culturable fungi from each plant on each medium. The estimators used (Chao 2, Jackknife 2, and ICE) are all non-parametric and incidence based, meaning they are based on counts of observations of a distinct fungus rather than number of individuals.^{156,157,159,162} Estimations of fungal populations must be incidence based as it is unknown whether two identical fungi growing from plant segments represent two individuals, one hyphal strand cut in half, or even multiple individuals that have undergone plasmogamy (fusion of cytoplasm).¹⁶¹ While there was variability between the three estimators, the number of distinct fungi estimated to be culturable on 2.4% PDA

was consistently greater than 2.0% MEA for every plant using each of the three species richness estimators (Table 12).

In terms of distinct isolates per sampling unit (plant segment) 2.4% PDA was consistently the more efficient of the two-isolation media. In future endophyte isolation projects where a single isolation medium is to be used 2.4% PDA is recommended over 2.0% MEA for recovering a greater number of morphologically distinct endophytic fungi without increasing sampling effort.

4.4 Impact of isolation medium on extract bioactivity

Extracts from a biological source are typically not pure but a mixture of many chemical compounds in varying concentrations.^{163–165} The complexity of these extracts creates a challenge when trying to isolate pure compounds, of interest due to novelty or biochemical activity.¹⁶³ One method of detecting potentially useful compounds in a complex mixture is through a method called bioactivity profiling.¹⁶⁶

Bioactivity profiling works by testing extracts against a panel of microorganisms to look for unique patterns of bioactivity that may indicate chemical novelty.¹⁶⁶ The NPRG has modified the bioactivity profiling method to work with a suite of eight test organisms.⁶⁴ No difference in the proportion of extracts showing antimicrobial activity was found between extracts from fungi isolated on 2.0% MEA and extracts from fungi isolated on 2.4% PDA overall, or for any of the eight test organisms ($\alpha = 0.05$). There is more to bioactivity profiling than simply presence/absence of activity and more work is needed to determine if different isolation media can lead to different outcomes for chemical discovery.

Antimicrobials are not the only use for new natural products, and other prioritization methods based on the chemical components of the extract such as NMR metabolomics or LC-HRMS spectroscopy have proven to be useful methods of detecting unknown structures.^{111,167} Further work on these extracts could involve comparing the likelihood of extracts from fungi isolated on 2.4% PDA versus 2.0% MEA to be outliers in a metabolomic study. The ultimate test of the effectiveness of the isolation media would be the discovery rate of new chemicals from fungi isolated on the two media.

4.5 Culture-independent analysis of endophytes in *Sarracenia purpurea*

The modified CTAB protocol was the only DNA extraction method which resulted in purified DNA meeting GeneWiz's sample submission requirements based on NanoDrop™ readings. After DNA extraction, a single sample of meta-DNA extracted from *S. purpurea* (which met the requirements) was sent for the specialized fungal diversity assay offered by GeneWiz. Due to the high proportion of plant sequences present in the results (Table 14) further metabarcoding was not performed on *S. purpurea* or the other two plants investigated.

While the experiment described above was successful in sequencing endophyte DNA extracted directly from host tissue there are some abnormalities. It should be expected that every strain cultured from the plant would also be detected in a highly sensitive culture-independent analysis, but this was not the case (Figure 8). There are two possible reasons for this.

Meta-barcoding fungal endophytes using the ITS region presents a unique challenge as the ITS region is also frequently used as a barcode marker for plants.¹⁶⁸⁻¹⁷⁰

The majority (99.3%) of the effective sequences detected in the fungal diversity assay in this study were from the host plant, *S. purpurea*. The earliest primer pair used for fungal barcoding (ITS 1 and ITS 4) was not designed to be specific to fungi.⁹⁵ Since then other primers have been developed that only amplify fungal DNA.^{94,104–106,120,121} In 2012 Toju et al. described the development of several primers with high fungal specificity and effective use in metabarcoding studies of fungi.¹⁰⁵ The presence of plant sequences in the results suggests that the primers used to amplify the ITS region for this study were not adequately fungal specific.^{94,95}

Beyond using up sequencing resources unnecessarily, the presence of plant DNA in such a high concentration can cause problems for the sequencing platform. Illumina technology uses a sequencing-by-synthesis technique where DNA fragments are bound to a flow cell and amplified using fluorescently tagged nucleotides.¹⁷¹ As the tagged nucleotides are added light is emitted and detected by photoreceptors in the sequencer.¹⁷¹ This technology is designed to sequence multiple strands of DNA simultaneously and works best with highly complex samples; a high concentration of a single sequence on the flow cell can cause rarer sequences to remain undetected.¹⁷²

To remedy these issues, ITS-based fungal diversity assays should be based on a fungal specific primer. Most sequencing services rely on researchers to provide their own primers for meta-barcoding studies. Due to their higher specificity for fungi the ITS 1F and ITS 4 primer pair or one of the primer pairs described in Toju et al. would be better candidates for metabarcoding plant-sourced fungi.^{104,105,120}

The other reason that fungi may remain undetected in a culture-independent analysis is harder to detect and remedy. The fungal cell wall is formed by a substance

called peptidoglycan, making it resistant to many DNA extraction techniques.¹⁷³ Consider the fungi isolated from the three plants; despite having pure cultures to work with, the DNA of eight of the 71 endophytes could not be successfully sequenced, even after several attempts using multiple DNA extraction procedures. DNA remaining unextracted in a culture-independent study means that not only do the affected strains of fungi remain unidentified but undetected altogether.

Even with a high-quality data set obtained with fungal-specific primers certain precautions need to be taken when interpreting the results of a meta-barcoding study. Although generally seen as a more powerful and thorough technique for fungal detection metabarcoding may still not contain sequences of every fungal strain present in a community due to issues with sample preparation.^{174,175} The issue of culture-dependency may then just become an issue of sequence-dependency.^{174,175} Furthermore, morphological dereplication based on physical characteristics would not be possible when working with sequence data alone. Although more fungal species may be uncovered using meta-barcoding the potential diversity within each species would remain unknown.

The intended use of culture-independent data in a natural products discovery context is to uncover unisolated microbial species such as the *Penicillium spinulosum* endophyte that was detected in the culture-independent analysis, but not isolated from *S. purpurea* (Figure 8). Targeting specific fungi during isolation using specially developed media, or cultivation *in situ* could result in the isolation of a previously unknown species.^{27,44,115,125}

4.6 Alternative methods of fungal endophyte isolation

While fungi could not be dereplicated morphologically across isolation media there were many cases of fungal species being isolated on 2.0% MEA that were not isolated on 2.4% PDA and vice versa, one notable instance being the isolation of *Colletotrichum lineola* 23 times from *P. elliptica* on 2.0% MEA, but never on 2.4% PDA (Table 6, Table 7). This suggests that even though 2.4% PDA was consistently the more efficient isolation medium there are fungi that may remain unisolated if only one medium is used. With unique fungi having been isolated on 2.0% MEA and 2.4% PDA, multiple media may be needed to capture all available diversity from a plant. The application of isolation techniques beyond surface sterilized plant segments may also lead to the isolation of unique fungi and possibly fungi that would be otherwise uncultivable.

Ultimately the isolation procedure should reflect the goal of the researchers. If one medium between 2.0% MEA and 2.4% PDA is to be chosen for an efficient endophyte isolation procedure with the goal of providing biological material for natural products discovery, all evidence points to 2.4% PDA being more effective for isolating the highest number of distinct fungi per unit of sampling effort.

The disparity between fungi that are detected in a culture-independent analysis and fungi that grow on artificial media is not an unsolvable one. Once called uncultivable, a more accurate term proposed for microbes making up the isolation gap has been proposed, yet-to-be-cultivated.³⁸ With the application of more advanced detection and cultivation techniques previously unisolated strains could be cultivated.^{25,38,44,45}

Dilution-to-extinction cultivation is a procedure that attempts to overcome the issue of dominant species by removing competition and separating individual cells.^{176,177} Dilution-to-extinction cultivation works by diluting homogenized plant material and isolating fungi in multi-well chambers in a high-throughput process.^{176,177} By diluting the homogenate, the colony forming unit (CFU) density of the homogenate is reduced removing chemical competition as well as allowing slower-growing fungi the space and nutrients to grow.^{176,177} This method has been shown to increase the culturable species richness of fungi from plant litter.¹⁷⁶

Artificial conditions used for isolating fungi in the laboratory may not be permissible to the growth of some strains of fungi.^{38,39,45,178} In the environment, fungi are exposed to a complex biochemical environment containing the metabolites of other microbes and their host. Some of these chemicals, referred to as growth factors, may be necessary for fungal growth.³⁸ Often these growth factors are not necessary for the fungus to survive but are necessary for it to grow. When the growth factors necessary for fungal proliferation are not present the fungi are considered viable-but-not-culturable and will remain unisolated until their growth conditions are met.³⁸

Microfluidic chips, such as the iChip, can be used to increase cultivability from a variety of environments using a combination of dilution-to-extinction and *in situ* methods.⁴⁴ The iChip consists of a plate with thousands of throughholes each filled with an agar plug.⁴⁴ The iChip can be submerged in a suspension of cells, capturing on average one cell per throughhole.⁴⁴ Initially developed for bacterial cultivation, the iChip could also be useful for isolating fungi or be modified to work *in situ* for cultivating microbes from soil communities.^{44,179} While the iChip has not been used to culture

filamentous fungi other microfluidic chips have been used to observe fungal growth *in situ*.¹⁷⁹

Another method of combatting the repeated isolation of dominant strains involves inhibiting growth overall. Fast-growing fungi are disproportionately affected by reduced nutrient density in growth media.^{125,180} Manipulating the concentration of conventional isolation media, including MEA, can increase culturable species diversity by allowing slower growing fungi the time and space to grow.^{125,180}

All the alternative isolation methods discussed so far are, like segment plating, blind processes. With the knowledge obtained from a culture-independent analysis researchers can target the isolation of specific fungi. Selective isolation media, isolation media specifically developed to meet the nutritional requirements of certain strains, are frequently employed in medical testing.^{75,77,81,123,181} Ideally a selective medium not only provides the conditions necessary for the targeted species to grow but also suppresses other strains.^{77,81,124} Even MEA and 2.4% PDA are selective to an extent, allowing fungi to grow while inhibiting the growth of most bacteria.^{75,83-85} Targeted isolation of specific environmental fungi can be more complicated. Attempts at targeted isolation using specially developed media have involved adjusting concentration, adding specific growth factors, adding antimicrobials to suppress other strains, and changing nutrient composition.¹⁸²

Other options for expanding the natural products research from endophytic fungi involve maximising the chemical potential of each biological source rather than collecting more sources. In a study on chemical yield from known producers of natural products chemical production was influenced by the composition of the fermentation

media.¹⁸³ Adding a stressor chemical such as ethanol to growth media has been found to affect chemical production.¹⁸³

While all of these isolation methods have strengths and weaknesses, the ideal microbial isolation procedure would be one that combines extinction cultivation to remove competition, with targeted isolation to capture fungi detected in a culture-independent analysis, and occurs *in situ* to permit biochemical interaction with the rest of the community. A microfluidic chip such as the iChip could be used to achieve this but has not been designed to work with filamentous fungi. Microfluidic chips have also not been designed to be embedded in plant tissue for the isolation of endophytes.

4.7 Conclusions and recommendations for future endophyte research

In this study endophytic fungi were isolated from *Pyrola elliptica*, *Pinus strobus*, and *Sarracenia purpurea* using two common isolation media for fungi, 2.0% malt extract and 2.4% potato dextrose. This is the first report of endophytic fungi from *P. elliptica*. Culture-dependency of endophyte isolation was investigated using two general-purpose isolation media. Potato dextrose agar led to the isolation of more distinct fungi than malt extract agar, and estimations of culturable species richness on 2.4% PDA exceeded 2.0% MEA.

Extracts from the endophytic fungi isolated were tested in antimicrobial susceptibility assays and no difference in the proportion of extracts from fungi isolated on the two media exhibiting antimicrobial activity was found.

In future endophyte isolation projects for natural products research 2.4% PDA should be chosen over 2.0% MEA for the isolation of a greater number of distinct fungi.

This recommendation is contingent on the idea that recovering a greater number of distinct fungi has positive implications for natural products discovery. The proportion of extracts exhibiting antimicrobial activity at 100 $\mu\text{g/mL}$ did not differ significantly by isolation medium.

Methods for conducting a culture-independent analysis were tested highlighting the importance of primer specificity. Preliminary results from a culture-independent analysis of *Sarracenia purpurea* found one example of a fungus present in the endophyte community, *Penicillium spinulosum*, that was not isolated on either medium. The ITS primers used in the fungal diversity assay, a proprietary set provided by GeneWiz, were not fungal specific, leading to the high amount of plant DNA sequences. Future work metabarcoding fungi from plants should use ITS primers with high specificity for fungi to avoid co-amplification of plant DNA.

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APPENDICES

Appendix I: Map of Plant locations

Locations of plants collected for endophyte isolation



Appendix II: Descriptions of endophytic fungi

Morphological descriptions of endophytic fungi under microscopy (400×) and colony morphology on four media

BRD3-078A – *Colletotrichum kahawae* I

Malt extract agar: Circular, dark green colony with a ring of white floccose on growing edge of culture, flat, filamentous margin, sterile. **Potato dextrose agar:** Circular, dark green colonies covered by thick, white floccose, flat, filamentous margin. **Cornmeal agar:** Circular, hyaline colony, thin white floccose, flat, filamentous margin, green spores. **Czapek-Dox agar:** Circular, white colony, smooth and flat surface, filamentous margin. **Microscopy:** septate hyphae.

BRD3-078B – *Alternaria alternata* I

Malt extract agar: Irregular shape, dark green colony with thin white floccose, flat, entire margin. **Potato dextrose agar:** Circular colony, green floccose, raised, entire margin with white edge. **Cornmeal agar:** Circular, dark green colony with thin white floccose throughout, flat, filamentous margin, sterile. **Czapek-Dox agar:** Circular colony, mostly white floccose with a dark green centre, raised, filamentous margin. **Microscopy:** Septate hyphae, ascospores (3 segments).

BRD3-078C – *Physalospora vaccinii* I

Malt extract agar: Filamentous colony, brown in centre fading to light grey on growing edge, flat, undulate margin, rough texture. **Potato dextrose agar:** Irregular shaped, light-brown colony, raised elevation, Undulate margin, rough texture. **Cornmeal agar:** Round light-brown colony, flat elevation, undulate margin, rough texture. **Czapek-Dox agar:** Yellow colony, does not grow large enough to discern shape, entire margin, flat elevation. **Microscopy:** Sterile, branching, hyaline, septate hyphae.

BRD3-078E – *Alternaria alternata* II

Malt extract agar: Irregular, dark-green colony with thin, white floccose, flat elevation, filamentous margin. **Potato dextrose agar:** Irregular, dark-green colony with white floccose, white and green rings around growing edge, raised elevation, entire margin. **Cornmeal agar:** Irregular, dark green colony with thin, white floccose, flat elevation, pigmented culture fades to hyaline at entire margin. **Czapek-Dox agar:** Irregular, white colony, entirely covered in thick, white floccose, raised elevation, filamentous margin. **Microscopy:** Hyaline, branching, septate hyphae, ascospores (3 segments)

BRD3-078F – *Alternaria alternata* III

Malt extract agar: Round, dark green colony with thin, white floccose fading to hyaline at growing edge, flat elevation, entire margin. **Potato dextrose agar:** Round, dark green colony covered by thick, white floccose with visible growth rings and white ring around growing edge, raised elevation, entire margin. **Cornmeal agar:** Round, dark-green colony covered by white floccose, flat elevation, filamentous margin. **Czapek-Dox agar:** Irregular, dark green colony completely covered by thick-white floccose, raised elevation, filamentous margin. **Microscopy:** Sterile, dark-pigmented, septate hyphae.

BRD3-078I – *Physalospora vaccinii* II

Malt extract agar: Mostly white, filamentous colony with some dark green segments, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, white colony, flat elevation, rough texture, undulate margin. **Cornmeal agar:** Irregular, segmented colony, segments light-brown or hyaline, flat elevation, undulate margin. **Czapek-Dox agar:** Punctiform, round colony, green in centre, surrounded by yellow ring, surrounded by outer ring fading from white to highly pigmented, flat elevation, entire margin. **Microscopy:** Large fusiform asci, each containing eight dark-pigmented ascospores arranged in rows of one or two, non-septate hyphae.

BRD3-078J – *Cladosporium* sp.

