Université de Montréal

Microbial endophytes and their interactions with cranberry plants

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Mémoire présenté en vue de l'obtention du grade de Maîtrise en Biochimie, option générale

Janvier 2023

Université de Montréal

Département de Biochimie et Médecine Moléculaire, Faculté de Médecine

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Ce mémoire intitulé

Microbial endophytes and their interactions with cranberry plants

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Résumé

Virtuellement toutes les plantes hébergent des champignons et des bactéries endosymbiontes (endophytes). Ces microorganismes façonnent le développement de leur hôte et peuvent inhiber des phytopathogènes. Au niveau moléculaire, les interactions plante-endophyte sont médiées par des molécules secrétées y compris des protéines et métabolites secondaires. Au cours des dernières années, la recherche d'endophytes a augmenté chez nombreux plantes, cependant chez les Ericaceae les endophytes ne sont pas bien connus. Alors, on s'est mis à investiguer les endophytes racinaires de la canneberge, une plante membre d'Ericaceae native de l'Amérique du Nord. On a échantillonné quatre plants provenant d'une ferme commerciale organique. Au total, 30 souches fongiques et 25 bactériens ont été isolés. Les bactéries *Pseudomonas* sp. EB212, *Bacillus* sp. EB213 et EB214; et les champignons *Hyaloscypha* sp. EC200, *Pezicula* sp. EC205 et *Phialocephala* sp. EC208 ont supprimé la croissance de cinq pathogènes de la canneberge, incluant *Godronia cassandrae*, un champignon causant la pourriture des fruits de la canneberge au Québec. EB213 a été capable de promouvoir légèrement la croissance de plantules de la canneberge. En performant des techniques microscopiques, on a constaté l'habileté de EC200, EC205 et EC208 à coloniser internement les racines des plantules de la canneberge. De plus, les génomes de ces champignons ont été séquencés, assemblés et annotés. Les analyses génomiques se sont concentrées sur les protéines secrétées et les groupes des gènes impliqués dans la biosynthèse (GGB). On a trouvé un large répertoire de gènes codant pour des enzymes qui métabolisent les carbohydrates et d'autres codant pour des protéases. Les deux groupes d'enzymes seraient utiles à dégrader de la matière organique pour libérer des nutriments. Aussi bien, ces enzymes pourraient faciliter la colonisation des racines de la plante hôte. De plus, on a prédit des nombreuses protéines effectrices qui assisteraient les endophytes à éviter l'activation du système immunitaire des plants. A noter que parmi les GGB inférés dans les génomes de EC200, EC205 et EC208, environ 90% ne sont pas caractérisés. Finalement, on a performé des analyses transcriptomiques pour élucider la réponse de EC200, EC205 et EC208 envers la présence de leur hôte, simulée par l'addition d'un extrait de canneberge au milieu de culture. Les conclusions majeures sont que les racines des plantes de la canneberge qui ont été échantillonnées sont dominées par des microorganismes avec

l'habileté d'inhiber des phytopathogènes ; et que les génomes de EC200, EC205 et EC208 codent pour un grand répertoire de protéines qui pourraient être liées aux interactions plante-endophyte.

Mots-clés : Keywords: endophytes de la canneberge, interactions plant-microbe, biocontrôle, promotion de la croissance des plantes, microscopie, génomique comparative, transcriptomique comparative, sécrétome, effectome, métabolites secondaires.

Abstract

Virtually all plants host fungal and bacterial endosymbionts (endophytes). These microbes shape plant development and may inhibit phytopathogens. At the molecular level, plant-endophyte interactions are mediated by secreted compounds, including proteins and secondary metabolites. While endophytes are increasingly studied in diverse plants, little is known about their presence in Ericaceae. Therefore, we set out to investigate the root endophytes of cranberry, an ericacean member native to North America. We sampled endophytes from four plants grown on an organic farm. In total, 30 fungal and 25 bacterial strains were isolated and identified. A subset of these, notably *Pseudomonas* sp. EB212, *Bacillus* sp. EB213 and EB214; and fungi *Hyaloscypha* sp. EC200, *Pezicula* sp. EC205, and *Phialocephala* sp. EC208, were tested for their ability to suppress phytopathogens. Altogether, they inhibited five cranberry pathogens, including *Godronia cassandrae*, an important cranberry fruit-rot agent in Quebec. EB213 was the only endophyte that increased the biomass of cranberry seedlings. Using microscopy techniques, we confirmed the ability of EC200, EC205, and EC208 to colonize cranberry roots internally. The genomes of these fungi were sequenced, assembled and annotated. Genomic analyses focused on secreted proteins and biosynthetic gene clusters (BGCs). We found an extensive repertoire of carbohydrate-active enzymes and proteases that could assist in recycling organic nutrients, rendering them accessible to plants; these enzymes may also facilitate root colonization. In addition, effector proteins were predicted; these molecules may assist endophytes to escape the plant immune system and favour colonization. We inferred 139 biosynthetic gene clusters (BGCs) across the three examined fungi. Remarkably, the product of around 90% of BGCs are unknown. Finally, transcriptomic analyses were performed to determine how EC200, EC205 and EC208 respond to the presence of cranberry, simulated by the addition of cranberry extract in the culture medium. The two major conclusions of this work are that the roots of the sampled cranberry plants are dominated by endophytes with

biocontrol abilities, and that EC200, EC205 and EC208 encode a broad repertoire of proteins that could be involved in plant-endophyte interactions.

Keywords: cranberry endophytes, plant-microbe interactions, biocontrol, plant growth-promotion, microscopy, comparative genomics, comparative transcriptomics, secretome, effectome, secondary metabolites.

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List of Abbreviations

16s rDNA: 16S ribosomal DNA

AA: auxiliary activities (CAZymes)

ABC: ATP-binding cassette transporters

AMF: arbuscular mycorrhizal fungi

ATP: adenosine triphosphate

BGCs: biosynthetic gene clusters

CAZymes: carbohydrate-active enzymes

CBM: carbohydrate-binding modules

CE: carbohydrate esterases

DSE: dark septate endophytes

EcMF: ectomycorrhizal fungi

EDTA: ethylenediaminetetraacetic acid

ErMF: ericoid mycorrhizal fungi

ETI: effector-triggered immunity

EtOH: ethanol

FCWDE: fungal cell wall degrading enzyme

GH: glycoside hydrolases

GO: gene ontology

GT: glycosyl transferases

ITS: internal transcriber space

KEGG: Kyoto Encyclopedia of Genes and Genomes

LysM: lysin Motif

MAPKS: mitogen-activated protein kinases

NRP: non-ribosomal peptide

NRPS: non-ribosomal peptide synthetase

OM: orchid mycorrhizal fungi

PCR: polymerase chain reaction

PCWDE: plant cell wall degrading enzyme

PDA: potato dextrose agar

PK: polyketide

PKS: polyketide synthase

PL: polysaccharide lyases

PTI: pattern-triggered immunity

QC: Quebec

SP: signal peptide

Tat: twin-arginine translocation motif

TSA: tryptic soy agar

WSC: cell wall integrity and stress response component

Para mi Madre (To my Mom)

Acknowledgments

I want to thank Professor B. Franz Lang, my director, for allowing me to do science in his laboratory and the cranberry fields. Without his financial support, guidelines and patience, this project would not have been possible. Likewise, I would like to thank Professor Gertraud Burger for being my co-director and always having optimal comments that enabled deep reflection to improve the understanding of nature.