Malt extract agar: Irregular, black colony fading to hyaline near growing edge, flat elevation, entire margin. **Potato dextrose agar:** Round, dark-green colony with ring of white floccose near growing edge, raised elevation with undulating segments, white outer ring, entire margin. **Cornmeal agar:** Round, dark-green colony with ring of white floccose near growing edge, flat elevation, rough texture, dark outer ring fading to smooth (entire) hyaline margin. **Czapek-Dox agar:** Irregular, dark-green colony, umbonate elevation, filiform margin, white ring around growing edge on reverse side. **Microscopy:** Septate, pigmented hyphae giving rise to oval conidia from sides.

BRD3-078K – *Godronia* sp. I

Malt extract agar: Punctiform, irregular, colony, green in centre with blue-green ring around growing edge, raised elevation, filiform margin. **Potato dextrose agar:** Round colony, green in centre surrounded by white ring, raised elevation, produces oily droplets, Raised elevation, entire margin. **Cornmeal agar:** Round, green colony fading to hyaline at growing edge, umbonate elevation, entire margin. **Czapek-Dox agar:** Round, light-green colony with ring of thick, white floccose in centre, raised elevation, filiform margin. **Microscopy:** Non-septate, hyaline hyphae.

BRD3-078L – *Alternaria alternata* IV

Malt extract agar: Round, white colony with thick, white floccose in centre, fading to hyaline on growing edge, raised elevation, entire margin, greenish-yellow centre on reverse side. **Potato dextrose agar:** Circular, dark-green colony, with white growing edge, raised elevation, entire margin. **Cornmeal agar:** Round, green colony with thin, white floccose fading to hyaline at growing edge, flat elevation, entire margin. **Czapek-Dox agar:** Round, white colony with thick, white floccose, raised elevation, filiform margin, dark pigmented centre fading to yellow then white on reverse side. **Microscopy:** Hyaline, non-septate hyphae with pigmented ascospores (four segments each).

BRD3-078M – *Dothiora* sp. II

Malt extract agar: Irregular, black colony with white outer ring, flat elevation, glistening texture, entire margin. **Potato dextrose agar:** Irregular, green colony covered by white floccose, raised elevation, entire margin. **Cornmeal agar:** Irregular, dark-green colony, flat elevation, undulate margin. **Czapek-Dox agar:** Round, dark-green colony with thin, white floccose and white ring around growing edge, flat elevation, entire margin. **Microscopy:** Dark-pigmented hyphae, frequent septate cross walls, ellipsoid conidia.

BRD3-079A – *Physalospora vaccinii* III

Malt extract agar: Irregular, hyaline culture, flat elevation, undulate margin. **Potato dextrose agar:** Irregular, grey colony, rough texture, flat elevation, smooth margin. **Cornmeal agar:** Irregular brown colony, dark in centre with lighter outer ring, flat elevation, filiform margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Large fusiform asci, each containing eight dark-pigmented ascospores arranged in rows of one or two, non-septate hyphae.

BRD3-079B – *Alternaria alternata* V

Malt extract agar: Irregular dark green colony with thin, white floccose, white ring around growing edge, flat elevation, entire margin. **Potato dextrose agar:** Round, dark-green colony with white ring around growing edge, very thin white floccose mostly concentrated near centre of colony, flat elevation, entire margin. **Cornmeal agar:** Round, dark-green colony with white floccose, growth rings visible, flat elevation, entire margin. **Czapek-Dox agar:** Irregular, white colony with thick, white floccose, raised elevation, filiform margin. **Microscopy:** Dark pigmented, septate hyphae with ascospores (four segments each).

BRD3-079E – *Cladosporium cladosporioides*

Malt extract agar: Irregular, dark-green colony, fading to hyaline at growing edge, rough surface, flat elevation, entire margin. **Potato dextrose agar:** Round, green colony, fading to white at growing edge, rough surface, wrinkled near centre, raised elevation, entire margin. **Cornmeal agar:** Irregular, dark-green colony, fading to hyaline at growing edge, flat elevation, entire margin. **Czapek-Dox agar:** Irregular, grey colony, rough texture, raised elevation, undulate margin, pink with dark centre on reverse side. **Microscopy:** Non-septate hyphae with ellipsoid spores.

BRD3-079G – *Physalospora vaccinii* IV

Malt extract agar: Irregular white colony, with scattered white spots, dull surface, flat elevation, undulate margin. **Potato dextrose agar:** Irregular, grey colony, rough surface, flat elevation, undulate margin. **Cornmeal agar:** Round, hyaline culture, pigmented yellow near centre, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Large fusiform asci, each containing eight dark-pigmented ascospores arranged in rows of one or two, septate hyphae.

BRD3-081C – *Encoeliopsis rhododendri*

Malt extract agar: Irregular, light green colony, rough surface, raised elevation, filiform margin, brown on reverse side with white ring around growing edge. **Potato dextrose agar:** Round, light green colony, rough texture, raised elevation, entire margin. **Cornmeal agar:** Irregular, green colony, rough surface, flat elevation, entire margin, dark green on reverse side. **Czapek-Dox agar:** Irregular, grey colony, rough surface, raised elevation, filiform margin, dark brown on reverse side, with white ring around growing edge. **Microscopy:** Hyaline, non-septate hyphae.

BRD3-081D – *Helotiales* sp.

Malt extract agar: Irregular, dark green colony fading to white at growing edge, smooth surface, flat elevation, entire margin. **Potato dextrose agar:** Round, white colony, smooth surface, flat elevation, entire margin. **Cornmeal agar:** Round hyaline colony, slight yellow pigmentation near centre, smooth surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, yellow colony, translucent at growing edge, butyrous surface, flat elevation, undulate margin. **Microscopy:** Septate hyphae.

BRD3-081G – *Candida pseudoglaebosa*

Malt extract agar: Punctiform, white yeast colony growing on top of agar, dull surface, raised elevation, undulate margin. **Potato dextrose agar:** Round, white, yeast colony growing on top of agar, flat elevation, dull surface, curled margin. **Cornmeal agar:** Punctiform, irregular, white yeast growing on top of agar, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, white yeast growing on top of agar, dull surface, flat elevation, filiform margin. **Microscopy:** Round, unicellular.

BRD3-081H – *Diaporthe* sp. I

Malt extract agar: Round, colony, red in centre surrounded by brown ring surrounded by hyaline growth to edge of plate, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular colony, multiple distinct rings of orange, green, brown, and white, thick white ring on outer edge, flat elevation, rough surface, filiform margin. **Cornmeal agar:** Round reddish-brown colony fading to hyaline near growing edge, thin white floccose, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Round, colony, dark brown in centre, surrounded by yellow-brown ring, green pigmentation of agar around colony, rough surface, flat elevation, filiform margin. **Microscopy:** Sterile, septate hyphae.

BRD3-081I – *Hypoxylon* sp. I

Malt extract agar: Round, translucent, white colony, growth rings visible, dull surface, flat elevation, entire margin. **Potato dextrose agar:** Round, opaque, white colony, growth rings visible, rough surface, flat elevation, entire margin. **Cornmeal agar:** Round colony, dark brown in centre with progressively lighter growth rings to nearly transparent at growing edge, flat elevation, rough surface, filiform margin. **Czapek-Dox agar:** Round, light yellow colony, flat elevation, dull texture, fades to hyaline filiform margin. **Microscopy:** Sterile, hyaline, non-septate hyphae.

BRD3-081J – *Hypoxylon* sp. II

Malt extract agar: Irregular, brown colony, lighter brown ring at growing edge, thin white floccose, rough surface, flat elevation, filiform margin, stains agar brown. **Potato dextrose agar:** Round, grey colony with white ring around growing edge, growth rings visible, white floccose, rough surface, flat elevation, filiform margin. **Cornmeal agar:** Round, brown colony, lighter brown ring near growing edge, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, translucent, red colony, fading to hyaline at growing edge, rough texture, flat elevation, filiform margin. **Microscopy:** Septate hyphae with ovoid conidia.

BRD3-082B – *Godronia* sp. II

Malt extract agar: Irregular, green colony, dull surface, raised elevation, filiform margin. **Potato dextrose agar:** Irregular, green colony, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, green colony, dull surface in centre, rougher around growing edge, raised elevation, entire margin. **Czapek-Dox agar:** Irregular, green colony, very dark green on reverse side, dull surface, raised elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-082C – *Hypoxylon* sp. III

Malt extract agar: Round, translucent colony, brown tint near centre, rough surface, flat elevation, entire margin. **Potato dextrose agar:** Irregular colony with four distinct rings, from inner to outer: dark green, light green, brown, and white, rough surface, flat elevation, filiform margin. **Cornmeal agar:** Irregular colony, dark brown in centre fading to white, translucent at growing edge, thin white floccose, rough texture, flat elevation, entire margin. **Czapek-Dox agar:** Irregular, translucent, white colony, dull surface, rough elevation, filiform margin. **Microscopy:** Sterile, non septate hyphae.

BRD3-082M – *Colletotrichum kahawae* II

Malt extract agar: Round, translucent colony, with white floccose, flat elevation, rough surface, filiform margin. **Potato dextrose agar:** Round colony, dark green near centre surrounded by white ring covered in thick, white floccose, raised elevation, rough surface, filiform margin. **Cornmeal agar:** Round green, translucent colony, flat elevation, rough surface, margin not visible. **Czapek-Dox agar:** Round, white colony with thick white floccose, raised elevation, rough surface, entire margin. **Microscopy:** Non-septate hyphae with ellipsoid conidia.

BRD3-082O – *Physalospora vaccinii* V

Malt extract agar: Irregular, brown colony, white ring near growing edge, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, grey colony, rough surface, raised elevation, undulate margin. **Cornmeal agar:** Irregular, translucent, brown colony, dull surface, flat elevation, undulate margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, non-septate hyphae.

BRD3-083A – *Physalospora vaccinii* VI

Malt extract agar: Irregular, dark green colony, white ring around growing edge, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Irregular, grey colony, segmented growth, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, green colony, fading to hyaline at growing edge, rough surface, flat elevation, margin not visible. **Czapek-Dox agar:** No growth observed. **Microscopy:** Branching, sterile, non-septate hyphae.

BRD3-083D – *Pseudoteratosphaeria* sp. I

Malt extract agar: Round, black colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, black colony, glistening surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, black colony, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Punctiform, irregular, black colony, raised elevation, entire margin. **Microscopy:** Dark, sterile, septate hyphae.

BRD3-083K – *Bryochiton* sp.

Malt extract agar: Round, black colony, rough surface, flat elevation, entire margin. **Potato dextrose agar:** Irregular, black colony, rough surface, raised elevation, entire margin. **Cornmeal agar:** Round, black colony, smooth surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, black colony, smooth surface, flat elevation, filiform margin. **Microscopy:** Sterile, dark, non-septate hyphae.

BRD3-083P – *Pseudoteratosphaeria* sp. II

Malt extract agar: Irregular, black colony, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Round, black colony, rough surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, black colony, smooth surface, flat elevation, undulate margin. **Czapek-Dox agar:** Punctiform, irregular, black colony, smooth surface, flat elevation, entire margin. **Microscopy:** Dark, sterile, septate hyphae.

BRD3-083W – *Ascomycota* sp. I

Malt extract agar: Round, brown colony, white ring around growing edge, rough surface, flat elevation, entire margin. **Potato dextrose agar:** Irregular, green colony, white ring around growing edge, rough surface, flat elevation, entire margin. **Cornmeal agar:** Round, brown colony, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, translucent, yellow colony, dull surface, flat elevation, margin not visible. **Microscopy:** Sterile, non-septate hyphae.

BRD3-083Y – *Pleosporales* sp.

Malt extract agar: Round, translucent, brown and white colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Round, green, colony, white ring around growing edge, rough surface, raised elevation, entire margin. **Cornmeal agar:** Round, green colony with white ring around growing edge, rough surface, raised elevation, entire margin. **Czapek-Dox agar:** Round, olive green colony, lighter outer ring, rough surface, flat elevation, filiform margin. **Microscopy:** Septate hyphae with ovoid conidia.

BRD3-084B – *Alternaria alternata* VI

Malt extract agar: Round, dark green colony, white floccose, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Round, dark green colony, white ring around growing edge, rough surface, raised elevation, entire margin. **Cornmeal agar:** Round, dark green, thin white floccose, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Round, white colony, thick white floccose, rough surface, raised elevation, filiform margin. **Microscopy:** Septate hyphae, with ovoid ascospores (4 segments).

BRD3-084F – *Pseudoteratosphaeria* sp. III

Malt extract agar: Punctiform, irregular, black colony, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Punctiform, irregular, black colony, rough surface, raised elevation, entire margin, distinct white outer ring around growing edge on reverse side. **Cornmeal agar:** Punctiform, irregular, black culture, rough surface near centre, smooth near growing edge, flat elevation, entire margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, dark, septate hyphae.

BRD3-084I – *Godronia* sp. III

Malt extract agar: Irregular, green colony, fading to white near growing edge, dull surface, raised elevation, filiform margin. **Potato dextrose agar:** Irregular, green colony, dull surface, raised elevation, undulating filiform margin. **Cornmeal agar:** Irregular, green colony, dark green outer ring, fading to translucent near growing edge, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, green colony, rough surface, raised elevation, entire margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-085E – *Alternaria alternata* VII

Malt extract agar: Round, green colony with thick white floccose covering centre of colony and translucent outer ring, rough texture, flat elevation, entire margin. **Potato dextrose agar:** Irregular, green colony, rough texture, raised elevation, entire margin. **Cornmeal agar:** Irregular, green colony with dark green outer ring, rough surface, raised elevation, undulating filiform margin. **Czapek-Dox agar:** Round, green colony, rough surface, raised elevation, filiform margin. **Microscopy:** Dark, septate hyphae, ovoid, segmented ascospores.

BRD3-085G – *Pseudoteratosphaeria* sp. IV

Malt extract agar: Irregular, black colony, faint white ring near growing edge, rough texture, raised elevation, filiform margin. **Potato dextrose agar:** Irregular, black colony, lighter in colour near centre, growth rings faintly visible, wrinkled surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, black colony, lighter near centre, rough texture in centre, smooth on growing edge, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, black colony, rough texture, raised elevation, entire margin. **Microscopy:** Sterile, dark, septate hyphae.

BRD3-085P – Yellow yeast

Malt extract agar: Punctiform, irregular, yellow yeast colony, glistening surface, flat elevation, undulating margin. **Potato dextrose agar:** Irregular, yellow, yeast colony, dull surface, flat elevation, undulating margin. **Cornmeal agar:** Punctiform, irregular, yellow yeast colony, dull surface, flat elevation, undulating margin. **Czapek-Dox agar:** Punctiform, yellow yeast colony, dull surface, flat elevation, finely undulating margin. **Microscopy:** Yellow, round, single cells.

BRD3-085R – *Hypoxylon* sp. IV

Malt extract agar: Irregular, translucent, white colony with white floccose, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, white colony covered by thick white floccose, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, white colony with white floccose, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, brown colony with light brown ring around growing edge, rough surface, flat elevation, entire margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-085T – *Leptospora rubella*

Malt extract agar: Round, dark green colony, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Irregular, greyish green colony, rough, wrinkled surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, brown colony with distinct growth rings, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, brown colony, rough surface, flat elevation, lobate margin. **Microscopy:** Non-septate hyphae, round spores.

BRD3-090A – *Pseudoteratosphaeria* sp. V

Malt extract agar: Irregular, dark grey colony, light grey ring around growing edge, rough surface, raised elevation, entire margin. **Potato dextrose agar:** Irregular, black colony, dull surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, grey and black colony, visible growth rings, rough surface, raised elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, black colony, dull surface, raised elevation, entire margin. **Microscopy:** Dark, septate hyphae with round conidia on ends.

BRD3-105A – *Colletotrichum fiorinae* I

Malt extract agar: Round, red colony, translucent ring around growing edge, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Round, red colony, green near centre, covered in thick, white floccose, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, pink colony, rough surface, raised elevation, filiform margin. **Czapek-Dox agar:** Irregular, pink colony, rough surface, raised elevation, filiform margin. **Microscopy:** Non-septate fungi, ellipsoid conidia.

BRD3-105B – *Colletotrichum fiorinae* II

Malt extract agar: Irregular, pink colony, translucent white ring around growing edge, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, pink colony, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, red colony, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, pink colony, rough surface, flat elevation, filiform margin. **Microscopy:** Non-septate fungi, ellipsoid conidia.

BRD3-105M – *Phyllosticta pyrolae* I

Malt extract agar: Irregular, grey colony, visible growth rings, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, grey colony, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, grey colony, visible growth rings, dull surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, grey colony, rough surface, raised elevation, filiform margin. **Microscopy:** Sterile, septate fungi.

BRD3-107A – *Daldinia decipiens*

Malt extract agar: Irregular, white colony, visible growth rings, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, green colony, dark green near centre, rough surface, flat elevation, filiform margin. **Cornmeal agar:** Round, white, translucent colony, agar stained brown near centre, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, non-septate hyphae.

BRD3-111A – Beige yeast

Malt extract agar: Irregular, opaque, white yeast colony, dull surface, flat elevation, undulate margin. **Potato dextrose agar:** Punctiform, irregular, brown yeast colony, dull surface, flat elevation, entire margin. **Cornmeal agar:** Punctiform, irregular, opaque, white, yeast colony, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, translucent, white, yeast colony, dull surface, flat elevation, entire margin. **Microscopy:** Round, single cells.

BRD3-111B – *Colletotrichum lineola*

Malt extract agar: Round, translucent, yellow colony, with white floccose near centre, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Round, green colony, white ring around growing edge, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, translucent, white culture, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Round green colony, covered by thick, white floccose, rough surface, raised elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-111E – *Phyllosticta pyrolae* II

Malt extract agar: Round, grey colony, translucent near growing edge, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, grey colony, yellow on reverse side, rough surface, raised elevation, entire margin. **Cornmeal agar:** Round, grey colony, visible growth rings, dull surface, flat elevation, filiform margin. **Czapek-Dox agar:** Rhizoid, grey colony, dull surface, flat elevation, filiform margin. **Microscopy:** Sterile, dark, septate hyphae.

BRD3-111J – *Gibberella acuminata*

Malt extract agar: Irregular, yellow, colony, dull surface, flat elevation, lobate margin. **Potato dextrose agar:** Irregular, white colony, slight yellow tint, yellow on reverse side, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, dull yellow colony, rough surface, crateriform elevation, filiform margin. **Czapek-Dox agar:** Irregular, white colony, distinct outer ring at growing edge, rough surface, raised elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-111K – *Colletotrichum fioriniae* III

Malt extract agar: Round, pink colony, with translucent outer ring, rough surface, raised elevation, filiform margin. **Potato dextrose agar:** Round, pink colony, green near centre, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, pink colony, thin white floccose, raised elevation, rough surface, filiform margin. **Czapek-Dox agar:** Irregular, pink colony, rough surface, raised elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-111N – *Hypoxylon fuscum*

Malt extract agar: Irregular, dull yellow colony, rough surface, flat elevation, undulate margin. **Potato dextrose agar:** Round, dull yellow colony, green near centre, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Round, yellow colony, darker in centre, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** Round, yellow, translucent colony, dull surface, flat elevation, filiform margin. **Microscopy:** Sterile non-septate hyphae.

BRD3-111T – *Chaetomium globosum*

Malt extract agar: Irregular, hyaline colony, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, dull yellow colony, growth rings visible, rough surface, flat elevation, filiform margin. **Cornmeal agar:** No growth observed. **Czapek-Dox agar:** Irregular, green colony, white near growing edge, dull surface, flat elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae with dark, round conidia.

BRD3-112E – White yeast I

Malt extract agar: Punctiform, irregular, white yeast colony, dull surface, flat elevation, lobate margin. **Potato dextrose agar:** Irregular, white yeast colony, dull surface, flat elevation, entire margin. **Cornmeal agar:** Irregular, white yeast colony, dull surface, flat elevation, lobate margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Round, single cells.

BRD3-113C – White filamentous

Malt extract agar: White floccose grows quickly to cover surface of plate, hyaline culture, flat elevation, margin not visible. **Potato dextrose agar:** Thick, white floccose grows to quickly fill plate, culture not visible through floccose, yellow tint on reverse side. **Cornmeal agar:** Thick, white floccose grows quickly to cover surface of plate, culture not visible through arial growth, margin not visible. **Czapek-Dox agar:** White floccose grows quickly to cover surface of plate, hyaline growth, flat elevation, margin not visible. **Microscopy:** Non-septate hyphae, ellipsoid conidia.

BRD3-113P – *Sordariomycetes* sp.

Malt extract agar: Round, black colony, white ring around growing edge, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, black colony with white ring near growing edge, wrinkled, leathery surface, filiform margin. **Cornmeal agar:** Round, grey colony, growth rings visible, dull surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, white colony, green near centre, dull surface, wrinkled near centre, flat elevation, entire margin. **Microscopy:** Non-septate hyphae with round conidia.

BRD3-113Y – White yeast II

Malt extract agar: Irregular, white yeast colony, glistening surface, raised elevation, entire margin. **Potato dextrose agar:** Irregular, white yeast colony, glistening surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, white yeast colony, glistening surface, raised elevation, undulate margin. **Czapek-Dox agar:** Punctiform, round, white yeast colony, glistening surface, raised elevation, entire margin. **Microscopy:** Round, single, yeast cells.

BRD3-115A – *Zasmodium fructigenum* I

Malt extract agar: Irregular, black colony, dull surface, flat elevation, entire margin. **Potato dextrose agar:** Irregular, grey colony, rough surface, raised elevation, entire margin. **Cornmeal agar:** Round, grey colony, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Irregular, grey colony, rough surface, raised elevation, entire margin. **Microscopy:** Sterile, dark, non-septate hyphae.

BRD3-121B – *Godronia* sp. IV

Malt extract agar: Irregular, dull green colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Round, green colony, rough surface, flat elevation, filiform margin, dark green on reverse side with white ring around growing edge. **Cornmeal agar:** Round, green colony, growth rings visible with dark green ring on growing edge, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, dull green colony, rough surface, crateriform elevation, lobate filiform margin, brown on reverse side. **Microscopy:** Sterile, septate hyphae.

BRD3-121F – *Ascomycota* sp. II

Malt extract agar: Round, grey colony, white floccose near centre, rough surface, raised elevation, entire margin. **Potato dextrose agar:** Round, olive green colony, rough surface, convex elevation, entire margin. **Cornmeal agar:** Round, grey colony, growth rings visible, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular colony, rough surface, raised elevation, filiform margin, dark green on reverse side. **Microscopy:** Sterile, non-septate hyphae.

BRD3-121J – *Pseudoteratosphaeria* sp. VI

Malt extract agar: Irregular, black colony, rough surface, raised elevation, undulate margin. **Potato dextrose agar:** Irregular, black colony, rough, wrinkled surface, raised elevation, entire margin. **Cornmeal agar:** Irregular, black colony, rough surface, raised elevation, entire margin. **Czapek-Dox agar:** Punctiform, black colony, rough surface, raised elevation, entire margin. **Microscopy:** Dark, septate hyphae with round conidia.

BRD3-124A – *Colletotrichum godetiae*

Malt extract agar: Round, dark green colony, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Round, olive green colony, rough surface, growth rings visible, crateriform elevation, filiform margin. **Cornmeal agar:** Round, green colony, white floccose, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, green colony, pink near growing edge, rough texture, raised elevation, filiform margin. **Microscopy:** Non-septate hyphae, ellipsoid conidia.

BRD3-125A – *Ramularia* sp.

Malt extract agar: Punctiform, irregular, dull yellow colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Punctiform, irregular, dull green colony, rough surface, raised elevation, entire margin. **Cornmeal agar:** Punctiform, irregular, dull yellow colony, rough surface, raised elevation, entire margin, fades agar to translucent. **Czapek-Dox agar:** Punctiform, irregular, dull green colony, rough surface, raised elevation, entire margin. **Microscopy:** Non-septate hyphae with obovoid spores.

BRD3-126B – Black filamentous

Malt extract agar: Irregular, black colony, white floccose near centre, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, grey colony, rough surface, raised elevation, filiform margin. **Cornmeal agar:** Irregular, grey colony, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Irregular black colony, rough surface, raised elevation, filiform margin. **Microscopy:** Dark, septate hyphae.

BRD3-126C – *Zasmidium fructigenum* II

Malt extract agar: Irregular, brown colony, rough surface, raised elevation, entire margin, stains agar brown. **Potato dextrose agar:** Round, brown colony, rough surface, raised elevation, entire margin, stains agar brown. **Cornmeal agar:** Round, grey colony, rough surface, flat elevation, entire margin. **Czapek-Dox agar:** Irregular, grey colony, rough surface, raised elevation, filiform margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-154A – White yeast III

Malt extract agar: Punctiform, yellowish yeast culture, dull surface, flat elevation, entire margin. **Potato dextrose agar:** Irregular, yellowish yeast colony, dull surface, flat elevation, undulate margin. **Cornmeal agar:** White, yeast colony, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, white, translucent yeast colony, dull surface, flat elevation, entire margin. **Microscopy:** Round, single yeast cells.

BRD3-154C – *Lophodermium pini-excelsae*

Malt extract agar: Irregular, white colony, segmented growth, dull surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, white colony, rough surface flat elevation, filiform margin, yellow on reverse side. **Cornmeal agar:** Round, translucent, yellow colony, dull surface, flat elevation, filiform margin. **Czapek-Dox agar:** Punctiform, white colony, rough surface, raised elevation, entire margin. **Microscopy:** Sterile, non-septate hyphae.

BRD3-154E – *Lophodermium* sp. I

Malt extract agar: Irregular, white colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, white colony, rough surface, raised elevation, undulate margin. **Cornmeal agar:** Irregular, translucent, white colony, dull surface, flat elevation, entire margin. **Czapek-Dox agar:** Punctiform, irregular, white colony, dull surface, flat elevation, entire margin. **Microscopy:** Sterile, septate hyphae.

BRD3-154F – *Lophodermium* sp. II

Malt extract agar: Irregular, translucent, brown colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, green colony, white near growing edge, rough surface, raised elevation, undulate margin. **Cornmeal agar:** Irregular, translucent, brown colony, rough surface, filiform margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, non-septate hyphae.

BRD3-154H – Brown filamentous

Malt extract agar: Irregular, light brown colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, brown colony, fading to white at growing edge, rough surface, flat elevation, filiform margin. **Cornmeal agar:** Irregular, white colony, dull surface, flat elevation, filiform margin. **Czapek-Dox agar:** Irregular, white colony, dull surface, flat elevation, undulate margin. **Microscopy:** Sterile hyphae.

BRD3-154J – *Naganishia liquefaciens*

Malt extract agar: Round, smooth yeast colonies, raised elevation, entire margin. **Potato dextrose agar:** Irregular, white yeast colony, wrinkled surface, raised elevation, entire margin. **Cornmeal agar:** Punctiform, white yeast colony, dull surface, flat elevation, undulate margin. **Czapek-Dox agar:** Punctiform, white yeast colony, dull surface, flat elevation, entire margin. **Microscopy:** Single, round yeast cells.

BRD3-154L – *Lophodermium nitens* I

Malt extract agar: Irregular, translucent, white colony, rough surface, flat elevation, filiform margin. **Potato dextrose agar:** Irregular, white colony, yellow near centre, rough surface, flat elevation, filiform margin. **Cornmeal agar:** No growth observed. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, septate hyphae.

BRD3-155A – *Lophodermium* sp. III

Malt extract agar: Irregular, white colony, yellow tint near centre, rough surface flat elevation, lobate margin. **Potato dextrose agar:** Irregular, white colony, rough surface, flat elevation, filiform margin. **Cornmeal agar:** Punctiform, irregular colony, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, septate hyphae.

BRD3-155C – *Lophodermium nitens* II

Malt extract agar: Round, white colony, rough surface, flat elevation, lobate margin. **Potato dextrose agar:** Irregular, light brown colony, rough surface, raised elevation, curled margin. **Cornmeal agar:** Irregular, translucent, white colony, rough surface, flat elevation, filiform margin. **Czapek-Dox agar:** No growth observed. **Microscopy:** Sterile, septate hyphae.

Appendix III: ITS sequences of endophytic fungi

ITS sequences of endophytic fungi used for fungal identification. Where possible, forward and reverse reads were aligned to create a consensus sequence.

>BRD3-078A (Forward sequence)

GCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGCAGGGT
CTCCGTGACCCTCCCGGCCTCCCGCCCCGGGCGGGTTCGGCGCCCGCCGGAG
GATAACCAAACCTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATA
ATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTTCGAGCG
TCATTTCAACCCTCAAGCTCTGCTTGGTGTGGGGCCCTACGGCTGACGTAGG
CCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTA
CGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAACCCCAATTTTCCA
AAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAAT
AAGCGGAG

>BRD3-078B (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTG
CTGAATTATTCACCCTTGTCTTTTGCCTACTTCTTGTTCCTTGGTGGGTTTCG
CCACCCTAGGACAAACATAAACCTTTTGTAAATTGCAATCAGCGTCAGTAAC
AAATTAATAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCA
TCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGT
TCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGGCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGG
AGCGCAGCACAAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGC
CTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATAACCCGCTGAACTTAAG
CATAT

>BRD3-078C (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTGCTGTAGGTGAACCTG
CAGCAGGATCATTAATAAACGAGCCTAAACGCTCAAAAACACCGCGAACAC
ACCTCTGTTGCCTCGGTGGTGACGGTCCGGGGAAGTCCCGGACTTCAGCCGC
CGGCGGCCCGAAAACCTACTATTTGATGGCACTCTGAGGCACTCTAAACGAGT
AAAAACTTTTCAGCAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCGCCGGCATTCCGGCGGGCATGCCTGTTTCGAGCGTC
ATTTCAACCCTCAGGCTCCGCCTGGTGTGGGGCCTTTTCGCATGAAAGCCCC
GAAAAGCAGTGGCGGGCTCGCAGCGGACCCGGGCGTAGTAGCACACCTCGC
TCAGGGCCCCGCCGCGGGCGCCGGCCGTGAAACCTCTTTTTACCCAAGGTTG
ANNNNNGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGG
AG

>BRD3-078E (Consensus sequence)

TTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTG
CTGAATTATTCACCCTTGTCTTTTTCGCTACTTCTTGTTTCCTTGGTGGGTTTCG
CCACCCTAGGACAAACATAAACCTTTTGTAAATTGCAATCAGCGTCAGTAAC
AAATTAATAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCA
TCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGT
TCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGGCGTCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGG
AGCGCAGCACAAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGC
CTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG
CATAT

>BRD3-078F (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGGA
TCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTTCGCTACTTCTTGTTTCCTTGGTGGGTTTCGCC
CACCCTAGGACAAACATAAACCTTTTGTAAATTGCAATCAGCGTCAGTAACA
AATTAATAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAA
GAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCAT
CGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTT
CGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGGCGTCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGG
AGCGCAGCACAAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGC
CTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG
CATAT

>BRD3-078I (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTTGCTGTAGGTGAACCTGCAGCAGGATCA
TTAAAAACGAGCCTGAACGCTCAAAAACACCGCGAACACACCTCTGTTGCC
TCGGTGGTGACGGTCCGGGGAAGTCCCGGACTTCAGCCGCCGGCGGCCCGAA
AACTACTATTTGATGGCACTCTGAGGCACTCTAAACGAGTAAAACTTTTCAG
CAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCGCCGCATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAG
GCTCCGCCTGGTGTGGGGCCTTTTCGCATGAAAGCCCCGAAAAGCAGTGGC
GGGCTCGCAGCGGACCCGGGCGTAGTAGCACACCTCGCTCAGGGCCCCGCCG
CGGGCGCCGGCCGTGAAACCTCTTTTACCCAAGGTTGACCTCGGATCAGGT
AGGAATACCCGCTGAACTTAAGCATAT

>BRD3-078J (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATC
ATTACAAGTGACCCCGGTCTAACCACCGGGATGTTTCATAACCCTTTGTTGTCC
GACTCTGTTGCCTCCGGGGCGACCCTGCCTTCGGGCGGGGGCTCCGGGTGGA
CACTTCAAACCTCTTTCGTAACCTTTGCAGTCTGAGTAACTTAATTAATAAATT
AAAACCTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG
AAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCCCTGGTATTCCGGGGGGGCATGCCTGTTTCGAGCGTCA
TTTCACCACTCAAGCCTCGCTTGGTATTGGGCAACGCGGTCCGCCGCGTGCCT
CAAATCGACCGGCTGGGTCTTCTGTCCCCTAAGCGTTGTGGAACTATTCGCT
AAAGGGTGTTCGGGAGGCTACGCCGTAACAACCCCATTTCTAAGGTTGAC
CTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT

>BRD3-078K (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
ATTAAGGAGTATTTGCGGGAAATCGAAAGAAAGTACCGCTCTCCCACCCGTG
ACTATATACTATGTTGCTTTCCGGGCTTCAACCCCGGAGAGGACCAAACCTCT
TGAATTTTACTGTCTGAGTACTATATAATAGTTAAAACCTTTCAACAACGGA
TCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTT
GGTATTCCGGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCAATCCCTTCGGG
GGTCTTGGGGCCTGGGATCTCCCAGCTCTTAAAATCAGTGGCGGTGCCTCTCG
GCTCTAAGCGTAGTAATTCTTCTCGCTATAGTCCCCGGGAGAACACTTGCCAT
AACCCCCACACTTTTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAA
CTTAAGCATATC