Thanks to Lise Forget, her critical thinking, troubleshooting and long reflective discussions were immensely helpful for the advancement of this project.

Thanks to Lila Salhi, who set out the basis for the endophytes research in cranberry. Thanks for always being willing to help and provide direction. Her support and delicious cranberry couscous had an immeasurable impact on the development of this project. Thanks to Matt Sarrasin, who handled the genomic annotation and was there to answer all kinds of questions, whether scientific or not. Thanks to Bhagya C. Thimmappa for bringing dynamism, fresh ideas and an optimistic point of view to the team. Her spontaneity, scientific curiosity and spicy dal mix made the time in the laboratory more enjoyable. Thanks to the three of them, who introduced me to and guided me in the fantastic world of bioinformatics

I am grateful to Andrea O. Amorim, whose unconditional support contributed to completing this project. Also, thanks to Paul Stretenowich and Savandara Besse for their support and the delicious meals they shared with me, especially on the gray days.

Finally, I want to thank my other friends and colleagues I met during this project's completion. Their names are in alphabetic order: Alexandra Leveille-Kunst, Amruta Sahoo, Karima Elazreg, Louis Gendron, Matus Valach, Musa Ozboyaci, Nazli Kocatuğ, Pedro do Couto Bordignon, Shamim Hasan and Yasmine Draceni.

Chapter 1 – Literature Review

1.1 Endophytes

Most eukaryotes host and interact with microorganisms that shape their development (Hardoim et al., 2015; Verma et al., 2021). That is, they live in symbiotic relationships. Plants make no exception; they are populated externally and internally by a broad array of microbial symbionts (Verma et al., 2021). Microorganisms attached to the plant surface are referred to as epiphytes. In contrast, those spending at least part of their lifecycle within vegetal tissues are known as endophytes, a term derived from the Greek prefixes "endo" (inside) and "phyton" (plant) (Hardoim et al., 2015).

Roots, the hidden half of plants, mediate nutrient and water uptake. Furthermore, microbes interacting with the roots, particularly endophytes, may significantly shape plant development and be critical players in plant adaptation to various environments. (Genre et al., 2020; Liu et al., 2017; Rodriguez et al., 2009; Schulz et al., 2006). For example, the emergence of land plants, believed to occur 460 million years ago, was probably facilitated by root endophytes, as suggested by molecular data and the fossil record (Provorov and Vorobyov, 2009; Redecker et al., 2000; Schüßler, 2002; Wilkinson, 2001).

In this study, we sought to investigate the root endophytes of cranberry, an ericaceous plant native to North America that grows under infertile and acidic soils (Vorsa and Johnson-Cicalese, 2012). Little is known about the microbial endophytes that could assist plant development under these harsh conditions. Furthermore, the molecular mechanisms that govern cranberry-microbe interactions remain largely elusive. The following sections will discuss what is currently known about root endophytes. We will describe the colonization process and the influence of endosymbionts on host fitness.

1.2 Root endophytes

Root endophytes comprise bacterial, archaeal, fungal, and protistic taxa. They may play different roles in plant development, ranging from mutualists to commensals to antagonists, depending on the host and hostage genotypes and environmental conditions (Hardoim et al., 2015; Rodriguez et al., 2009; Santoyo et al., 2016; Schulz et al., 2006). Here we will focus on fungi and bacteria that form neutral or mutualistic relationships with their host.

1.2.1 Fungal root endophytes

Most root endophytes are facultative endosymbionts that colonize the roots internally and extend their hypha beyond the rhizosphere, forming a plant-soil interface that facilitates nutrient exchange (Berch et al., 2002; Parniske, 2008; Sathiyadash et al., 2020). Their internal structures are restricted to the root apoplast, comprising the space from the root plasma membrane to the rhizoplane on the root surface (Sattelmacher, 2001).

Among fungal root endophytes, arbuscular mycorrhizal fungi (AMF) are the most studied and best-understood endomycorrhizal symbionts. They are estimated to colonize about 70% of all vascular plants (Lee et al., 2013; Smith and Read, 2010). AMF were named after their signature intracellular structure, tree-resembling (arbuscules) hyphae formed in the inner cortical cells of their host (Parniske, 2008). Without exception, AMF belong to the monophyletic Glomeromycota.

Other mycorrhizal fungal guilds are more host-specific but poorly defined from the phylogenetic point of view, i.e., they are mainly identified based on morphological traits. For example, orchid mycorrhizal fungi (OMF) and ericoid mycorrhizal fungi (ErMF) associate mostly with members of Orchidaceae and Ericaceae. Most OMF belong to Basidiomycota, with rare species in Ascomycota (Jiang et al., 2019; Sathiyadash et al., 2020), whereas ErMF belong mainly to Ascomycota, yet with several members in Basidiomycota (Vohník, 2020). The distinct morphologic traits of OMF are intracellular coiled structures called pelotons formed in cortical cells of their hosts (Sathiyadash et al., 2020), remotely similar to ErMF that develop thick hyphal coils in epidermal cells (Vohník, 2020).

Ectomycorrhizal fungi (EcMF) are characterized by a dense hyphal sheath (mantle) surrounding the root surface and an intercellular network of branched hyphae between epidermal and cortical cells (Hartig net) (Anderson and Cairney 2007; Johnson and Gehring 2007). EcMF colonize mainly woody plants in boreal forests, and like ErMF, and OMF, they are multiphyletic, belonging to Basidiomycota and Ascomycota.

Finally, the term dark septate endophyte (DSE) refers to an indistinct collection of diverse Ascomycota (Helotiales, Xylariales, and Pleosporales) with low host-specificity. These fungi are identified by melanized septate hyphae (Knapp et al., 2018; Lukešová et al., 2015; Rodriguez et al., 2009). They colonize intercellular regions of roots and occasionally form dense intracellular structures (microsclerotia). DSE may also form structures resembling ericoid mycorrhizal fungi hyphal coils (Lukešová et al., 2015; Yang et al., 2018).

1.2.2 Bacterial root endophytes

The term 'bacterial root endophyte' commonly groups together rhizobia, rhizobacteria and plant-growth-promoting bacteria. Rhizobia comprise only the proteobacterial genera *Rhizobium*, *Mesorhizobium*, *Ensifer* and *Bradyrhizobium*, clearly defined by nodule formation at the roots of legumes (plant family Fabaceae), as a consequence of nitrogen fixation (Willems, 2006). The terms plant-growth-promoting bacteria and rhizobacteria are used in a broader sense, including rhizobia and other Proteobacteria (*Burkholderia* and *Pseudomonas*), Actinobacteria (*Streptomyces* and *Microbacterium*) and Firmicutes (*Bacillus*) (Liu et al., 2017; Santoyo et al., 2016). Bacterial endophytes colonize intercellularly and intracellularly epidermal and cortical root cells. However, in contrast to fungi that are confined to the root apoplast, bacteria may trespass to the cytoplasm (Afzal et al., 2019; Kandel et al., 2017; Liu et al., 2017), establishing a more intimate interaction with their host.