>BRD3-078L (Consensus sequence)

GGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCAT
TACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCTGAA
TTATTCACCCTTGTCTTTTTCGTAACCTTTGTAATTGCAATCAGCGTCAGTAACAAATT
ATAAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCCTTTGGTATTCCAAAGGGCATGCCTGTTTCGAG
CGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGCGTCTTGTCTCTAGCTTT
GCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCG
CAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGCCTTTT
TTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCA

>BRD3-078M (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGA
TCATTAAAGAGTAAGGGTCTCTGGCCCGAACCTCCAACCCTCTGTCGTTATAA
CTACTTCGTTGCTTTGGCGGGACCGCGAGGGTCCTCCCGAGCGCGCCGGTCTC
CGGACAGGCGAGCGCCCGCCAGAGTCTAACCAAACCTCTTGTTTTTAAACCAG
TCGTCTGAGTATAAAATTTTAATTAAATTA AAACTTTCAACAACGGATCTCTT
GGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATT
CCGAGGGGCATGCCTGTTTCGAGCGTCATTACACCACTCAAGCACTGCTTGGT
ATTGGGCATCGTCCGTCGTAAAGGCGGGCGTGCCTCGAAGACCTCGGCGGGG
TTTCTCAAACCTTCGGGCGTAGTAGAGTTAAATCAAACGTCTTATAAGCTTGG
TGAGATCTCATTGCCGTTAAACCTTTTATATATTTTCAGGTTGACCTCGGATC
AGGTAGGGATACCCGCTGAACTTAAGCATATC

>BRD3-079A (Consensus sequence)

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ATCATTAAAAACGAGCCTAAACGCTCAAAAACACCGCGAACACACCTCTGT
TGCCTCGGTGGTGACGGTCCGGGGAAAGTCCCGGACTTCAGCCGCCGGCGGCC
CGAAAACACTATTTGATGGCACTCTGAGGCACTCTAACGAGTAAAACTT
TCAGCAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCGCCGGCATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACC
CTCAGGCTCCGCCTGGTGTGGGGCCTTTTCGCATGAAAGCCCCGAAAAGCA
GTGGCGGGCTCGCAGCGGACCCGGGCGTAGTAGCACACCTCGCTCAGGGCCC
CGCCGCGGGCGCCGGCCGTGAAACCTCTTTTTACCCAAGGTTGACCTCGGAT
CAGGTAGGAATACCCGCTGAACTTAAGCATAT

>BRD3-079B (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTG
CTGAATTATTCACCCTTGCTTTTTGCGTACTTCTTGTTTTCTTGGTGGGTTTCG
CCACCCTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAAC
AAATTAATAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCA
TCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGT
TCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGGCGTCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGG
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CTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG
CATATC

>BRD3-079E

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CCGACTCTGTTGCCTCCGGGGCGACCCTGCCTTCGGGCGGGGGCTCCGGGTG
GACACTTCAAACCTTTGCGTAACCTTTGCAGTCTGAGTAAACTTAATTAATAAA
TTAAAACCTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTC
ATTTCAACCACTCAAGCCTCGCTTGGTATTGGGCAACGCGGTCCGCCGCGTGCC
TCAAATCGACCGGCTGGGTCTTCTGTCCCCTAAGCGTTGTGGAACTATTCGC
TAAAGGGTGTTCGGGAGGCTACGCCGTAACAACCCCATTTCTAAGGTTGA
CCTCGGATCAGGTAGGGATACCCGCTGAACCTTAAGCATAT

>BRD3-079G (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTNNCTTGGTCNNNTAGAGGAAGTAAA
AGTCGTAACAAGGTTGCTGTAGGTGAACCTGCAGCAGGATCATTAAAAACG
AGCCTGAACGCTCAAAAACACCGCGAACACACCTCTGTTGCCTCGGTGGTGA
CGGTCCGGGGAAGTCCCGGACTTCAGCCGCCGGCGGCCCGAAAACACTATT
TGATGGCACTCTGAGGCACTCTAAACGAGTAAAACCTTTCAGCAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCGGGC
ATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAGGCTCCGCCTG
GTGTTGGGGCCTTTTCGCATGAAAGCCCCGAAAAGCAGTGGCGGGCTCGCAG
CGGACCCGGGCGTAGTAGCACACCTCGCTCAGNGCCCCGCCGCGGGCGCCGG
CCGTGAAACCTCTTTTACCCAAGGTTGACCTCGGATCAGGTAGGAATACCCG
CTGAACCTTAAGCATATCA

>BRD3-081C (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
ATTAAGGAGTATTTGCGGGAAATCGAAAGAAAGTACCGCTCTCCACCCGTG
ACTATACTATGTTGCTTTCCGGGCTTCAACCCCGGAGAGGATCAAACCTCTT
GAATTTACTGTCTGAGTACTATATAATAGTTAAAACCTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTG
GTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCAATCCCTTCGGGG
GTCTTGGGGCTTGGGATCTCCAGCTCTTAAAATCAGTGGCGGTGCCTTTCGG
CTCTAAGCGTAGTAATTCTTCTCGCTATAGTCCCCGGGAGAACACTTGCCATA
ACCCCATACTTTTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC
TAAGCATAT

>BRD3-081D (Consensus sequence)

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTATCCCTACCTGATCCGAG
GTCAAACCTTAGAAAGTTGGGGGTTGATGGCTAGCGTCCGCCGAGTCCCTATA
GCGAGGATATTTACTGCGCTCAGGGCCTCGACGGCACCGCCACTGATTTTAA
GGCCCGCCAGGTGAGTGGCGAAGCCCAATACCAAGCCAGGCTTGAGGGTTGT
AATGACGCTCGAACAGGCATGCCCTCGGAATRCCAAGGGGCGCAATGTGCG
TTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATT
TCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTT
AACTATTATATAGTACTCAGACGACACTAATAATCAGGGTTTTGGGTCCTCTG
GCGGGCGCTCACCAGCCGGGGCCGGTGGTCGGGCGTGAGCCTGACGGCCCGC
CAAAGCAACAAAGTGATAATAACACAGGGTGGGAGATCTACCNNTAAGGGC
ATGAACTCTGTAATGATCCCTCCGCAGGTTACCTACGGAGACCTTGTTACGA
CTTTTACTTCTCTAAATGACCAAG

>BRD3-081G (Consensus sequence)

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AACGTGTTTTTTAATAAACTATTACTTTGGTTTGGCTAAGAAATTAGTTG
AGCCAGAGGTGATTTAACTTCAATTTTATTGAATTGTTATTTTAATTTTATGT
CAATTTGTTGATTAAATTCAAACAATCTTCAAACCTTTCAACAACGGATCTC
TTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAAT
TGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCACCCTCTGGTA
TTCCAGAGGGTATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTTGGGTTTG
GTATTGAGTGATACTCTTAGTCGAACTAGGCGTTTGCTTGAAATATATTGGCA
CGAGTAGTGTTGAACAGTGTTGTCTGAACATCAATGTATTAGGTTTATCCAAC
TCGTTGAAGCGTTTAGGTATTACTATTCTTCATTAGGCTTTGCCTTATAAAAC
ACAAACAAGTTTGACCTCAAATCAGGTAGGATTACCCGCTGAACTTAAGC

>BRD3-081H (Consensus sequence)

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AGCGGAGGGATCATTGCTGGAACGCGCCCCGGCGCACCCAGAAACCCTTGT
GAACTTATACCCATACCGTTGCCTCGGCGCAGGCCGGCCTCTGCTGCAGAGG
CCCCCTGGAGACAGGGAGCAGCCAGCCGGTGGCCAACCAAACCTCTGTTTCTA
CAGTGAATCTCTGAGTAAAAAACATAAATGAATCAAACCTTTCAACAACGG
ATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTC
TGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCGTGG
CTTGGTGATGGGGCACTGCCCGTAAAAGGGCAGGCCCTGAAATCTAGTGGCG
AGCTCGCCAGGACCCCGAGCGTAGTAGTTATATCTCGCTCTGGAAGGCCCTG
GCGGTGCCCTGCCGTTAAACCCCAACTTCTGAAAATTTGACCTCGGATCAG
GTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAG

>BRD3-081I (Consensus sequence)

GGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCAT
TACTGAGTTCTACAAAACTCCCAACCCTTTGTGAACCTTACCGTCGTTGCCT
CGGCGCTGAGCGGGCGGCTACCCTGGAGAGCTTTTCCGGGAGCCACCTACCCT
GTAGGTGGCTACCCTGGAGCTACCCTGTAGTAGTTTGC ACTGTACGCTCCGCC
GGCGGACCTCTACTCTGTTTTGTATAGTGTATCTCTGAAACCTATAACTTA
ATACGTTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCATATTGCGCCCATTAGTATTCTAGTGGGCATGCCTATTCGAG
CGTCATTTCAACCCTTACGCCCTGTTGCGTAGTGTGGGACCCTACAGGCCCG
TAAAGGGCCCTGTAGCTCCCCAAAGGTAGTGGCGGTGTTAGGTGCACTCGTA
GCGTAGTAAATCTTTTCTCGCTCCTGCAGTGTACCTAGGGCCTGCCGTGAAAC
CCCTTATACCTTCTAGTGGTTGACCTCGGATTAGGTAGGAATACCCGCTGAAC
TTAAGCATATC

>BRD3-081J (Consensus sequence)

GGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCAT
TACTGAGTTCTACAAAACTCCCAACCCTTTGTGAACCTTACCGTCGTTGCCT
CGGCGCTGAGCGGGCGGCTGCCCTGGAGAGCTTTTCCGGGAGCCACCTACCCT
GTAGGTGGCTACCCTGGAGCTACCCTGTAGTAGTTTGC ACTGTACGCTCCGCC
GGCGGACCTCTACTCTGTTTTGTATAGTGTATCTCTGAAACCTATAACTTA
ATACGTTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAC
GCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCATATTGCGCCCATTAGTATTCTAGTGGGCATGCCTATTCGAG
CGTCATTTCAACCCTTACGCCCTGTTGCGTAGTGTGGGACCCTACAGGCCCG
TAAAGGGCCCTGTAGCTCCCCAAAGGTAGTGGCGGTGTTAGGTACTACTCGTA
GCGTAGTAAATCTTTTCTCGCTCCTGCAGTGTACCTAGGGCCTGCCGTGAAAC
CCCTTATACCTTCTAGTGGTTGACCTCGGATTAGGTAGGAATACCCGCTGAAC
TTAAGCATAT

>BRD3-082B (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCA
TTAAGGAGTATTTGCGGGAAATCGAAAGAAAGTACCGCTCTCCCACCCGTGA
CTATATACTATGTTGCTTTCCGGGCTTCAACCCCCGGAGAGGACCAAACCTTT
GAATTTATTACTGTCTGAGTACTATATAATAGTTAAAACCTTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTG
GTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCAATCCCTTCGGGG
GTCTTGGGGCCTGGGATCTCCAGCTCTTAAAATCAGTGGCGGTGCCTCTCGG
CTCTAAGCGTAGTAATTCTTCTCGCTATAGTCCCCGGGAGAACACTTGCCATA
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TTAAGCATATCA

>BRD3-082C (Consensus sequence)

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CCCACCCGTGACTATATACTATGTTGCTTTCCGGGCTTCAACCCCGGAGAGG
ACCAAACCTCTTGAATTTATTACTGTCTGAGTACTATATAATAGTTAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCCTTGGTATTCCGGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCA
ATCCCTTCGGGGGTCTTGGGGCTTGGGATCTCCAGCTCTTAAATCAGTGGC
GGTGCCTCTCGGCTCTAAGCGTAGTAATTCTTCTCGCTATAGTCCCCGGGAGA
ACACTTGCCATAACCCCCACACTTTTAAGGTTGACCTCGGATCAGGTAGGGAT
ACCCGCTGAACCTAAGCATATCAATAAGCGGAGGA

>BRD3-082M (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGG
ATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACTATAACTGTT
GCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCGGGCGG
GTCGGCGCCC GCCGGAGGATAACCAAACCTCTGATTTAACGACGTTTCTTCTGA
GTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGCCAGCATTCTGGCGGG
CATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTGGGGCC
CTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCC
TTTGCCTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTA
AAACCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTG
AACTTAAGCATAT

>BRD3-082O (Consensus sequence)

TCTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTGCTGTAGGTGAAC
CTGCAGCAGGATCATTAAAAACGAGCCTAAACGCTCAAAAACACCGCGAA
CACACCTCTGTTGCCTCGGTGGTGACGGTCCGGGGAAGTCCCGGACTTCAGC
CGCCGGCGGCCCGAAAACACTATTTGATGGCACTCTGAGGCACTCTAAACG
AGTAAAAACTTTTCAGCAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCCGCCGGCATTCCGGCGGGCATGCCTGTTCGAGC
GTCATTTCAACCCTCAGGCTCCGCCTGGTGTGGGGCCTTTTCGCATGAAAGC
CCCGAAAAGCAGTGGCGGGCTCGCAGCGGACCCGGGCGTAGTAGCACACCT
CGCTCAGGGCCCCGCCGGCGCCGGCCGTGAAACCTCTTTTACCCAAGG
TTGACCTCGGATCAGGTAGGAATACCCGCTGAACCTAAGCATATCA

>BRD3-083A (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTGCTGTAGGTGAACCTGCAGCAGGATC
ATTAAAAACGAGCCTAACGCTCAAAAACACCGCGAACACACCTCTGTTGC
CTCGGTGGTGACGGTCCGGGGAAAGTCCCGGACTTCAGCCGCCGGCGGCCCGA
AACTACTATTTGATGGCACTCTGAGGCACTCTAAACGAGTAAAACTTTCA
GCAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCGCCGGCATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTC
AGGCTCCGCCTGGTGTGGGGCCTTTTCGCATGAAAGCCCCGAAAAGCAGTG
GCGGGCTCGCAGCGGACCCGGGCGTAGTAGCACACCTCGCTCAGGGCCCCGC
CGCGGGCGCCGGCCGTGAAACCTCTTTTTACCCAAGGTTGACCTCGGATCAG
GTAGGAATACCCGCTGAACTTAAGCATATC

>BRD3-083D (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACTGAGTGAGGGCTCCGGCCCAACCTCCAACCCCATGTGAATCCGA
CCTCTGTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACC
ACTCAACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACCTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACACCA
CTCAAGCCTGGCTTGGTATTGGGCACGGCGGCTTCGCGGCCGCCCGCCTCAA
AGTCTCCGGCTGGACCGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTT
GCGAGTACGGGACAGTCCGTGGCCGTTAAACCCCCCATGAAAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC

>BRD3-083K (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTCTCCGTAGGTGAACCTGCGGAGGGAT
CATTACTGAGTGAGGGCCCCCGGGCCCGACCTCCAACCCTATGTCTACCGAC
CCTGTTGCCTCGGGGGTGACCCGGCGCCCGTTCCCCGGGCGCCCCGGGGCCC
CCGGCGGACCCCTCAACGCTGCATCTGTGCGTCCGAGTCATATTTGAATCAAT
CAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCCGTGGTATTCCGCGGGGCATGCCTGTTTCGAGCGTC
ATTTACCAATCAAGCCTCGCTTGGTATTGGGCGTCCGCGGCGGCAACGCCG
CGCGCCCCAATGTCTCCGGCTGAGCCGCCTGTCTCTAAGCGTTGTGGTCTAAC
TGTTTCGCTTTGGAGTATGGGCTGGATCATCGCCGTTAAACACCCCATCAAAGG
TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT

>BRD3-083P (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACTGAGTGAGGGCTCCGGTCCAACCTCCAACCCCATGTGAATCCGA
CCTCTGTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACC
ACTCAACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACACCA
CTCAAGCCTGGCTTGGTATTGGGCGAGGCGGCTTTGCGGCCGCCCGCCTCAA
AGTCTTCGGCTGGACTGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTT
GCGAGTACGGGACAGCCCGTGGCCGTTAAACCCCCCATGAAAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA

>BRD3-083W (Consensus sequence)

AAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
AAAAGTTTACGTCCTACTTGCCTCCGTGCAAAGGATAAGATTCCACGCGTG
AAACAGCGAGCTCTTCGGAGCGGTGCTGGGCCTGCCCTGTGAACCGAACCC
ACCTGCTTCTCGGAGCGGTGTGCCATTAGGGATCAGGATCAGTGCAGCTTGC
GGTGCCTCTAGTAATAGAGCCTTCGGGATGGATATACTTAACCCGTGTTTAC
CTTCTTTGTTGCTTTGGCGGGTTCGCTCTAAGGCGTTGGCCTCGGCTAAACCG
TACCCGCCAGAGAACTCAAACTCTTTTGTTTTAGTGACGTCTGAGTACTATA
TAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTTCGA
GCGTCATTATGACCAATCAAGCCTGGCTTGGTGTGGGGTGCAGCGGTCCCGC
GGCCCTTAAAATCAGTGGCGGTGCCGTCCGGCTCTAAGCGTAGTAAATTTCTT
CGCTATAGTGTCCGGGTGGTTACCTGCCAGAACCCCCCAATCTCACGGTTGAC
CTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCA

>BRD3-083Y (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGA
TCATTACCTTTCTATGCAGAAGTATTGTGTAGGGCAACCTCACAATACTCCCT
GTATAACCACCCTTGTCTTTTGCGCACTCATATTTCTCGGCAGGCTTGCCTGCC
GATTGGACAATTTATACTATTTTAAATTTTGAATCAGCGTCTGAAATAATTAA
TAATTACAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTTCGAGCG
TCATTTGTACCTTCAAGCTCTGCTTGGTGTGGGGTGTGTTGTCCTTCTCATGTGG
TTGGACTCGCCTTAAAGTAATTGGCAGCCAGTGTTTTGGTTTTGAAGCGCAGC
ACAAGTCGCGATTCTAGCTAACATCACCAGCTTCCAGCAAGCCTTTATTACCT
TTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCAT

>BRD3-084B (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGA
TCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGC
TGAATTATTCACCCTTGTCTTTTGCCTACTTCTTGTTTCCTTGGTGGGTTTCGCC
CACCCTAGGACAAACATAAACCTTTTGTAAATTGCAATCAGCGTCAGTAACA
AATTAATAATTACAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAA
GAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCAT
CGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTT
CGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTGGGCGTCTTGTCTCTA
GCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGG
AGCGCAGCACAAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTAAGC
CTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAG
CATATC

>BRD3-084F (Consensus sequence)

AGAGGAAGTAAAAGNCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGA
TCATTACTGAGTGAGGGCTCCGGCCCAACCTCCAACCCCATGTGAATCCGAC
CTCTGTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACCA
CTCAACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACACCAC
TCAAGCCTGGCTTGGTATTGGGCACGGCGGCTTCGCGGCCGCCCGCCTCAA
GTCTCCGGCTGGACCGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTTG
CGAGTACGGGACAGTCCGTGGCCG

>BRD3-084I (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG
ATCATTAAAGGAGTATTTGCGGGAAATCGAAAGAAAGTACCGCTCTCCCACCC
GTGACTATATACTATGTTGCTTTCCGGGCTTCAACCCCGGAGAGGACCAAAC
TCTTGAATTTATTACTGTCTGAGTACTATATAATAGTTAAAACCTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
CTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCAATCCCTTC
GGGGGTCTTGGGGCCTGGGATCTCCAGCTCTTAAAATCAGTGGCGGTGCCT
CTCGGCTCTAAGCGTAGTAATTCTTCTCGCTATAGTCCCCGGGAGAACTTG
CCATAACCCCCACACTTTTAAGGTTGACCTCGGATCAGGTAGGGATACCCGC
TGAACCTAAGCATATCAAT

>BRD3-085E (Consensus sequence)

GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCAT
TACCGAGTGCGGGCCCCTCGTGGCCCAACCTCCCACCCTTGTCTCTATACACC
TGTTGCTTTGGCGGGCCACCGGGGCCACCTGGTCGCCGGGGGACGTTTCGTC
CCCGGGCCC GCGCCC GCGAAGCGCTCTGTGAACCCTGATGAAGATGGGCTG
TCTGAGTACTATGAAAATTGTCAAACCTTCAACAATGGATCTCTTGGTTCCG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGG
GGCATGCCTGTCCGAGCGTCATTTCTGCCCTCAAGCACGGCTTGTGTGTTGGG
TGCGGTCCCCCGGGGGCCTGCCCGAAAGGCAGCGGCGACGTCCGTCTGGTC
CTCGAGCGTATGGGGCTTTGTACTCGCTCGGGAAGGACTGGCGGGGGTTGG
TCACCACCACAAAATTTTACCACGGTTGACCTCGGATCAGGTAGGAGTTACC
CGCTGAACTTAAGCATAT

>BRD3-085G (Consensus sequence)

GGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCAT
TACTGAGTGAGGGCTCCGGTCCAACCTCCAACCCCATGTGAATCCGACCTCT
GTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACCACTCA
ACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACTTTCAAC
AACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACCACTCAAG
CCTGGCTTGGTATTGGGCGAGGCGGCTTTGCGGCCGCCCGCCTCAAAGTCTTC
GGCTGGACTGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTTGCAGTA
CGGGACAGCCCGTGGCCGTTAAACCCCCCATGAAAGGTTGACCTCGGATCAG
GTAGGGATACCCGCTGAACTTAAGCATATC

>BRD3-085P

No sequence obtained

>BRD3-085R (Consensus sequence)

GTTTCCTCGGCGTGCGCANNNGGATGCCCGCGGGGGATCCGGGCCTGCCCTG
CAGCTACCCCGTAGCCGCGCGGCAAGGCCGGATCCTCAGCACACGGCGCCCG
TCAAGGTCCCGCCGAAGTACCCCAAACCTCTGTTTTACGTGGAATTCTGAATGC
TTCAACTAAATAAGTTAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCG
WTGRWGAACGCAGCGRAATGSGATMAKTARWGTGAATATSCARAAAYTCWSY
SAATCATCANNATCTTTGAACGCACATTGCGCCATTAGTATTCTAGTGGGCAT
GCCTATTCGAGCGTCATTTCAACCCTTAAGCCCGCGTTGCTTAGCGTTGGGAG
TCTGCGGGTCACCCCGCAGCTCCTGAAAACCATTGGCGGAGTCAGGGAGCAC
TCTAAGCGTATTACACTTTTTATTTTTTGTCTCGCTTTGGACGTTGCCCGGCC
CCACGCCGTAAAACACCCCATACCAAATGTTGACCTCGAATTAGGTAGGAAT
ACCCGCTGAACTTAAGCATATCAA

>BRD3-085T (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG
ATCATTAAAAAATCAGTAGCTTGCTACTGTGCTGGGGGAGATGGGAAAGTCC
TGGCTGAAAAGTCGGCGTACCGGTACCTGCACCCCTACGATACCCTTGTCTTT
TGAGCACTTATGTTTCCTTGGTAGGCTTGCCTGCCAACAGGACAAACTTTAAA
CCTTTTTAAATATCAATCAGCGTCTGAATTATAATTAATAATTACAACCTTTCA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCCTTGGTATTCCATGGGGCATGCCTGTTTCGAGCGTCATTTGTACCCTC
AAGCTATGCTTGGTGTGGGTGATTGTCCAGCCTGTATGGCCAGGACTCGCCT
TAAAGTAATTGGCAGCCAGTGTTTGGTAGAAAGCGCAGCACATTTTGCAT
TACTCCAATAATATTAGCAACCATAAAGCTCATTTATCACTTTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

>BRD3-090A (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGG
ATCATTACCGAGTGAGGGCTCCGGCCCAACCTCCAACCCCATGTGAATCCGA
CCTCTGTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACC
ACTCAACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACTT
TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACACCA
CTCAAGCCTGGCTTGGTATTGGGCGAGGCGGCTTTGCGGCCGCCCGCCTCAA
AGTCTTCGGCTGGACTGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTT
GCGAGTACGGGACAGCCCGTGGCCGTTAAACCCCCCATGAAAGGTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

>BRD3-105A (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCT
TCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCC
ACCACGGGGACGGGGCGCCCCGCCGGAGGAAACCAAACCTCTATTTACACGAC
GTCTCTTCTGAGTGGCACAAGCAAATAATTAACAACTTTTAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCA
TTCTGGCGAGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGG
TTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCC
CGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGGGATCCGGAGGGAC
TCTTGCCGTTAAACCCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGA
ATACCCGCTGAACTTAAGCATATCAAT

>BRD3-105B (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCT
TCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCC
ACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACCTCTATTTACACGAC
GTCTCTTCTGAGTGGCACAAGCAAATAATTTAAACTTTTAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCA
TTCTGGCGAGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGG
TTTTGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCC
CGGAGCCTCCTTTGCGTAGTAACCTAACGTCTCGCACTGGGATCCGGAGGGAC
TCTTGCCGTTAAACCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGA
ATACCCGCTGAACTTAAGCATA

>BRD3-105M (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCA
TACTGAACTAGTAATTCTCTGAAAGGTCGCCGGTACCCGGTCCCCCCCCTAA
ACAAGGGGGGCCCGGGGAAGGTCCTCTCACACCCTTGTGTACCTTACCATGTT
GCTTTGGCGGGCCGACCCGGTTTCGACCCGGGCGGCCGGCCCGCCCCAGCCCT
CACCGGCCAGGACGTCAGGCTAAGCGCCCGCCAGTATACAAAACCTCAAGCG
ATTATTTTCGTGTAGTTCTGATAAATTATTCAATTAATTTAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
TCTGGTATTCGGGAGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTC
TGCTTGGTATTGGGCGACGTCCGCTGCCGGACGCGCCTCGAAGACCTCGGCG
ACGGCGTCTTAGCCTCGAGCGTAGTAGTAACATCTCGCTTTGGAGTGCTAGGC
GTCGGCCGCCGACAATCGACCTTCGGTCTATTACTTCCAAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATAT

>BRD3-107A (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACCGAGTTATCTAAACTCCCAACCCTATGTGAACCTTACCGTCGTTGC
CTCGGCGGGCCCGCGCTTACCCGGTAGCTACCCTGTAGCTACCCGGTAGGTGC
GCTACAGGCCCCGCCGGTGGACTACTAAACTCTGTTTTAATACTGTATCTCTGA
ATGCTTCAACTTAATAAGTTAAACTTTCAACAACGGATCTCTTGGTTCTGGC
ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGC
ATGCCATTCGAGCGTCATTTCAACCCTTAAGCCTAGCTGCTTAGTGTTGGGA
ATCTGCCCCGTAACCTACGGGGCAGCTCCCTAAAGTCATCGGCGGAGTTAGG
GCATACTCTAAGCGTAGTAATATTCTTCTCGCTTCTGTAGTTGTCCTGGCGGC
TTGCCGTTAAACCCCTATATTTTCTAGTGGTTGACCTCGGATTAGGTAGGAA
TACCCGCTGAACTTAAGCATATC

>BRD3-111A

No sequence obtained

>BRD3-111B (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACTGAGTTACCGCTCTACAACCCTTTGTGAACATACTAACTGTTGCT
TCGGCGGGCAGAGGTTCCCTCGCGGAACCCCTCCCGGTGACGCCCTCACGGG
CGTCGCGCCCGCCGGAGGATACCAAACCTCTATTTTAACGACGTTTCTTCTGAG
TGGCACAAGCAAATAATTA AAAACTTTTAACAACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACATTGCGCCCCGCCAGCATTCTGGCGGGCA
TGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTGGGGCCCT
ACGGTTAAACGTAGGCCCTTAAAGGTAGTGGCGGACCCTCTCGGAGCCTCCT
TTGCGTAGTAACCTAACGTCTCGCACTGGGATTTCGGAGGGATTCTAGCCGTAA
AACCCCAATTTTTTAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAA
CTTAAGCATATC

>BRD3-111E (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
ATTACTGAACTAGTAATTCTCTGAAAGGTGCGCCGGTACCCGGTCCCCCCCCTA
ACAAGGGGGCCCGGGGAAGGTCCTCTCACACCCTTGTGTACCTTACCATGT
TGCTTTGGCGGGCCGACCCGGTTTCGACCCGGGCGGCCGGCGCCCCAGCCC
TCACCGGCCAGGACGTCAGGCTAAGCGCCCCGCCAGTATACAAAACCTCAAGCG
ATTATTTTCGTGTAGTTCTGATAAATTATTCAATTAATTA AAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
TCTGGTATCCGGAGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTC
TGCTTGGTATTGGGCGACGTCCGCTGCCGGACGCGCCTCGAAGACCTCGGCG
ACGGCGTCTTAGCCTCGAGCGTAGTAGTAACATCTCGCTTTGGAGTGCTAGGC
GTCGGCCGCGGACAATCGACCTTCGGTCTATTACTTCCAAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA

>BRD3-111J (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACTTAATGTTGCC
TCGGCGGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCAGAGGACCC
AAACTCTAATGTTTCTTATTGTAACCTTCTGAGTAAAACAAACAAATAAATCAA
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCCGCTGGTATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTT
CAACCCTCAAGCCCCGGGTTTGGTGTGGGGATCGGCTCTGCCCTTCTGGGC
GGTGCCGCCCCCGAAATACATTGGCGGTCTCGCTGCAGCCTCCATTGCGTAGT
AGCTAACACCTCGCAACTGGAACGCGGCGCGGCCATGCCGTAAAACCCCAAC
TTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATAT
C

>BRD3-111K (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATC
ATTACTGAGTTACCGCTCTATAACCCTTTGTGAACGTACCTAACCGTTGCTTC
GGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGCCGGCCCCAC
CACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACCTCTATTTACACGACGTC
TCTTCTGAGTGGCACAAGCAAATAATTTAAAACCTTTTAACAACGGATCTCTTGG
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCT
GGCGAGCATGCCTGTTGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTTT
GGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGGA
GCCTCCTTTGCGTAGTAACCTAACGTCTCGCACTGGGATCCGGAGGGACTCTTG
CCGTTAAACCCCAAATTCTTTACAGGTTGACCTCGGATCAGGTAGGAATACC
CGCTGAACCTTAAGCATAT

>BRD3-111N (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACTGAGTTCTTACAAACTCCAACCCTTTGTGAACCATAACCAACTGTTG
CCTCGGCGCGAGCTGCGGCTGCTTGGTAGCTACCCGGTAGTCACCTACCCGG
GAGCTACCCGGTAGCGTCTGCGTACAGGCCCGCCGAAGGACCACCAAACCTCT
GTTTGACAGTGTATTCTGAATGCTTCAACTAAATAGTTAAAACCTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
ATTAGTATTCTAGTGGGCATGCCTATTCGAGCGTCATTTGACCCTGAAGCCC
TAGTTGCTTCGCGTTGGGACTCTACTGGCTACCCTGTAGTTCCCTAATGACAG
TGGCGGAGTTCAGGTGTACTCTCAGCGTAGTAATTCATCTCGCTTTTGCAGT
AGCCTGGTCGCCGGCCGTAAAACCCCTATTTTCTAGTGGTTGACCTCGGATT
AGGTAGGAATACCCGCTGAACCTTAAGCATATC

>BRD3-111T (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACAGAGTTGCAAAACTCCCTAAACCATTGTGAACGTTACCTATACCGT
TGCTTCGGCGGGGCGGCCCGGGGTTTACCCCCGGGCGCCCCCTGGGCCCCAC
CGCGGGCGCCCGCCGGAGGTCACCAAACCTTTGATAATTTATGGCCTCTCTG
AGTCTTCTGTACTGAATAAGTCAAAACCTTTCAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCAGCATTCTGGCG
GGCATGCCTGTTGAGCGTCATTTCAACCATCAAGCCCCGGGCTTGTGTTGG
GGACCTGCGGCTGCCGAGGCCCTGAAAAGCAGTGGCGGGCTCGCTGTCGCA
CCGAGCGTAGTAGCATAACATCTCGCTCTGGTCGCGCCGCGGGTTCCGGCCGTT
AAACCACCTTTTAACCAAGGTTGACCTCGGATCAGGTAGGAAGACCCGCTG
AACTTAAGCATAT

>BRD3-112E

No sequence obtained

>BRD3-113C

No sequence obtained

>BRD3-113P (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATC
ATTAAAGAGATCATGCCCTCACGGGTAGACCTCCCACCCTTTGTTTACAATGC
CTTTGTTGCTTTGGCGGGCCCGTTTGGCCCCGCGCTGAACAACCGGCCCCCGGC
TGGTCAGTGCCCCGCCAGAGAACCGAAAACCTCTGAATTAAATGTCGTCTGAGT
ACTATGTAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGGGGGGCATGCCT
GTTGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCTACACCCGA
CTGGGTGGGCCTTAAAATCAGTGGCGGTGCCATCTGGCTCTAAGCGTAGTAA
TTCTTCTCGCTCTGGAGATCTAGGTGTTTGGCTTGCCAGCAACCCCAATTTATC
AAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA

>BRD3-113Y

No sequence obtained

>BRD3-115A (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTG
CGGAGGGATCATTACTGAGTGAGGGTCTCCGGGCCCGACCTCCAACCCTTTT
GTGAACCACCTCGTTGCTTCGGGGGCGACCCTGCCGTTTCGCGGCGTGGGGCC
CCCGGAGCCCATCAAACCCTGCGTAACTAAGTCGTCGGAGTTTAAACAAATT
AAACAAAACCTTCAACAACGGATCTCTTGGTTCGGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGGCATGCCTGTCCGAGC
GTCATTACACCAATCAAGCCTGGCTTGGTATTGGGCGTCGCGGGTCTGACCCG
CGCGCCTTAAAGTCTCACCGGCTGAGCGGCGTCGTCTCTAAGCGTTGTGGAA
ACTATTCGCGGAGAGGAGGCGCGGCGTGGCCGTTAAACACCCCATCAAAGGT
TGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA

>BRD3-121B (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG
ATCATTAAAGGAGTATTTGCGGGAAATCGAAAGAAAGTACCGCTCTCCCACCC
GTGACTATATACTATGTTGCTTTCCGGGCTTCAACCCCCGGAGAGGACCAAAC
TCTTGAATTTACTGTCTGAGTACTATATAATAGTTAAAACCTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
CTTGGTATTCCGGGGGGGCATGCCTGTTTCGAGCGTCATTAATACCAATCCCTCC
GGGGGTCTTGGGGCTTGGGATCTCCAGCTCTTAAAATCAGTGGCGGTGCCTC
TCGGCTCTAAGCGTAGTAATTCTTCTCGCTATAGTCACCGGGAGAACACTTGC
CATAACCCCCACACTTTTAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCT
GAACTTAAGCATAT

>BRD3-121F (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGA
TCATTAAAAAGTTATGTCCTACTTGCCTCCGTGCAAAAGGATAAGATTCCACG
TGTGAAACAGTGAGCTCTTTGGAGCGAAGCTGGGCCAGCCCCTGTGAACCGA
AACCGCCTGCTTCTCGGAGCGTGGTGTATTAGGCGATCAGGATCAGTGACG
CTTGCGGTGCGCTCTAGTAATAGGGCCTTTCGGGATGAATATACTTAACCCGTG
TCTACCTTCTTTGTTGCTTTGGCGGGTTCGCCTCTAAGGCGTTGGCTTCGGCTAA
CCGTACCCGCCAGAGGATTCAAACCTCTTTTGTTTTAGTGACGTCTGAGTACTA
TATAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGAGGGGCATGCCTGTTC
GAGCGTCATTATGACCAATCAAGCCTGGCTTGGTGTGGGGTTCGCGGGTCCCG
CGGCCCTTAAACTCAGTGGCGGTGCCGCCCGGCTCTAAGCGTAGTAAATTTTC
CTCGCTATAGCGTCCGGGTGGTTACCCGCCAGAACCCCCCAATCTTACGGTTG
ACCTCGGATCAGGTAGGGATAACCCGCTGAACTTAAGCATATCA

>BRD3-121J (Consensus sequence)

GAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGAT
CATTACCGAGTGAGGGCTCCGGCCCAACCTCCAACCCCATGTGAATCCGACC
TCTGTTGCCTCGGGGGCGACCCGGCCCTGCGCCGGGGCCCCCGGTGGACCAC
TCAACTCTGCATCTTTGCGTCTGAGTATGATATTTGAATCAATCAAACTTTC
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGAT
AAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACAT
TGCGCCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTACACCACT
CAAGCCTGGCTTGGTATTGGGCGAGGCGGCTTTGCGGCCGCCCGCCTCAAAG
TCTTCGGCTGGACTGACCGTCTCTAAGCGTTGTGACTTCATTGGACCGCTTGC
GAGTACGGGACAGCCCGTGGCCGTTAAACCCCCCATGAAAGGTTGACCTCGG
ATCAGGTAGGGATAACCCGCTGAACTTAAGCATATCAA

>BRD3-124A (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGA
TCATTACTGAGTTACCGCTCTACAACCCTTTGTGAACATACTAACCGTTGCT
TCGGCGGGCAGGGGAAGCCTCTCGTGGGCGGACCCTCCCGGCGCCGGCCCCG
TCACGGGGGCGGAGCGCCCCGCCGGAGGAAACCAAACCTCTATTTACACGACGT
CTCTTCTGAGTGGCACAAGCAAATAATTAACAACTTTTAACAACGGATCTCTTG
GTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTC
TGCGGAGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGTTT
TGGGGCCCCACGGCCGACGTGGGCCCTTAAAGGTAGTGGCGGACCCTCCCGG
AGCCTCCTTTGCGTAGTAACCTAACGTCTCGCACTGGGATCCGGAGGGACTCTT
GCCGTTAAACCCCCAAATTTTTTTCAGGTTGACCTCGGATCAGGTAGGACTACC
CGCTGAACTTAAGCATAT

>BRD3-125A (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATC
ATTACTGAGTTAGGGAGCAATCCCGACCTCCAACCCTTTGTGAACGCATCATG
TTGCTTCGGGGGCGACCCTGCCGTTTCGCGGCATTCCCCCGGAGGTCATCAA
AACACTGCATTCTTACGTCGGAGTAAAAAGTTAATTTAATAAAACTTTCAACA
ACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTACCACTCAAG
CCTCGCTTGGTATTGGGCGTTCGCGAGTCTCTCGCGTGCCTCAAAGTCTCCGGC
TGTCTTGATTCAATTCCAGCGTTGTGGCAACTATTTTCGCAGTGGAAACGAGT
CGATGTGGCCGTTAAATCTTTCAAAGGTTGACCTCGGATCAGGTAGGGATAC
CCGCTGAACTTAAGCATATCAATA

>BRD3-126B

No sequence obtained

>BRD3-126C (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTG
CGGAGGGATCATTACTGAGTGAGGGTCTCCGGGCCCGACCTCCAACCCTTTT
GTGAACCACCTCGTTGCTTCGGGGGCGACCCTGCCGTTTCGCGGCGTGGGGCC
CCGGAGCCCATCAAACCCTGCGTAACTAAGTCGTCGGAGTTTAAACAAATTA
AACAAAACCTTTCAACAACGGATCTCTTGGTTTCGGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCG
TCATTACACCAATCAAGCCTGGCTTGGTATTGGGCGTCGCGGGTCTGACCCGC
GCGCCTTAAAGTCTCACCGGCTGAGCGGCGTCGTCTCTAAGCGTTGTGGAAA
CTATTCGCGGAGAGGAGGCGCGGCGTGGCCGTTAAACACCCCATCAAAGGTT
GACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA

>BRD3-154A

No sequence obtained

>BRD3-154C (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTAGAGTTT
TGCCTACTACAGCACGCCTACCCGGCGCTTGCTAGTCTCGCGAGAGGGCGACA
CGCTAAATGTGCGGAGAGTCCTGGCTCGCCAGGCAATCCGCAGTCACTTTCG
GGTGATTCAGAGACTCAATGAGCGTGGGTTCTTTTTAAAGAGCTTGAGATAG
AGCCCGTCCTCGCTGGTCACAGCGGGGATTCAACGGAACCTGCGGAAGGATC
ATTAAAGAATAAACGGGCCTCCGGGCCCCCTATTCTCACCCCTATGTTTACCAC
ACTTTGTTGCCTTGGCGCACTGCGCCAGCGGATCAAACCCTTGAATCATTGC
TGTCTGAGTACTATATAATAGTTAAAACCTTTCACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAA
TTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGG
GGGCATGCCTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGGG
CTCGCCCTGTAGGGCTTGCCTCAAAGTCAGTGGCGGCTACCGTCCGACCTTCA
GCGCAGTACTATCCGTCGCTGTTAGGGAAGGCCTATAACCCGTCATCACAAC
CCCCACTTTACAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTA
AGCATATCAATAA

>BRD3-154E (Consensus sequence)

AGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTCAAGTTTTGCCTACCA
CAGCAGCCCCCGCTGCTAGTCTCCTAGTGAGGCGACACGCTCAATTTGCGGA
GAGGTCCTCTTCTGAGGAAAATCCGCAGCCCATCTCCCTTACCCGGAGGTCG
GTTTCAGAGGCTCAATGAGTGTGGGTTTCAGCGCAGCTGAGCTTAAGATAGAGT
CCGTCTTGGCTGGCAACAGCGAAGATTCAACGGAACCTGCGGAAGGATCATT
AAAGAATACATGGCCTTCGGGTCTATTCTCACCCCTTTGTTTACCAAAACTCT
TGTTGCCTTGGCGCATTTATGCGCCAAAGGAATCAAACCCTTGAATCTCTGCT
GTCTGAGTACTATATAATAGTTAAAACCTTTCACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTCCTGGTATTCCGGGAG
GCATGCCTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCT
CGCCTTCTTTGGCCTGCCTCAAATCAGTGGCGGCACAGTCCGATCCTCAAGC
GCAGTAATACACGACGCTTGCCGGTGAAGGTTGCTGCTCCAGAAACCCCCCA
CAAATAAGGTTGACCTCGAATCAGGTAGGGATACCCGCTGAACTTAAGCA
TATCAATAAGCGGAG

>BRD3-154F (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTCAAGTCTTGCCTACC
ACAGCAGCCCCGGCTGCTAGTCTCTTTAGTGAGGCGACACGCTCAATTTGCG
GAGAGGTCTCTTCTGAGGAAAATCCGCAGCCCATCTCCCTTACCCGGAGGT
CGGTTCAGAGGCTCAATGAGTGTGGGTTCAGCGCAGCTGAGCTTAAGATAGA
GTCCGTCTTGGCTGGCAACAGCAAAGATTCAACGGAACCTGCGGAAGGATCA
TTAAAGAATACATGGCCTTCGGGTCTATTCTCACCCCTTTGTTTACCAAAACT
CTTGTTGCCTTGGCGCATTTCGTGCGCCAAAGGAATCAAACCCTTGAATCTCTG
CTGTCTGAGTACTATATAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTCCTGGTATTCCGG
GAGGCATGCCTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGG
GCTCGCCTCCTTTGGCCTGCCTCAAATCAGTGGCGGCACAGTCCGATCCTCA
AGCGCAGTAATACACGACGCTTGCCGGTGAAGGTTGCTGCTCCAGAAACCCC
CCACAAACTAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA
GCATA

>BRD3-154H

No sequence obtained

>BRD3-154J (Consensus sequence)

TGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAANNNG
CGGAAGGATCATTAATGATTAAGTGTCTGTGAGCTTGCTCACAGACTTATCA
TATCCATAACACCTGTGCACTTGTGCGATGGCTTAGTGAAGACCGCAAGGTT
GGATCTATCCATCTACTTTACATAACAATTTAGTAACAAATGTAGTCTTATTA
TAACATAATAAAAACCTTCAACAACGGATCTCTTGGCTCTCGCATCKATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACCTTGCCTCCCTGGTATTCCGGGGAGCATGCCTGTTT
GAGTGTCATGAAAACCCTCAACCTTAGATTGGTTAACACCTTTCTTTGGCTTG
GATTTGGACGTTTGCCGATGATAAGTCGGCTCGTCTTAAAAGTAATAGCTGG
ATCTGTCTCGCGACATGGTTTGAAGTGGCGTAATAAGTATTTGCTAAGGACA
TCTTCGGATGGCCGCGTTGCAAGACTAAAGACCGCTTTCTAATCCATTGATCT
TCGGATTAATATTCTTGACATCTGGCCTCAAATCAGGTAGGACTACCCGCTGA
ACTTAAGCATATCA

>BRD3-154L (Forward sequence)

CCGGCTGCTAGTCTCCTAGTGAGGCGANNNGCTCAATTTGCGGAGAGGTCCT
CCTTCTGAGGAAAATCCNNNNNCCACCTCCCCTACCCGGAGGTCGGTTCNNN
GGCTCAATGAGTGTGGGTTCTNGCGCAGNTGANNNTANGATAGAGTCCGTCT
TGGCTGGCAACAGCGAAGATTCAACGGAACCTGCGGAAGGATCATTAAGA
ATACATGGCCTTCGGGTCTATTCTCACCCCTTGTGTTACCAAACTCTTGTTGC
CTTGGCGCATTTCGTGCGCCAAAGGAATCAAACCCTTGAATCTCTGCTGTCTGA
GTACTATATAATAGTTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACCGNGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCTCCTGGTATTCCGGGAGGCATGC
CTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCTCGCCTC
CTTTGGCCTGCCTCAAATCAGTGGCGGCACAATCCGATCCTCAAGCGCAGT
AATACACGACGCTTGCTGGTGAAGGTTGCTGCTCCAGAAACCCCCACAAC
TAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA
ATAAGCGGA

>BRD3-155A (Consensus sequence)

AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTCAAGTCTTGCCTACCACA
GCAGCCCCGGCTGCTAGTCTCCTAGTGAGGCGACACGCTCAATTTGCGGAGA
GGTCCTCCTTCTGAGGAAAATCCGCAGCCCACCTCCCCTACCCGGAGGTCGG
TTCAGAGGCTCAATGAGTGTGGGTTCTAGCGCAGCTGAGCTTAAGATAGAGTC
CGTCTTGGCTGGCAACAGCGAAGATTCAACGGAACCTGCGGAAGGATCATTA
AAGAATACATGGCCTTCGGGCCCTATTCTCACCCCTTGTGTTACCAAACTCTT
GTTGCCTTGGCGCATTTCGTGCGCCAAAGGAATCAAACCCTTGAATCTCTGCTG
TCTGAGTACTATATAATAGTTAAAACCTTTCAACAACGGATCTCTTGGTTCTGG
CATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCTCCTGGTATTCCGGGAGG
CATGCCTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCTC
GCCTCCTTTGGCCTGCCTCAAATCAGTGGCGGCACAGTCCGATCCTCAAGC
GCAGTAATACACGACGCTTGCTGGTGAAGGTTGCTGCTCCAGAAACCCCCAC
AACTAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCAT
ATCAT

>BRD3-155C (Consensus sequence)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTCAAGTCTTGCCTACC
ACAGCAGCCCCGGCTGCTAGTCTCCTAGTGAGGCGACACGCTCAATTTGCGG
AGAGGTCCTCCTTCTGAGGAAAATCCGCAGCCCACCTCCCCTACCCGGAGGT
CGGTTTCAGAGGCTCAATGAGTGTGGGTTTCAGCGCAGCTGAGCTTAAGATAGA
GTCCGTCTTGGCTGGCAACAGCGAAGATTCAACGGAACCTGCGGAAGGATCA
TTAAAGAATACATGGCCTTCGGGCCCTATTCTCACCCCTTTGTTTACCAAAAC
CTTGTTGCCTTGGCGCATTTCGTGCGCCAAAGGAATCAAACCCTTGAATCTCTG
CTGTCTGAGTACTATATAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTC
TGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCTCCTGGTATTCCGG
GAGGCATGCCTGTTTCGAGCGTCATTACAACCCTCAAGCTCTGCTTGGTATTGG
GCTCGCCTCCTTTGGCCTGCCTCAAATCAGTGGCGGCACAGTCCGATCCTCA
AGCGCAGTAATACACGACGCTTGCTGGTGAAGGTTGCTGCTCCAGAAACCCC
CCACAAACTAAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAA
GCATATCA

Appendix IV: Accession numbers of ITS sequences from endophytic fungi

GenBank accession numbers of ITS sequences from endophytic fungi

Endophyte code	Taxonomic identifier	Accession number
BRD3-078A	<i>Colletotrichum kahawae</i> I	MZ571366
BRD3-078B	<i>Alternaria alternata</i> I	MT133260
BRD3-078C	<i>Physalospora vaccinii</i> I	MT133261
BRD3-078E	<i>Alternaria alternata</i> II	MT133262
BRD3-078F	<i>Alternaria alternata</i> III	MT133263
BRD3-078I	<i>Physalospora vaccinii</i> II	MT133264
BRD3-078J	<i>Cladosporium</i> sp.	MT133265
BRD3-078K	<i>Godronia</i> sp. I	MT133266
BRD3-078L	<i>Alternaria alternata</i> IV	MT133267
BRD3-078M	<i>Dothiora</i> sp. II	MZ571367
BRD3-079A	<i>Physalospora vaccinii</i> III	MT133268
BRD3-079B	<i>Alternaria alternata</i> V	MT133269
BRD3-079E	<i>Cladosporium cladosporioides</i>	MZ571368
BRD3-079G	<i>Physalospora vaccinii</i> IV	MT133270
BRD3-081C	<i>Encoeliopsis rhododendri</i>	MT133271
BRD3-081D	<i>Helotiales</i> sp.	MT133272
BRD3-081G	<i>Candida pseudoglaebosa</i>	MZ571369
BRD3-081H	<i>Diaporthe</i> sp. I	MZ571370
BRD3-081I	<i>Hypoxylon</i> sp. I	MT133273
BRD3-081J	<i>Hypoxylon</i> sp. II	MT133274
BRD3-082B	<i>Godronia</i> sp. II	MT133275
BRD3-082C	<i>Hypoxylon</i> sp. III	MZ571371
BRD3-082M	<i>Colletotrichum kahawae</i> II	MT133276
BRD3-082O	<i>Physalospora vaccinii</i> V	MT133277
BRD3-083A	<i>Physalospora vaccinii</i> VI	MT133278
BRD3-083D	<i>Pseudoteratosphaeria</i> sp. I	MT133279
BRD3-083K	<i>Bryochiton</i> sp.	MT133280
BRD3-083P	<i>Pseudoteratosphaeria</i> sp. II	MT133281
BRD3-083W	<i>Ascomycota</i> sp. I	OL435705
BRD3-083Y	<i>Pleosporales</i> sp.	MZ571372
BRD3-084B	<i>Alternaria alternata</i> VI	MT133282
BRD3-084F	<i>Pseudoteratosphaeria</i> sp. III	MT133283
BRD3-084I	<i>Godronia</i> sp. III	MT133284
BRD3-085E	<i>Alternaria alternata</i> VII	MT133285
BRD3-085G	<i>Pseudoteratosphaeria</i> sp. IV	MT133286
BRD3-085P	Yellow yeast	No sequence obtained
BRD3-085R	<i>Hypoxylon</i> sp. IV	MT133287
BRD3-085T	<i>Leptospora rubella</i>	MZ571373
BRD3-090A	<i>Pseudoteratosphaeria</i> sp. V	MT133288