1.2.3 Endophytic communities and their secretomes

Bacterial and fungal endophytes coexist inside the plant (van Overbeek and Saikkonen, 2016), with multilateral interactions between microbes and between microbes and the host. Thus, elucidating the molecular mechanisms behind such associations is challenging. Advances in 'omics' are fundamental to understanding plant-endophyte exchanges. Proteomic data demonstrate

that secreted proteins are involved in host colonization and nutrient mobilization (Doré et al., 2015; Khatabi et al., 2019; Vincent et al., 2012). Similarly, metabolomic data indicate that extracellular secondary metabolites assist in nutrient uptake, regulate phytohormones and inhibit microbes (Barúa et al., 2019; Mehmood et al., 2019; Rungin et al., 2012). Then, genomics reveals the genes that code for secreted proteins and the enzymes involved in synthesizing secondary metabolites (Knapp et al., 2018; Martino et al., 2018; Miyauchi et al., 2020).

Across all domains of life, most secreted proteins contain an N-terminal cleavable signal peptide (SP) that allows transport within the cell and outside (José Juan Almagro Armenteros et al., 2019; Caccia et al., 2013; Green and Mecsas, 2016). Although the SP sequence is only moderately conserved, it has four invariant features: (i) it is 15–30 amino acids long, (ii) it contains a positively charged amino terminus, (iii) a hydrophobic core, and (iv) a polar carboxylic terminus (Caccia et al., 2013).

In fungi, nascent proteins containing an SP are translocated toward the endoplasmic reticulum. Afterwards, they are folded and may undergo distinct modifications such as glycosylation, disulphide bridge formation, phosphorylation, and subunit assembly. Then, vesicles guide them to the Golgi compartment, where further alterations may occur. Finally, they are packed into secretory vesicles that transport them through the plasma membrane to the extracellular space (Conesa et al., 2001).

Bacterial secreted proteins contain either an SP or the twin-arginine translocation motif (Tat). Proteins with an SP are transported to the periplasm by the general secretory pathway (sec), while Tat-containing proteins are translocated by the homonym pathway. Then, in gram-negative bacteria, proteins are guided to the extracellular space by specialized secretory systems, including type 2, type 3, type 4 and type 6. In contrast, in gram-positive bacteria, proteins are released by passive diffusion through the peptidoglycan layer.

Efflux pumps are a series of well-conserved transporter membrane proteins. They are present in eukaryotes and prokaryotes and mainly perform the secretion of secondary metabolites. These molecular carriers are grouped into four classes: ATP-binding cassette transporters (ABC), major

facilitator superfamily; small multidrug resistance; and resistance nodulation determinants. While ABC transporters require ATP hydrolysis as an energy source, the others are powered by the membrane electrochemical gradient (Martín et al., 2005).

The role of secreted proteins and secondary metabolites in plant-endophyte interactions is described in more detail in the following sections.

1.3 Endophyte colonization and plant defense

1.3.1 Secreted Carbohydrate-Active enZymes (CAZymes) and proteases as colonization agents

Root-endophyte interactions commonly start in the rhizosphere, where microbes compete for plant-secreted nutrients (Verma et al., 2021). Transition of the microbe to the root endosphere may be more efficient for acquiring plant-derived nutrients. However, penetrating the root cell wall and overcoming the plant's innate immune system is challenging. It is currently debated whether plants selectively assist microbial colonization. On the other hand, it is acknowledged that fungal and, to a lesser extent, bacterial endophytes deploy a series of degradative enzymes that break down the plant cell wall. This would open the port of entry for the endophytes to access the root endosphere (Liu et al., 2017).

CAZymes synthesize, degrade, and modify glycosidic bonds. They are grouped into six functional classes, each containing multiple families: Glycoside Hydrolases (GHs), Glycosyl Transferases (GTs), Polysaccharide Lyases (PLs), Carbohydrate Esterases, the non-catalytic Carbohydrate-Binding Modules (CBMs) and certain redox enzymes which team up with CAZymes known as Auxiliary Activities (AAs) (Lombard et al., 2014; Yin et al., 2012). Numerous members of these families degrade cellulose, hemicellulose, pectin, and lignin of the plant cell wall and, therefore, are referred to as plant cell-wall degrading enzymes (PCWDE). There is evidence that PCWDE are essential for the establishment of endosymbiosis. For example, experiments with the fungus *Laccaria bicolor* and poplar seedlings revealed that the fungal pectinase GH28 was bound to the microbe-plant interface. Moreover, GH28-knocked-down strains of *L. bicolor* were significantly impaired in colonizing the roots of the poplar host (Zhang et al., 2022).

Proteases may target plant cell-wall structural proteins and are classified depending on the functional group in their active site: aspartic, cysteine, glutamic, metallic, serine, or threonine proteases (Chandrasekaran et al., 2016). A recent report indicates that the secreted serine proteases S10, aspartic A1, and glutamic G01 were highly upregulated in the fungus *Oidiodendron maius* when in symbiosis with blueberry seedlings, suggesting a crucial role of these proteases in endosymbiotic interactions (Martino et al., 2018).

1.3.2 Plant pattern-triggered immunity (PTI)

The plant immune system is elicited after sensing the presence of microbes and damage of the root cell wall (Jones and Dangl, 2006). Plant transmembrane receptor proteins sense glucan, chitin, and peptidoglycan, which are components of fungal or bacterial cell walls. Moreover, plant receptors can also identify oligogalacturonides and cyclodextrins released during the degradation of the plant cell wall (Newman et al., 2013). Plant receptor proteins activate mitogen-activated protein kinase cascades that trigger defensive responses such as the synthesis and secretion of reactive oxygen species, salicylic and abscisic acid, proteinases, chitinases, glucanases, inhibitors of PCWDE, activated *via* transcriptomic reprogramming (Sperschneider et al., 2018; Vincent et al., 2020; Yuan et al., 2021).

1.3.3 Endophytic effectors

To overcome the plant immune system, endophytes secrete proteins termed effectors. These proteins can mask the microbial patterns recognized by PTI or regulate defense pathways of the plant in the root cytoplasm. Effector proteins are structurally diverse; some are small (<300 aa) and cysteine-rich (>3%), others lack sequence similarity to known proteins, and tend to be strain-specific, which makes it difficult to identify them (Kristianingsih and MacLean, 2021; Sperschneider et al., 2016).

Fungal endophytes secrete effectors in the root apoplast or into the cytoplasm, by a yet unknown mechanism. In contrast, bacterial symbionts utilize the type 3 secretory system to inject effectors into the host cytoplasm directly (Kamoun, 2006; Sperschneider et al., 2017). Most known fungal apoplastic effectors contain the domain LysM and sequester chitin oligosaccharides to prevent the

release of microbial compounds recognized by PTI (Lucke et al., 2020). In addition, fungal and bacterial cytoplasmic effectors may inhibit plant defensive pathways in various cellular compartments. For instance, the effector chorismite mutase, secreted by *Ustilago maydis* in symbiosis with maize, targets the chloroplast and reduces the synthesis of salicylic acid (Lo Presti et al., 2015). Likewise, *Pseudomonas syringae* secretes proteins HopI1 and HopN1 that target Hsp70 and PsbQ in *Arabidopsis thaliana*, and thus reduce the levels of salicylic acid and reactive oxygen species (Deslandes and Rivas, 2012).