GenBank accession numbers of ITS sequences from endophytic fungi (continued)

Endophyte code	Taxonomic identifier	Accession number
BRD3-105A	<i>Colletotrichum fioriniae</i> I	MT133289
BRD3-105B	<i>Colletotrichum fioriniae</i> II	MT133290
BRD3-105M	<i>Phyllosticta pyrolae</i> I	MZ571374
BRD3-107A	<i>Daldinia decipiens</i>	MZ571375
BRD3-111A	Beige yeast	No sequence obtained
BRD3-111B	<i>Colletotrichum lineola</i>	MT133291
BRD3-111E	<i>Phyllosticta pyrolae</i> II	MZ571376
BRD3-111J	<i>Gibberella acuminata</i>	MZ571377
BRD3-111K	<i>Colletotrichum fioriniae</i> III	MT133292
BRD3-111N	<i>Hypoxylon fuscum</i>	MT133293
BRD3-111T	<i>Chaetomium globosum</i>	MZ571378
BRD3-112E	White yeast I	No sequence obtained
BRD3-113C	White filamentous	No sequence obtained
BRD3-113P	Sordariomycetes sp.	MZ571379
BRD3-113Y	White yeast II	No sequence obtained
BRD3-115A	<i>Zasmidium fructigenum</i> I	MZ571380
BRD3-121B	<i>Godronia</i> sp. IV	MZ571381
BRD3-121F	<i>Ascomycota</i> sp. II	OL435706
BRD3-121J	<i>Pseudoteratosphaeria</i> sp. VI	MT133294
BRD3-124A	<i>Colletotrichum godetiae</i>	MT133295
BRD3-125A	<i>Ramularia</i> sp.	MZ571382
BRD3-126B	Black filamentous	No sequence obtained
BRD3-126C	<i>Zasmidium fructigenum</i> II	MZ571383
BRD3-154A	White yeast III	No sequence obtained
BRD3-154C	<i>Lophodermium pini-excelsae</i>	MT133296
BRD3-154E	<i>Lophodermium</i> sp. I	MT133297
BRD3-154F	<i>Lophodermium</i> sp. II	MT133298
BRD3-154H	Brown filamentous	No sequence obtained
BRD3-154J	<i>Naganishia liquefaciens</i>	MZ571384
BRD3-154L	<i>Lophodermium nitens</i> I	MZ571385
BRD3-155A	<i>Lophodermium</i> sp. III	MT133299
BRD3-155C	<i>Lophodermium nitens</i> II	MT133300

Appendix V: ITS Sequences from culture-independent analysis

Sequences of OTUs identified in DNA metabarcoding of *S. purpurea*

OTU 1 (*S. purpurea*):

GGGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCTGAAGCCATTAGGCCG
AGGGCACGTCTGCCTGGGCGTCAGCCATTGTGTCGCTATTCCCAATTACCCCA
CCTAAGAGTCGGTGAGTAATGTGTAAGGCGTATATTGGCCCTCCGTGCGCAC
TCGTCAACGGTTGGCCTAAAAATGAGTTCTTGACGACACACGTCACGATAAG
TGGTGGTTAACAAACGTTGCATCCTATCGTGCGTGCCTATGTTGCCATGAGTT
GGCTCATATGATTAACCCAAAGTGTGACTACAATGCACCGATTGCGACC
CCAGGTCAGACGGGACGACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGA
CC

OTU 2 (*Pseudoteratosphaeria* sp.):

GTGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGG
GCATGCCTGTTTCGAGCGTCATTACACCACTCAAGCCTGGCTTGGTATTGGGCG
AGGCGGCTTTGCGGCCGCCCGCCTCAAAGTCTTCGGCTGGACTGACCGTCTCT
AAGCGTTGTGACTTCATTGGACCGCTTGCAGTACGGGACAGCCCGTGGCCG
TTAAACCCCCCATGAAAGGTTGACCTCGGATCAGGTAGGGATAACCCGCTGAA
CTTAAGCATATCAATAAGCGGAGGAGC

OTU 3 (*Colletotrichum* sp.):

TTGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGG
GCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTGGGGC
CCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTC
CTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGT
AAAACCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCT
GAACTTAAGCATATCAATAAGCGGAGGAC

OTU 4 (*S. purpurea*):

AGGTGAATCATCGAGTCTTTGAACGCAAGTTGGGCCTGAAGCCATTAGGCCG
AGGACTCGTCTGCCTGGGCGTCAGCCATTGTGTCGCTATTCCCAATTGCCCA
CCTAAGAGTCGGTGAGCAATGGGCAAGGCGTATATTGGCCCTCCGTGCGCAC
TCGTCAACGGTTGGCCTAAAAATGAGTTCTTGACGACACACGTCACGATAAG
TGGTGGTTAACAAATCGTTGCATCCTATCGTGCGTGCCCATGTTGCCACAAGT
TGGCTCATATGATTAACCTGAAGTGTGACTACAATGCACCGATTGCGACC
CCAGGTCAGACGGGACGACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGA
AT

OTU 5 (*S. purpurea*):

TGGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCTGAAGCCATTAGGCCG
AGGGCACGTCTGCCTGGGCGTCAGCCATTGTGTGCTATTCCCAATTACCCCA
CCTAAGAGTCGGTGAGTAATGTGTAAGGCGTATATTGGCCCTCCGTGCGCAC
TCGTACGATAAGTGGTGGTTAACAAACGTTGCATCCTATCGTGCCTGCTAT
GTTGCCATGAGTTGGCTCATATGATTAACCCAAAGTGTTGTGACTACAATGCA
CCGATTGCGACCCAGGTCAGACGGGACGACCCGCTGAGTTTAAGCATATCA
ATAAGCGGAGGATC

OTU 6 (*Penicillium spinulosum*):

ATGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGG
GCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGC
TCCGTCCCCCGGGGACGGGTCCGAAAGGCAGCGGGCGGCACCGAGTCCGGTC
CTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCCAGC
CGACAACCAATCATCCTTTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCC
GCTGAACTTAAGCATATCAATAAGCGGAGGAAC

OTU 7 (*S. purpurea*):

CGGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCTGAAGCCATTAGGCCG
AGGGCACGTCTGCCTGGGCGTCAGCCATTGTGTGCTATTCCCAATTACCCCA
CCTAAGAGTCGGTGAGTAATGTGTAAGGCGTATATTGGCCCTCCGTGCGCAC
TCGTAAACGGTTGGCCTAAAAATGAGTTCTTGACGACACACGTTGCATCCTAT
CGTGCCTGCTATGTTGCCATGAGTTGGCTCATATGATTAACCCAAAGTGTTG
TGACTACAATGCACCGATTGCGACCCAGGTCAGACGGGACGACCCGCTGAG
TTTAAGCATATCAATAAGCGGAGGAAT

OTU 8 (*S. purpurea*):

TGGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCTGAAGCCATTAGGCCG
AGGGCACGTCTGCCTGGGCGTCAGCCATTGTGTGCTATTCCCAATTGCCCA
CCTAAAGTCGGTGAGCAATAGGCATGGCGTATATTGGCCCTCCGTGCGCACT
CGTCAACGGTTGGCCTAAAAATGAGTTCTTGACGACACACGTCGCGATAAGT
GGTGGTTAACAAATCGTTGCATACTATCGTGCCTGCCCATGTTGCCACAAGTT
GGCTCATATGATTAACCCGAACTGTTGTGACTACAATGCACTGATTGCGACCC
CAGGTCAGACGGGACGACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGA
AT

OTU 9 (*Candida glabrosa*):

CCGTGAATCATCGAATCTTTGAACGCACATTGCACCCTCTGGTATTCCAGAGG
GTATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCTTTGGGTTTGGTATTGAGT
GATACTCTTAGTCGAGGCGTTTGTGAAATATATTGGCACGAGTAGTGTTGA
ACAGTGTTGTCTGAACATCAATGTATTAGGTTTATCCAACCTCGTTGAAGCGTT
TAGGTATTACTATTCTTCATTAGGCTTTGCCTTATAAAACACAAACAAGTTT
ACTCAAATCAGGTAGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAG
GAC

Appendix VI: Accession numbers of sequences from culture-independent analysis

GenBank accession numbers of ITS sequences obtained during culture-independent analysis of *S. purpurea*

OTU Number	Taxonomic identification	Accession number
1	<i>Sarracenia purpurea</i>	MZ612797
2	<i>Pseudoteratosphaeria</i> sp.	MZ612798
3	<i>Colletotrichum</i> sp.	MZ612799
4	<i>Sarracenia purpurea</i>	MZ612800
5	<i>Sarracenia purpurea</i>	MZ612801
6	<i>Penicillium spinulosum</i>	MZ612802
7	<i>Sarracenia purpurea</i>	MZ612803
8	<i>Sarracenia purpurea</i>	MZ612804
9	<i>Candida pseudoglaebosa</i>	MZ612805

Appendix VII: Extracts from endophytic fungi

Volume of liquid endophyte culture extracted, yield, and corresponding code for extracted product used in antimicrobial susceptibility assays

Endophyte code	Volume extracted (mL)	Extract mass (mg)	Extract Code
BRD3-078A	300	13.8	BRD4-007-01
BRD3-078B	300	3.6	BRD4-006-01
BRD3-078C	900	16.6	BRD4-009-01.2
BRD3-078E	300	3.0	BRD4-036-01
BRD3-078F	600	15.1	BRD4-034-01.2
BRD3-078I	1000	4.0	BRD4-045-01.2
BRD3-078J	800	11.7	BRD4-019-01.2
BRD3-078K	900	17.9	BRD4-022-01.2
BRD3-078L	300	2.5	BRD4-001-01
BRD3-078M	900	81.2	BRD4-053-01.2
BRD3-079A	600	11.8	BRD4-012-01.2
BRD3-079B	300	2.5	BRD4-003-01
BRD3-079E	600	6.2	BRD4-005-01.2
BRD3-079G	300	4.6	BRD4-014-01
BRD3-081C	300	8.3	BRD4-024-01
BRD3-081D	900	76.5	BRD4-037-01.2
BRD3-081G	300	3.4	BRD4-015-01
BRD3-081H	300	6.1	BRD4-017-01
BRD3-081I	300	3.2	BRD4-030-01
BRD3-081J	300	4.4	BRD4-046-01
BRD3-082B	300	4.3	BRD4-021-01
BRD3-082C	300	5.6	BRD4-008-01
BRD3-082M	300	4.3	BRD4-013-01
BRD3-082O	300	4.0	BRD4-011-01
BRD3-083A	600	9.4	BRD4-010-01.2
BRD3-083D	300	2.3	BRD4-041-01
BRD3-083K	500	3.9	BRD4-054-01.2
BRD3-083P	1000	6.0	BRD4-051-01.3
BRD3-083W	300	5.7	BRD4-050-01
BRD3-083Y	600	7.5	BRD4-004-01.2
BRD3-084B	700	14.6	BRD4-040-01.2
BRD3-084F	900	2.4	BRD4-042-01.2
BRD3-084I	300	5.4	BRD4-025-01
BRD3-085E	300	3.2	BRD4-016-01
BRD3-085G	600	4.4	BRD4-052-01.2
BRD3-085P	300	2.7	BRD4-047-01

Volume of liquid endophyte culture extracted, yield, and corresponding code for extracted product used in antimicrobial susceptibility assays (continued)

Endophyte code	Volume extracted (mL)	Extract mass (mg)	Extract Code
BRD3-085R	300	11.0	BRD4-023-01
BRD3-085T	900	8.0	BRD4-029-01.2
BRD3-090A	600	3.5	BRD4-055-01.2
BRD3-105A	600	9.1	BRD4-020-01.2
BRD3-105B	900	8.1	BRD4-039-01.2
BRD3-105M	600	12.6	BRD4-018-01.2
BRD3-107A	300	2.5	BRD4-059-01
BRD3-111A	600	3.7	BRD4-048-01.2
BRD3-111B	1000	3.5	BRD4-061-01.3
BRD3-111E	600	11.8	BRD4-002-01.2
BRD3-111J	600	11.4	BRD4-035-01.2
BRD3-111K	300	2.7	BRD4-027-01
BRD3-111N	300	8.0	BRD4-026-01
BRD3-111T	300	4.2	BRD4-032-01
BRD3-112E	900	2.4	BRD4-043-01.2
BRD3-113C	300	3.2	BRD4-028-01
BRD3-113P	600	12.7	BRD4-038-01.2
BRD3-113Y	1000	3.0	BRD4-056-01.3
BRD3-115A	1000	2.7	BRD4-049-01.3
BRD3-121B	300	2.4	BRD4-033-01
BRD3-121F	600	5.1	BRD4-062-01
BRD3-121J	900	8.0	BRD4-167-01
BRD3-124A	600	3.5	BRD4-060-01
BRD3-125A	1000	11.3	BRD4-168-01
BRD3-126B	600	7.4	BRD4-066-01
BRD3-126C	900	9.0	BRD4-169-01
BRD3-154A	900	14.7	BRD4-064-01
BRD3-154C	900	23.9	BRD4-044-01.2
BRD3-154E	900	2.3	BRD4-057-01.2
BRD3-154F	300	37.1	BRD4-058-01.2
BRD3-154H	900	60.0	BRD4-173-01
BRD3-154J	500	2.9	BRD4-063-01
BRD3-154L	900	13.6	BRD4-170-01
BRD3-155A	900	11.3	BRD4-171-01
BRD3-155C	1000	11.7	BRD4-172-01

Appendix VIII: Antimicrobial susceptibility assays

* Denotes significant difference from negative control ($\alpha = 0.05$) in unpaired t-test for all data sets

Average percent inhibition of microbial growth by extracts from fungi isolated from *P. elliptica* on MEA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-018-01.2	-2.8 (1.1)	-10.7 (1.4)	4.9 (2.5)	-8.7 (3.7)	3.0 (0.1)*	0.6 (0.8)	-2.5 (1.1)	7.2 (2.2)
BRD4-020-01.2	-8.8 (2.0)	-17.9 (4.0)	-2.6 (2.1)	-3.1 (2.9)	3.4 (4.1)	11.1 (3.2)*	-0.5 (1.7)	11.7 (2.0)
BRD4-028-01	-3.8 (1.2)	-31.2 (3.3)	2.0 (1.2)	7.8 (2.6)	7.0 (3.4)	1.8 (2.5)	3.5 (0.6)	92.6 (1.7)*
BRD4-038-01.2	-7.2 (0.2)	-0.5 (3.0)	-4.4 (1.5)	-4.6 (2.8)	5.4 (1.8)	10.5 (2.0)*	-0.5 (1.7)	0.4 (0.9)
BRD4-039-01.2	-2.3 (1.7)	1.6 (0.6)	-2.9 (1.1)	-9.4 (6.1)	-3.5 (4.6)	8.5 (0.8)*	2.1 (1.2)	99.8 (0.1)*
BRD4-056-01.3	-37.9 (5.1)	-10.7 (1.4)	25.4 (0.7)*	2.8 (1.4)	-1.2 (6.6)	4.1 (3.6)	1.5 (2.6)	-18.2 (1.4)
BRD4-060-01	5.6 (1.1)	-3.1 (0.8)	4.8 (1.8)	22.1 (3.5)*	-1.9 (0.6)	6.5 (1.3)	0.0 (1.5)	-1.4 (2.0)