1.3.4 Plant effector-triggered immunity

The plant immune system has evolved to detect microbial effectors. The mechanism that allows effector recognition is called effector-triggered immunity (ETI) and relays on intercellular and intracellular proteins carrying nucleotide-binding and leucine-rich repeat domains (Jones and Dangl, 2006). The ETI and PTI pathways overlap (Yuan et al., 2021). For instance, ETI is regulated by mitogen-activated protein kinase cascades that result in transcriptomic reprogramming and the synthesis of reactive oxygen species, salicylic and jasmonic acids. However, ETI can also lead to a hypersensitive response and localized programmed cell death to avoid microbial proliferation (Thulasi Devendrakumar, Li, and Zhang, 2018; Irieda et al., 2019; Yuan et al., 2021).

1.4 Impact of endophytes on plant development

1.4.1 Plant growth promotion mechanisms

1.4.1.1 Nutrient Uptake

The soil is the primary source of nutrients for plants. It can contain macronutrients like nitrogen and phosphorus, and micronutrients like iron, manganese, and copper. In natural ecosystems, most nutrients are insoluble or trapped in decaying organic matter and, therefore, inaccessible to plants (Rana et al., 2020; Wei et al., 2022). Microorganisms associated with plants are able to solubilize and mineralize these nutrients (Verma et al., 2021).

Nutrient uptake by fungal and bacterial endophytes is facilitated by diverse proteins and secondary metabolites they secrete. Proteases and plant and fungal cell-wall degrading enzymes catalyze the depolymerization of organic nitrogen bound to plant and microbial necromass (Cabello et al.,

2009; Wei et al., 2022). Then, the ammonification process can mineralize released small peptides and amino acids (Kieloaho et al., 2016). In addition, some bacteria may also secrete nitrogenases that fixate atmospheric nitrogen into ammonia (Cabello et al., 2009; Sickerman et al., 2019).

Phosphatases, secreted by fungi and bacteria, liberate phosphorous by hydrolyzing phospho-ester and phosphoanhydride bonds of organic sources like inositol phosphate esters, phospholipids, nucleic acids, phosphate linked to sugars and derivatives of phosphoric acid (Mehta et al., 2019; Rana et al., 2020; Tarafdar et al., 2001). Siderophores are a low-molecular-weight secondary metabolite, that chelate iron and form soluble ferric complexes, which, in turn, plants can assimilate (Albelda-Berenguer et al., 2019).

Examples of endophytes able to promote plant growth include the fungus *Piriformospora indica,* which was shown to facilitate phosphorous uptake in maize seedlings (Kumar et al., 2011). Also, the fungus *O. maius* increases the biomass and nitrogen levels as demonstrated in seedlings of *Rhododendron fortune* (Wei et al., 2016). Finally, *Bacillus velezensis* is able to secrete phosphatases and siderophores, improving phosphorous and iron uptake in sugar cane under greenhouse conditions (Z. Wang et al., 2020).

1.4.1.2 Phytohormones

Phytohormones such as auxin, cytokinin, and gibberellin, serve as signaling molecules in cell division and regulate plant growth and fruit production (Ali et al., 2017; Kalra and Bhatla, 2018; Kieber and Schaller, 2014). Fungal and bacterial endophytes may modulate phytohormone levels or produce phytohormones themselves. For example, *Trichoderma* sp. Synthesizes and controls cytokinin levels in *Arabidopsis* Seedlings (Bean et al., 2021). Likewise, *Bacillus velezensis* and *Bradyrhizobium diazoefficiens* produce auxin and gibberellin, promoting the growth of wheat and soybean seedlings (Nett et al., 2022; Talboys et al., 2014).

1.4.2 Biocontrol mechanisms

Endophytes may also favour plant fitness by suppressing microbes that are detrimental to the host. Microbial inhibition, known as biocontrol, is mainly due to the secretion of secondary metabolites and lytic enzymes.

1.4.2.1 Secondary metabolites

A broad spectrum of endophyte-produced secondary metabolites has an antibiotic effect (Gross and E. Loper, 2009; Lucke et al., 2020). Non-ribosomal peptides (NRPs), polyketides (PKs), and terpenes are the most widespread biocontrol compounds (Belbahri et al., 2017; Gross and E. Loper, 2009; Knapp et al., 2018; Martino et al., 2018; Miyauchi et al., 2020). Secondary metabolites are assembled by specialized enzymes encoded in biosynthetic gene clusters (BGCs) alongside with transport and regulatory genes (Medema et al., 2011).

PKs are polymers of carboxylic acid derivates such as acetyl-CoA and malonyl-CoA. They are assembled by multidomain polyketide synthases as follows (Weissman 2009). The synthesis initiates when an acyl-carrier domain recognizes the starter unit. Next, the chain is elongated by a ketosynthase domain through a decarboxylative Claisen condensation giving place to a β-ketothioester. In some cases, the β-ketone may undergo modifications by accessory enzymatic domains such as ketoreductase, dehydratase, and enoyl reductase. Then, the molecule is released by either hydrolysis of the thioester bond, cyclization, transesterification, peptide bond formation, or macrolactonisation (D. Walker et al., 2021; Schümann and Hertweck, 2006; Weissman, 2009). PKs may be assembled by a single multi-domain synthase or a complex composed of multiple enzymes. Also, the synthesis process may be linear or iterative (Weissman, 2009). Table 1 summarizes the distinct types of polyketide synthases.

PKs with antimicrobial properties include fengycin B and the mycotoxin assembled by *Bacillus* spp. And *Fusarium* spp., respectively (Chen et al., 2018; Palazzini et al., 2007) (Fatema et al., 2018).

Table 1. ***Different types of polyketide synthases found in fungi and bacteria.***

Type of PKS	Mode of operation	Type of product	Organism
I	modular	Reduced	bacteria
I	iterative	Aromatic and reduced	fungi
II	iterative	Aromatic	bacteria
III	iterative	Aromatic	fungi and bacteria

NRPs are polymers of proteinogenic and non-proteinogenic amino acids (Finking and Marahiel, 2004). They are assembled by either a single multidomain synthetase or a complex, similar to PKS (Reimer et al., 2018). NRPs synthesis proceeds as follows. An amino acid is recruited and activated by an adenylation domain. Subsequently, the monomer is transferred onto the thiolation domain, also known as a peptidyl-carrier protein. Then, the condensation domain performs the chain elongation by catalyzing the amide bond between the monomers. Finally, a terminal thioesterase domain catalyzes the release of the nascent NRP (Duban et al., 2022; Reimer et al., 2018). Optional modifications may also occur before the molecule is released. For example, epimerization, methylation, or formylation can be performed by domains distributed across or outside the modules. Examples of NRPs with antimicrobial properties are the well-known penicillins synthesized by *Penicillium* spp. and surfactin assembled by *Bacillus* spp. (Süssmuth and Mainz, 2017).

Terpenes, also known as isoprenoids, are the most diverse natural compounds (Oldfield and Lin, 2012). They are built from the isomers isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which contain five carbons (C5) in their structure. (Barúa et al., 2019; Oldfield and Lin, 2012). Successive condensations of DMAPP and IPP form linear isoprenyl diphosphate compounds. Then, a single terpene synthase transforms these precursors into terpenes with a distinct number of carbons: hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40) (Oldfield and Lin, 2012). Examples of antimicrobial terpenes include trichodermin synthesized by *Trichoderma* spp. (Barúa et al., 2019; Khan et al., 2020; Shentu et al., 2014) and the volatile compound beta-cubebene produced by *Streptomyces* spp. (Ayed et al., 2021)

1.4.2.2 Lytic enzymes for biocontrol

CAZymes and proteases are able to degrade glycoconjugates and structural proteins of fungal pathogens. For example, glucanases purified from *B. velezensis* ZJ20 disrupts the growth of pathogens *Cryphonectria parasitica*, *and Cylindrocladium quinqueseptatum* (Xu et al., 2016). Similarly, the aspartic proteases P6281 and P6281 secreted by the fungus *Trichoderma harzianum* inhibit several pathogens including *Botrytis cinerea*, the agent of grey mold in apples, oranges, and cucumber (Deng et al., 2018).

1.5 Cranberry and endophytes

Cranberry (*Vaccinium macrocarpon* Aiton), also known as American cranberry or large cranberry, is an evergreen shrub, member of Ericaceae, and native to eastern North America (Vorsa and Johnson-Cicalese, 2012). Cranberry plants produce small reddish berries that are used in the human diet. The plant has been cultivated intensively for the last two hundred years, while the use of the fruit can be traced back more than five hundred years. For instance, pre-Columbian cultures used cranberries as a food source and medicine (Bakshi et al., 2019). The Algonquins called the berry "atoqua," from which the *Québecois* word "atoca" is derived. The name 'cranberry' was given to the plant by early colonizers referring to the resemblance of the cranberry flowers with the head and bill of the crane (Bakshi et al., 2019).

Contrary to a common misconception, cranberry plants do not grow underwater, but they are flooded to collect the berries and protect them in winter. Instead, cranberries grow in moist, well-drained soils (Neto and Vinson, 2011). The presence of sand and organic matter makes cranberry soil infertile and too acidic for most other plants. In such harsh conditions, plant nutrition is limited since nitrogen and phosphorous are primarily bound to complex compounds in decaying matter (Cairney and Meharg, 2003; Wei et al., 2022). Cranberry can survive in poor soils, and one reason for that is that they associate with root endophytes that can depolymerize organic sources and render nutrients accessible to the plant, as observed in other Ericaceae.

Ericaceous plants are commonly colonized by Helotiales fungi such as *Hyaloscypha aggregate*, *O. maius*, and members of the *Phialocephala-Acephala* complex (Lukešová et al., 2015; Wei et al., 2022). These fungi are known to improve plant nutrition and alleviate abiotic stress (Cairney and Meharg, 2003; Kosola et al., 2007; Wei et al., 2022). Remarkably, Helotiales emerged approximately at the same period as Ericaceae, about 66-72 Ma ago, suggesting co-evolution of the host and symbiont (Martino et al., 2018; Salhi et al., 2022; Schwery et al., 2015).

Fungi found in Ericaceae are often classified based on morphologic traits of hypha inside the host's root (Mitchell and Read, 1981; Sauer et al., 2002) or on an agar plate (Kosola and Workmaster, 2007; Sadowsky et al., 2012; Scagel, 2003; Stackpoole et al., 2008), and designated ericoid mycorrhizal fungi or dark septate endophytes (see section 1.2.1). However, such terms lack

phylogenetic consistency and could lead to confusion (Salhi et al., 2022). Therefore, we designate these endophytes collectively ericoid fungi or Ericaceae-associated fungi, appealing simply to their plant host. Further classification requires molecular approaches, which is even more important for bacterial endophytes as they lack distinctive morphological traits.

Recently our laboratory made significant advances in the research of cranberry root endophytes (Elazreg, 2020; Salhi et al., 2022). Hundreds of fungi and bacteria have been isolated from conventionally-farmed plants of Stevens, Mullica Queen, and Scarlet Knight cultivars. The microorganisms were identified via ribotyping and classified into Proteobacteria, Firmicutes and Actinobacteria, and five classes across Ascomycota. Isolates *Lachnum* sp. EC5 and *Bacillus velezensis* EB37 stand out due to their biocontrol and plant growth promotion abilities. Nevertheless, the presence of ericoid fungi was scarce, and no members of the *Hyaloscypha* aggregate were found (Salhi et al., 2022).

In the current work, we aim to explore the endophytic community of cranberry plants farmed under an organic agriculture scheme. We intend to elucidate the molecular mechanisms underlying cranberry-endophyte interactions in organic plants, as well as compare the microbial profile of plants cultivated under different agricultural practices.

1.6 Hypothesis

Organic cranberry agriculture grows the plants in the absence of chemical fungicides. Therefore, we hypothesize (i) that Ericaceae-associated fungi have more opportunities to proliferate, and (ii) that fungal and bacterial endophytes support fitness in organic cranberry plants by assisting in nutrient uptake and pathogen control.

1.7 Objectives

The objectives of this research project are:

1- Isolate and identify fungal and bacterial root-endophytes from the cranberry plant cultivar Scarlet Knight, planted in a commercial organic field in the region of Lanaudière, Quebec, Canada.

2- Screen endophytes for their biocontrol and plant growth promotion abilities.

3- Sequence and analyze selected endophyte genomes and transcriptomes, focusing on genes encoding secreted proteins and enzymes catalyzing secondary metabolite synthesis that may be involved in plant-endophyte interactions.

Chapter 2 – Material and Methods

2.1 Cranberry plant sampling

Four healthy and vigorous plants harvested from an organic cranberry field (cultivar Scarlet Knight) in Ste Emelie de l'Énergie, QC, Canada were examined. Spots of about 15 cm^2 were considered individual plants. Samples were stored in a cold room until the isolation of microbes.

2.2 Endophyte isolation and culture purification.

Fungal and bacterial endophytes were isolated from surface-sterile roots of the sampled plants. Root surface sterilization was performed as described elsewhere (Schulz *et al.*, 1993) with minor modifications. Roots were stripped and then washed twice using running tap water, first for about 2-3 min and the second for 16-24 h. Next, under laminar airflow, stripped roots were immersed for 2 min in a solution containing 2% of detergent (Neutrad, Decon Lab Inc), then for 2 min in a solution of 0.79% sodium hypochlorite and 0.1% Tween 80, and finally for 10 sec in 70% ethanol. Afterwards, root strips were rinsed three times in sterile distilled water. Subsequently, 1 cm root fragments were placed in Petri dishes with either potato dextrose agar (PDA) pH 7 or tryptic soy agar (TSA) pH 7 for isolating fungi and bacteria, respectively. PDA and TSA were prepared according to the manufacturer's recommendations, and pH was adjusted with NaOH. Petri dishes were incubated at room temperature for up to six weeks. Bacterial and fungal colonies were transferred to new plate up to five times until pure cultures were observed.