Average percent inhibition of microbial growth by extracts from fungi isolated from *P. elliptica* on PDA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-002-01.2	-42.0 (5.3)	-23.0 (2.5)	-4.4 (0.3)	3.6 (2.0)	2.8 (3.0)	1.0 (1.1)	0.1 (0.4)	-1.4 (2.4)
BRD4-026-01	3.6 (1.4)	59.3 (2.4)*	8.9 (1.2)*	2.0 (1.3)	-29.5 (3.2)	22.6 (2.0)*	3.1 (2.1)	41.1 (4.9)*
BRD4-027-01	-0.6 (0.8)	14.0 (0.9)*	2.1 (0.7)	-7.5 (1.4)	3.6 (5.2)	10.1 (2.9)*	6.2 (2.0)	99.3 (0.0)*
BRD4-032-01	1.6 (2.2)	16.6 (1.9)*	-5.8 (2.4)	41.4 (2.1)*	-7.0 (5.1)	9.1 (0.8)*	1.5 (0.9)	4.1 (1.0)*
BRD4-035-01.2	-36.4 (5.4)	-12.0 (0.4)	-9.0 (1.8)	65.7 (1.8)*	6.0 (2.5)	4.0 (4.3)	5.8 (2.3)	25.9 (3.5)*
BRD4-043-01.2	16.4 (5.0)*	-4.1 (1.6)	-2.2 (0.2)	88.3 (1.2)*	4.4 (5.1)	7.1 (1.4)	-0.8 (1.1)	1.3 (1.9)
BRD4-048-01.2	11.4 (1.4)*	30.2 (3.4)*	0.8 (1.3)	98.8 (0.0)*	-4.2 (1.3)	6.0 (1.3)	6.4 (1.6)	-6.0 (2.9)
BRD4-059-01	0.3 (2.0)	3.6 (1.5)	-2.7 (1.6)	39.7 (8.2)*	3.8 (1.0)*	2.8 (1.7)	-0.7 (1.0)	-3.7 (2.7)
BRD4-061-01.3	2.9 (3.5)	-9.3 (3.6)	-0.0 (0.3)	14.8 (2.4)*	-3.9 (6.4)	-1.3 (2.4)	3.3 (0.5)	-15.5 (9.5)
BRD4-168-01	-29.6 (3.5)	-3.0 (3.9)	3.8 (0.4)	97.9 (0.1)*	3.6 (1.6)	-4.1 (6.6)	-1.3 (1.8)	99.2 (0.2)*

Average percent inhibition of microbial growth by extracts from fungi isolated from *P. strobus* on MEA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-171-01	0.4 (4.0)	6.4 (2.9)	101.9 (0.1)	73.0 (3.5)*	-1.5 (1.0)	16.7 (0.6)*	67.0 (1.1)*	99.5 (0.1)*
BRD4-172-01	5.0 (5.0)	-8.4 (3.0)	-14.6 (1.6)	26.9 (0.6)*	11.5 (1.7)*	4.1 (1.7)	-2.7 (1.0)	-15.4 (0.7)

Average percent inhibition of microbial growth by extracts from fungi isolated from *P. strobus* on PDA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-044-01.2	-3.8 (2.2)	-5.3 (2.4)	-3.0 (0.8)	68.2 (2.1)*	13.1 (2.4)*	11.3 (1.0)*	-4.8 (0.7)	3.4 (4.2)
BRD4-057-01.2	-36.1 (6.8)	0.9 (0.7)	18.4 (1.2)*	86.9 (2.2)*	6.0 (2.6)	15.5 (1.1)*	4.4 (0.7)*	-2.7 (1.8)
BRD4-058-01.2	-193.7 (4.6)	-18.8 (4.0)	88.3 (3.8)*	93.9 (1.4)*	4.3 (6.5)	1.2 (3.8)	-0.3 (1.0)	-18.5 (2.6)
BRD4-063-01	-16.3 (4.0)	-9.6 (4.2)	33.1 (2.2)*	80.7 (1.5)*	-4.4 (7.2)	4.2 (0.8)*	6.2 (0.2)*	-19.8 (2.4)
BRD4-064-01	-49.1 (2.3)	2.2 (2.5)	31.4 (0.9)*	17.9 (1.0)*	-0.4 (2.6)	2.5 (3.1)	0.7 (1.6)	-11.9 (4.8)
BRD4-170-01	-7.7 (3.9)	-13.5 (2.4)	-14.8 (0.2)	87.4 (0.4)*	36.9 (0.4)*	12.4 (1.6)*	1.8 (2.8)	-18.9 (4.2)
BRD4-173-01	-147.5 (9.2)	-10.1 (3.8)	111.9 (4.6)*	83.6 (0.9)*	-1.5 (1.1)	-0.9 (1.3)	-0.5 (0.3)	-16.2 (7.2)

Average percent inhibition of microbial growth by extracts from fungi isolated from *S. purpurea* on MEA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-003-01	-6.3 (0.5)	-9.4 (0.8)	-8.0 (0.6)	2.4 (1.1)	12.7 (4.7)	-5.3 (0.8)	-0.4 (0.9)	-4.3 (1.4)
BRD4-004-01.2	-9.2 (2.0)	-3.1 (3.3)	-3.0 (0.8)	20.4 (3.0)*	12.4 (3.6)*	-3.4 (1.2)	0.9 (1.1)	21.5 (2.6)*
BRD4-005-01.2	-5.1 (2.8)	-26.0 (3.4)	-4.3 (1.3)	5.6 (0.7)*	6.9 (2.2)*	8.5 (2.5)*	-0.3 (1.3)	1.4 (3.1)
BRD4-008-01	-9.6 (2.2)	33.2 (2.2)*	4.6 (1.5)	-6.0 (1.0)	-13.5 (1.7)	-7.1 (2.8)	-3.1 (0.7)	26.8 (2.0)*
BRD4-010-01.2	-8.1 (0.1)	-23.4 (8.8)	-8.7 (1.9)	1.9 (0.7)	11.1 (1.3)*	3.8 (0.2)*	-0.4 (2.8)	-2.9 (2.7)
BRD4-011-01	-12.0 (4.8)	-17.4 (11.5)	-9.1 (0.5)	43.4 (0.2)*	3.3 (4.3)	4.5 (2.1)	-6.0 (1.1)	3.5 (1.2)
BRD4-012-01.2	6.6 (0.4)	-17.8 (1.6)	-2.7 (2.7)	-1.0 (2.8)	4.2 (2.3)	-0.9 (1.5)	-2.3 (1.5)	8.3 (0.1)
BRD4-013-01	-8.5 (1.4)	-28.2 (11.2)	-2.6 (4.2)	7.7 (0.5)*	-11.0 (2.0)	7.5 (0.2)*	-1.5 (1.1)	-1.7 (2.7)
BRD4-014-01	-9.1 (1.1)	-24.3 (3.6)	-0.6 (2.0)	13.1 (1.9)*	-2.1 (2.6)	-2.4 (1.3)	-0.9 (1.5)	9.0 (7.4)
BRD4-021-01	5.9 (0.0)	40.9 (2.2)*	7.1 (1.7)*	-0.6 (3.2)	25.1 (0.4)*	3.1 (1.6)	5.0 (2.6)	101.1 (0.1)*
BRD4-025-01	11.0 (1.6)*	56.7 (0.8)*	2.9 (0.5)	21.5 (6.8)*	21.0 (0.6)*	-4.7 (4.9)	2.0 (0.3)	101.0 (1.0)*
BRD4-040-01.2	-26.0 (1.5)	38.6 (1.2)*	-7.9 (0.1)	95.0 (0.1)*	11.2 (1.7)*	6.9 (1.3)	0.0 (1.5)	45.5 (3.8)*
BRD4-041-01	-24.9 (1.5)	-17.1 (2.0)	-7.6 (2.5)	3.2 (0.8)*	1.5 (2.7)	1.2 (4.0)	0.0 (1.6)	-10.0 (1.2)
BRD4-042-01.2	15.9 (2.6)*	16.9 (1.0)*	-1.6 (2.2)	95.5 (1.9)*	15.9 (0.4)*	7.7 (2.9)	1.6 (0.3)	-4.8 (0.4)
BRD4-049-01.3	-23.1 (4.7)	-24.2 (1.1)	22.4 (1.0)*	4.3 (0.6)*	1.9 (3.4)	4.1 (0.6)	3.8 (2.0)	5.5 (1.9)
BRD4-050-01	-32.2 (2.3)	-28.7 (4.7)	-5.6 (1.8)	1.5 (0.6)	0.8 (7.7)	0.6 (1.9)	1.1 (3.7)	-8.5 (1.4)
BRD4-051-01.3	1.5 (9.0)	99.1 (4.3)*	-4.6 (2.9)	87.4 (0.2)*	1.5 (2.0)	23.0 (0.6)*	4.7 (1.9)	-3.2 (3.1)
BRD4-054-01.2	-3.6 (5.9)	-17.1 (2.6)	-3.1 (0.9)	7.6 (2.2)	-1.1 (2.1)	10.3 (1.0)*	-5.0 (0.2)	-10.8 (3.5)
BRD4-066-01	-36.7 (1.9)	-14.0 (3.9)	-5.6 (0.7)	10.0 (3.2)*	9.4 (2.9)*	13.2 (1.1)*	-2.7 (1.5)	-16.1 (5.7)
BRD4-169-01	-3.3 (4.0)	38.1 (2.8)*	9.5 (1.1)*	73.4 (1.1)*	6.6 (2.0)*	9.8 (2.7)*	0.9 (0.5)	-12.1 (1.8)

Average percent inhibition of microbial growth by extracts from fungi isolated from *S. purpurea* on PDA (\pm SEM)

Extract Code	<i>E. faecium</i>	<i>S. aureus</i>	<i>M. smegmatis</i>	<i>M. tuberculosis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
BRD4-001-01	4.0 (4.8)	15.0 (2.7)*	-5.9 (1.4)	26.5 (6.9)*	8.1 (2.1)*	26.4 (0.9)*	1.0 (0.4)	4.1 (0.9)*
BRD4-006-01	-0.7 (1.7)	19.7 (1.6)*	3.2 (2.0)	19.9 (2.5)*	9.6 (1.0)*	-6.4 (0.4)	1.7 (0.4)	30.9 (4.0)*
BRD4-007-01	-4.5 (1.8)	-15.0 (6.2)	-9.6 (0.9)	-9.5 (0.7)	0.9 (1.4)	-2.4 (0.6)	0.2 (1.5)	-10.4 (1.8)
BRD4-009-01.2	-39.8 (2.2)	0.3 (1.4)	1.6 (0.8)	-0.1 (3.2)	3.0 (6.1)	-4.4 (1.1)	2.7 (1.8)	5.0 (1.6)*
BRD4-015-01	-8.0 (0.4)	-23.0 (1.7)	-2.0 (1.3)	5.8 (0.8)*	3.1 (4.3)	-0.9 (4.8)	1.1 (0.5)	102.0 (0.1)*
BRD4-016-01	-0.3 (2.9)	34.5 (2.4)*	-6.3 (0.6)	60.0 (1.2)*	1.5 (2.9)	14.3 (2.4)*	3.8 (0.8)*	96.9 (0.2)*
BRD4-017-01	-7.7 (1.0)	-15.7 (3.1)	-0.3 (0.5)	11.5 (2.1)*	12.6 (1.2)*	-7.2 (2.0)	4.7 (1.2)*	81.5 (4.2)*
BRD4-019-01.2	1.3 (6.2)	-24.6 (3.6)	0.6 (2.3)	-11.8 (1.9)	6.1 (5.2)	-2.3 (1.2)	-5.3 (1.7)	-1.1 (2.1)
BRD4-022-01.2	-30.5 (2.2)	71.3 (1.9)*	2.0 (2.2)	16.6 (1.5)*	29.4 (0.7)*	20.8 (4.8)*	4.1 (0.9)	99.3 (0.1)*
BRD4-023-01	-11.6 (1.8)	5.7 (2.8)	-3.2 (2.0)	-16.2 (2.7)	-3.5 (1.5)	-3.0 (2.3)	-2.4 (0.9)	16.9 (2.9)*
BRD4-024-01	13.0 (2.0)*	61.7 (9.0)*	15.9 (1.0)*	-5.0 (1.4)	22.5 (0.7)*	20.9 (1.9)*	0.8 (0.9)	102.5 (1.3)*
BRD4-029-01.2	14.1 (0.9)*	-21.4 (1.1)	-6.6 (1.9)	2.5 (3.4)	0.6 (2.5)	-10.2 (1.9)	-1.1 (0.4)	-6.3 (2.3)
BRD4-030-01	14.3 (3.9)*	23.4 (1.6)*	2.1 (1.4)	13.3 (3.6)*	13.4 (5.7)	24.1 (1.1)*	-0.4 (1.0)	13.1 (1.7)*
BRD4-033-01	5.9 (0.7)	26.4 (1.7)*	-7.7 (0.7)	79.9 (1.2)*	22.4 (1.3)*	17.3 (1.0)*	0.3 (2.0)	98.3 (0.2)*
BRD4-034-01.2	-29.3 (3.5)	-8.2 (1.9)	-13.2 (1.6)	51.6 (1.1)*	-1.0 (1.3)	-1.6 (2.7)	0.8 (1.4)	-2.3 (2.2)
BRD4-036-01	6.9 (0.9)*	76.0 (3.8)*	-4.8 (1.4)	51.3 (4.4)*	10.7 (1.5)*	27.2 (2.4)*	6.4 (0.8)*	5.7 (1.2)
BRD4-037-01.2	-55.9 (2.4)	5.3 (2.2)	-8.2 (1.4)	-23.9 (1.5)	2.3 (2.0)	-6.1 (0.8)	3.8 (1.2)	14.0 (1.4)*
BRD4-045-01.2	4.3 (2.5)	-39.9 (2.0)	-2.6 (0.3)	26.7 (1.0)*	-8.2 (3.2)	2.3 (2.8)	7.6 (0.5)*	-13.8 (0.8)
BRD4-046-01	7.0 (2.7)	37.1 (1.7)*	8.9 (0.8)*	-1.5 (0.6)	-35.9 (1.5)	-8.0 (7.8)	-1.3 (0.1)	1.1 (2.1)
BRD4-047-01	10.8 (1.5)	-6.5 (4.5)	0.9 (1.8)	16.2 (2.9)*	0.9 (3.1)	5.9 (0.2)	1.1 (0.7)	-4.7 (2.4)
BRD4-052-01.2	-2.1 (4.9)	10.33 (8.2)	-4.0 (2.8)	94.6 (0.0)*	-5.4 (5.5)	6.7 (0.5)	3.5 (1.8)	-2.2 (2.1)
BRD4-053-01.2	-9.7 (3.4)	-3.5 (1.7)	10.4 (2.2)*	0.2 (1.0)	1.4 (0.3)*	-5.6 (2.3)	-7.0 (2.0)	-5.9 (0.8)
BRD4-055-01.2	-27.6 (2.4)	0.4 (1.4)	-2.2 (1.8)	35.5 (8.2)*	-0.5 (3.5)	0.7 (1.8)	1.8 (1.6)	-6.1 (1.5)
BRD4-062-01	-22.3 (3.9)	-10.8 (1.1)	-0.5 (0.4)	27.5 (8.5)*	-8.5 (2.4)	11.0 (1.2)*	4.6 (0.2)*	1.0 (4.7)
BRD4-167-01	-1.6 (5.2)	-15.1 (1.7)	-0.1 (0.6)	-5.4 (2.6)	1.3 (0.9)	4.6 (2.3)	-2.1 (1.7)	-19.0 (2.2)

Curriculum Vitae

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Publication:

Zhu, D., Hunter, C.D., Baird, S.R., **Davis, B.R.**, Bos, A., Geier, S.J., Vogels, C.M., Decken, A., Gray, C.A., and Westcott, S.A. 2017. Synthesis and antimicrobial properties of cyclic fluorodiamines containing boronate esters. *Heteroatom Chemistry* 28(6): e21405.

Conference presentations:

- 2017** **Maritime Natural Products Conference: University of Prince Edward Island University, Charlottetown, PE; oral presentation**
Title: Isolation of endophytes from the roots of Canadian medicinal plants.
Authors: Bradley R Davis, John A Johnson, Christopher A Gray
- 2018** **Maritime Natural Products Conference: Dalhousie University, Halifax NS; oral presentation**
Title: Estimates of endophyte species richness depend on culture conditions
Authors: Bradley R Davis, John A Johnson, Christopher A Gray
- 2019** **Maritime Natural Products Conference: University of New Brunswick – Saint John, Saint John, NB; oral presentation**
Title: Making the best use of databases for fungal species identification by DNA barcoding
Authors: Bradley R Davis, John A Johnson, Christopher A Gray