2.3 Biocontrol assays

Six fungal pathogens were used to test the endophyte's biocontrol ability. Four cranberry pathogenic strains, *Alternaria alternata* IS2, *Peniophora* sp. IS5, *Diaporthe* sp. IS7 and *Penicillium* sp. IS8, were kindly provided by Dr Richard Bélanger (*Centre de recherche et d'innovation sur les vegétaux, Université Laval*). *Colletotrichum* sp. EC77 and *Godronia cassandrae* EC82 come from our laboratory collection. *In vitro* dual culture confrontations were performed as described elsewhere (Yin et al., 2013) with minor modifications. Briefly, a pathogen and an endophyte were placed 50 mm away from each other on Petri dishes with yeast extract agar

and 2.5% glycerol media (pH 7). In control plates, endophytes were replaced by sterile distilled water. Bacterial endophytes were inoculated at the same time as the pathogens. In contrast, fungal endophytes were inoculated seven days in advance because of their slow growth rate. Four replicates were prepared for each endophyte and control. Petri dishes were incubated at room temperature, and measurements of pathogen growth were taken after 3, 6, 15, and 30 days. The inhibition index I was calculated as described elsewhere (Colombo et al., 2019):

$$\mathrm{I} = \frac{R1-R2}{R1} x100$$

where R1 is the pathogen's radial growth in control plates, and R2 is the pathogen radial growth in the screening experiment.

2.4 DNA isolation, PCR and Sanger sequencing

Fungal and bacterial endophytes were identified by ribotyping. In this technique, the fungal internal transcriber space (ITS) rDNA and the bacterial 16s rDNA regions are PCR-amplified followed by sequencing of the amplicon. While we directly used colonies for bacteria, fungal samples were treated differently. First, the mycelium was collected from Petri dishes and placed in 1.5 ml tubes over a layer of glass beads (425-600 µm; Sigma). Then, 50 µl of TE (2 ml Tris 100mM + 5 µl EDTA 5mM) was added to the tube; the samples were vigorously crushed with a plastic pestle to open fungal cells mechanically. Afterwards, an SDS-protein K protocol was used to extract genomic DNA. Briefly, 150 µl of TE, 4 µl of 20% SDS, and 4 µl of proteinase K were added to the samples and then incubated at 37°C for 30 min. Next, the tubes were centrifuged at 9,000 x g for 20 min. The supernatant was transferred, and protein precipitation was performed with ¼ volume of 5 M NaCl. Subsequently, samples were vortexed and incubated on ice for 1 h. Afterwards, the tubes were centrifuged for 10 min at 9,000 x g, and the supernatant was transferred to a new tube. DNA was precipitated by adding EtOH/AMC (95% ethanol/0.5 M ammonium acetate) (2.5 times the transferred volume) and samples were placed on ice for 20 min and centrifuged for 15 min at 9,000 x g. The supernatant was discarded. DNA pellets were washed with 175 µl of 70% ethanol; then, the samples were gently mixed and centrifuged for 5 min at 9,000 x g. Finally, ethanol was discarded, and DNA pellets were dissolved in 21 µl of TE. Purified DNA was either stored at 4°C or -20°C.

Fungal and Bacterial PCRs were performed in a Bio-Rad thermal cycler using BioBasic Taq polymerase following the manufacturer's instructions. The universal primers BMBC-Fwd (5'-GTACACACCGCCCGTCG-3') and ITS4-rev (5'-TTCCTCCGCTTATTGATATGC-3') (Ihrmark et al., 2012) were used for fungi. Primers 27-Fwd (5'-AGAGTTTGATCCTGGCTCAG-3') and LP58-Rev (5′-AGGCCCGGGAACGTATTCAC-3') were used for bacteria. PCR products were verified by gel agarose electrophoresis; the band sizes were determined using a 1-kbp molecular marker. Amplicon purification was performed using the PCR Clean-Up System (Promega, USA) according to the manufacturer's recommendations and then bi-directionally Sanger sequenced at the *Institut de recherche en immunologie et en cancérologie de l'Université de Montréal* with the primers mentioned above. Forward and reverse sequences were assembled using Phrap v.1.090518 (Gordon, 2003) with default parameters, visualized using Consed v.27.0 (Gordon, 2003), trimmed by quality score (> 30), and primer sequences were manually removed. Species identification was performed using BLASTN against the NCBI ITS or 16S RefSeq database with default settings (http://www.ncbi.nlm.nih.gov/BLAST).

2.5 Plant growth promotion assays

Cranberry seeds were manually extracted from berries of the Scarlet Knight and Stevens cranberry cultivars. Seeds were surface sterilized using the protocol mentioned above. Sterilized seeds were inoculated for germination in Petri dishes with plant minimum mineral growth medium (MM; see the composition in Appendix 1), adjusted to pH 5.5 with KOH. Seeds were monitored daily for contamination. After two weeks, germinated seedlings were placed in sterile culture boxes (ten plants per box) with MM media. Seedlings were inoculated two weeks after transplantation with either an endophyte suspension or sterile distilled water. Endophyte inoculation was performed as follows. Fungi were grown in liquid media for six days. Then, the mycelium was collected, grounded in a sterilized blender, and adjusted to suspensions of 10^{-4} CFU per ml. Aliquots of 350-µl endophyte suspension were inoculated in the seedling boxes. Four replicates were prepared for each endophyte, plus four control boxes. Seedling boxes were incubated at room temperature at 16 h light cycles for 40 days. Afterwards, stem size was measured with a ruler. Stems and roots were dehydrated in an oven at 70 °C for 24 h, and the dry mass was weighed using a microbalance.

2.6 DNA isolation and whole-genome sequencing

The fungal endophytes *Hyaloscypha* sp. EC200, *Pezicula* sp. EC205 and *Phialocephala* sp. EC208 were inoculated in liquid yeast extract medium supplemented with 2.5% glycerol and incubated for six days. Then, the mycelium was crushed in a mortar, in presence of liquid nitrogen. Afterwards, DNA was purified following the QIAGEN Genomic-tip 20/G protocol. Illumina MiSeq paired-end sequencing was performed with a read length of 300 bp. Library preparation and Illumina sequencing were outsourced to the Montreal Genome Quebec Innovation Center platform.

2.7 *De novo* genome assembly, structural and functional annotation

Illumina MiSeq reads were trimmed of adapter sequences using Trimmomatic v0.35 (Bolger et al., 2014) and corrected using the k-mer-based error corrector Rcorrector v1.0.4 (Song and Florea, 2015)). *De novo* nuclear genome assembly was performed with the SPAdes assembler v3.15.0. (Bankevich et al., 2012). Functional and structural annotation was performed by M. Sarrasin, a member of the Lang laboratory, using a home-made pipeline (EUKKANOT), as described elsewhere (Gray et al., 2020). Protein names were assigned from the first best Blast hit against the UniProt-reviewed database. Furthermore, domain-specific information was inferred from HMM-based searches of the Pfam database against the translated gene model sequences. Genome completeness and gene duplications were analyzed with BUSCO v.4.1.4 with default settings using the Helotiales database (helotiales_odb10) (Seppey et al., 2019).

2.8 Annotation of secretome, effectome and biosynthetic gene clusters

The secretome was predicted based on N-terminal signal peptides, excluding transmembrane and endoplasmic reticulum proteins. The signal peptide was detected using PrediSi v1.0.5 (Hiller et al., 2004), SignalP v5.0 (José Juan Almagro Armenteros et al., 2019) and TargetP v2.0 (Jose Juan Almagro Armenteros et al., 2019). Only proteins detected by at least two tools were considered further. Transmembrane proteins were inferred with TMHMM v2.0 (Krogh et al., 2001) and Phobius v1.01 (Käll et al., 2004). Reticulum proteins were predicted using PS-Scan (de Castro et al., 2006) and Prosite motif 'PS00014'. Proteins with functional annotations related to the membrane or cytoplasmic compartments were excluded.

Secreted CAZYmes were predicted by the dbCAN software v9 (Zhang et al., 2018). Proteins detected by at least two tools within dbCAN were considered as CAZYmes. Secreted proteases were predicted after blasting the secretome with the MEROPS database (Rawlings et al., 2016) and the Hotpep-protease software v1.0 (Busk, 2020). The best hit was retained, and proteins with an e-value <0.00001 were considered proteases. Putative effectors were inferred by analyzing the secretome with EffectorP3 (Sperschneider and Dodds, 2022), deepredeff (Kristianingsih and MacLean, 2021) and FunEffector_pred (C. Wang et al., 2020). Only proteins predicted by at least two tools were considered candidate effectors. Effector candidates were submitted to the LOCALIZER v1.0.4 (Sperschneider et al., 2017) tool to infer their possible target in the plant cytoplasm. Conserved domains of effector proteins were identified using the NCBI CDD service (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). Biosynthetic gene clusters (BGCs) were predicted using AntiSMASH v.6.1.0 (Blin *et al*., 2019). Homology inference of the BGCs from the three fungi was examined using cblaster (Gilchrist et al., 2021).

Default parameters were used for all the software except Antismash, for which the following workflow was employed: strict detection, KnownClusterBlast, ClusterBlast, SubClusterBlast, MIBiG cluster comparison, ActiveSiteFinder, RREFinder, Cluster Pfam analysis, Pfam-based GO term annotation, TIGRFam analysis and Cluster-border prediction based on transcription factor binding sites (CASSIS).

2.9 RNA Isolation and sequencing

Fungal RNA was extracted from cultures grown in the presence or absence of a homemade cranberry extract that was prepared as follows. Cranberry plants, including roots, aerial tissues and berries, were mixed and boiled to produce a homogeneous solution. Then, the mix was sterilized in an autoclave at 121 °C for 15 min, followed by two centrifugation steps for 20 min at 2000 x g, discarding precipitated particles. Cranberry extract was added to yeast extract liquid media enriched with glycerol 2.5 % at pH 7 (YG), at a final proportion of 20%.

The fungal endophytes *Hyaloscypha* sp. EC200, *Pezicula* sp. EC205 and *Phialocephala* sp. EC208 were cultured as follows. 400 µl of fungal suspension (1000 CFU ml^{-1}) were inoculated in 50 ml

of YG with or without cranberry extract and incubated at room temperature under constant agitation at 300 rpm for seven days. Afterwards, mycelia were collected and weighed. Around 30-50 mg of fungal material was disrupted as described above. RNA purification was performed using the RNeasy mini kit (Qiagen, GmbH), following the manufacturer's instructions. RNA-seq libraries preparation and sequencing were outsourced to the Montreal Genome Quebec Innovation Center platform. The raw reads obtained with the Illumina HiSeq TM 2000 platform were quality-filtered (Q>30), trimmed and corrected using the same methods described earlier. Clean RNA reads were aligned to the reference genome using STAR aligner v2.7.3a (Dobin et al., 2013).

2.10 Differentially expressed genes

Fungal RNA data from the two conditions (with and without cranberry extract) was used to calculate differentially expressed genes. The statistical analyses were performed using the R package v3.6.1. (Team, 2019) Limma-voom v.3.32.10 (Ritchie et al., 2015). RNA reads were normalized using the transcripts-per-million metrics obtained with Rsem v1.3.3 (Li & Dewey, 2011). As the false discovery rate (FDR), we used the P-value threshold based on the Benjamini-Hochberg procedure to account for multiple significance tests (Benjamini & Hochberg, 1995). Significant differentially expressed genes were selected with a cut-off of FDR ≤ 0.05 and log2 fold change ≥ 1 or ≤ 1.

2.11 GO terms and KEGG enrichment analysis

Go terms, and KEGG pathways were determined by analyzing the proteomes with EggNOG v5.0 (Huerta-Cepas et al., 2019). Then the enrichment analysis for GO terms was performed with the gseGO algorithm from the R package ClusterProfiler (Yu et al., 2012). GO-term redundancy elimination was manually performed based on a directed acyclic graph produced with ClusterProfiler. Most informative GO terms were retained. KEGG enrichment analysis was performed using the GSEA algorithm from ClusterProfiler. For KEGG and GO terms enrichment analysis, the P-value was adjusted using the Benjamini-Hochberg approach (see above), setting the value at 0.05.

2.12 Microscopy

For microscopic inspection of endophytes, cranberry seedlings were inoculated with EC200, EC205 and EC208 (see above). One month after inoculation, control and inoculated plants were collected, roots were stripped and washed in distilled water to remove the media, and then stained with solophenyl flavine followed by safranin, as described elsewhere (Knight and Sutherland, 2011). Stained samples were mounted in 50% (v/v) glycerol and examined with a Nikon eclipse Ts2R microscope under bright field and epifluorescence. Images obtained from epifluorescence were then processed using the NIS elements online deconvolution test site (https://deconv.laboratory-imaging.com/process). For each endophyte, three host plants from three independent culture boxes were examined.

2.13 Statistical analysis of the biocontrol and plant growth promotion assays

The inhibition index was determined by biocontrol assays, while the stem length, stem dry weight and root dry weight were obtained from the plant growth promotion assays. These data were analyzed by the Shapiro methodology to assess their statistical normality. Normal data were analyzed with ANOVA and Tukey HSD as post-hoc method. In contrast, non-parametric data were analyzed following the Kruskal-Wallis approach and Dunn's test (Colombo et al., 2019; Paulissen et al., 2004). For all tests, statistical significance was established as $p \leq 0.05$. All procedures were performed using Rstudio (v.1.2.1335© 2009-2019 Rstudio, Inc).

Chapter 3 – Results

3.1 Isolation of fungal and bacterial endophytes

In this study, we investigated the root endophytes from the cranberry cultivar Scarlet Knight. For that, four healthy and vigorous plants were harvested from an organic commercial field in the Lanaudière region of Quebec. In total, 30 fungi and 25 bacteria were isolated and identified via ribotyping (Tables 2-3 and Figs. 1-2).

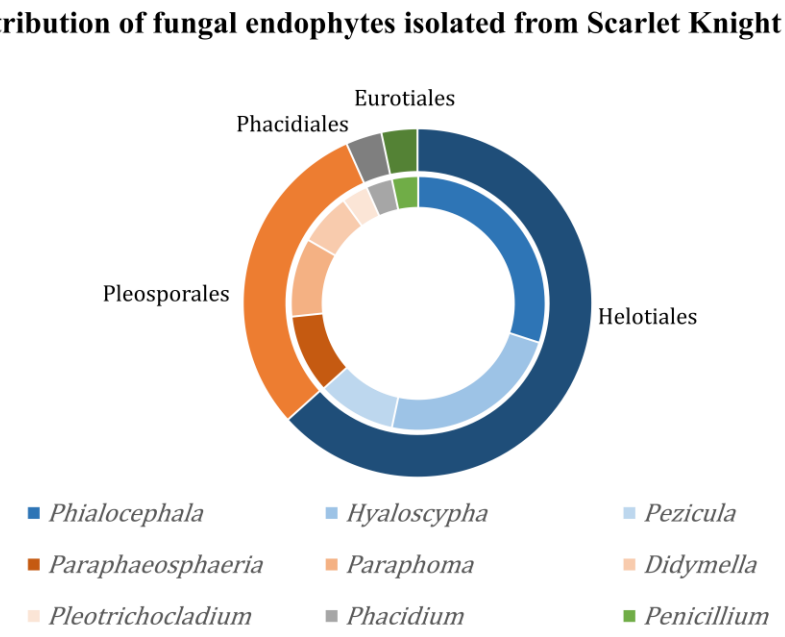

Figure 1. ***Taxonomic distribution of fungal endophytes isolated from Scarlet Knight cranberry roots***.
The identity of fungal endophytes was determined by amplifying the ITS rDNA region and comparing it to the ITS RefSeq database from the NCBI. The outer ring shows the order level and the inner ring the genera. The Helotiales genera Phialocephala, Hyaloscypha and Pezicula account for 63.3 % of the isolates (blue), while the Pleosporales genera Paraphaeosphaeria, Paraphoma, Dydymella and Pleotrichocladium represent 30 % (brown). Phacidium (Phacidiales, grey) and Penicillium (Eurotiales, green) represent 3.3 % each.

The root-dwelling fungal symbionts (mycobionts) belong to nine genera and four orders across Ascomycota (Fig. 1). *Phialocephala* sp., *Hyaloscypha* sp., and *Pezicula* sp., all members of Helotiales, were the most dominant among the fungal isolates. The description of each isolate and details of the Blast analysis are shown in Table 2. The bacterial isolates were identified within 12

genera and seven orders across Proteobacteria, Firmicutes and Actinobacteria (Fig. 2). The genus *Pseudomonas* sp. Was the most frequent among the bacterial community. The description of each isolate and details of the Blast analysis are listed in Table 3.

For the following data analysis, we selected representative strains of the dominant genera among our isolates. For comparison, we added data from microorganisms reported in the literature to have plant growth promotion and biocontrol abilities.

Taxonomic distribution of bacterial endophytes isolated from Scarlet Knight cranberry roots

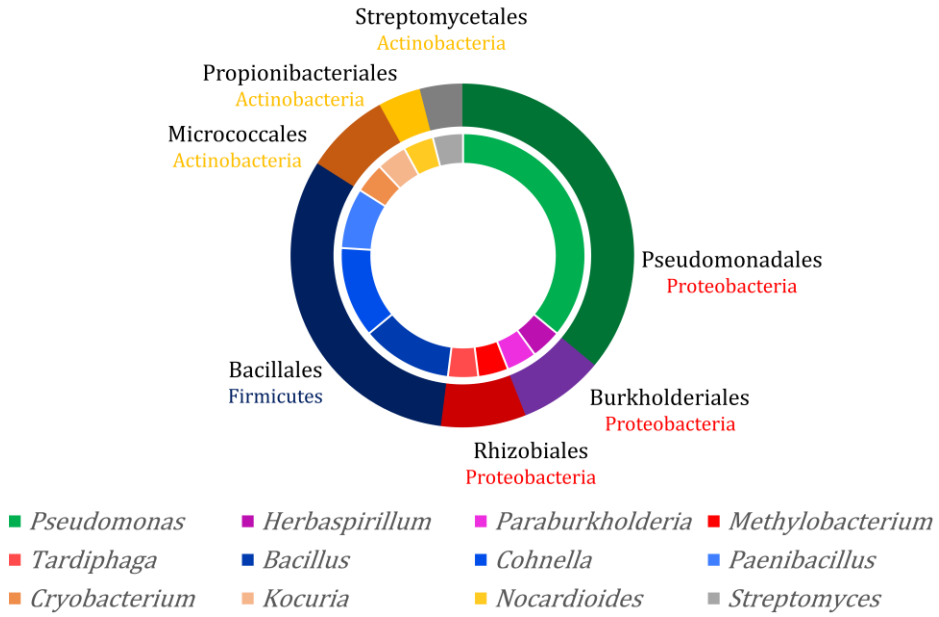

Figure 2. ***Taxonomic distribution of bacterial endophytes isolated from Scarlet Knight cranberry roots***.
The identity of bacterial endophytes was resolved by amplifying the 16S rDNA region and comparing it to the 16S RefSeq database from the NCBI. The outer ring shows the order level. The phyla Proteobacteria, Firmicutes and Actinobacteria are indicated below each order label. The inner ring shows the genera. The distribution in percentage is as follows. Pseudomonas (Pseudomonales, green), 36%. Herbaspirillum and Paraburkholderia (Burkholderiales, purple), 8%. Methylobacterium and Tardiphaga (Rhizobiales, red) 8%. Bacillus, Conhella, and Paenibacillus (Bacillales, blue), 32%. Cryobacterium and Kocuria (Micrococcales brown), 8%. Norcadioides (Propionibacteriales, yellow), 4%. Streptomyces (Streptomycetales, grey), 4%.

Table 2. ***Description and BLAST results of fungal endophytes isolated from Scarlet Knight cranberry roots.***

Strain	Phylum	Order	*Genus*	Closest NCBI ID	% Identity
EC200	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC201	Ascomycetes	Phacidiales	*Phacidium*	NR_137977.1	98.5
EC202	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	98.6
EC203	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	97.8
EC204	Ascomycetes	Eurotiales	*Penicillium*	NR_169981.1	94.8
EC205	Ascomycetes	Helotiales	*Pezicula*	NR_155611.1	99.2
EC206	Ascomycetes	Pleosporales	*Paraphoma*	NR_156556.1	99.6
EC207	Ascomycetes	Pleosporales	*Paraphaeosphaeria*	NR_145167.1	98.1
EC208	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	98.0
EC209	Ascomycetes	Pleosporales	*Didymella*	NR_136125.1	98.5
EC210	Ascomycetes	Pleosporales	*Didymella*	NR_136125.1	98.4
EC211	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	98.7
EC212	Ascomycetes	Pleosporales	*Pleotrichocladium*	NR_155696.1	99.5
EC213	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.8
EC214	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	98.4
EC215	Ascomycetes	Pleosporales	*Paraphoma*	NR_154373.1	97.4
EC216	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	98.0
EC217	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	97.7
EC218	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC219	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC220	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC221	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC222	Ascomycetes	Helotiales	*Hyaloscypha*	NR_121313.1	91.1
EC223	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	97.8
EC224	Ascomycetes	Helotiales	*Pezicula*	NR_155611.1	99.2
EC225	Ascomycetes	Helotiales	*Pezicula*	NR_155611.1	99.2
EC226	Ascomycetes	Pleosporales	*Paraphoma*	NR_156556.1	99.6
EC227	Ascomycetes	Pleosporales	*Paraphaeosphaeria*	NR_145167.1	98.1
EC228	Ascomycetes	Pleosporales	*Paraphaeosphaeria*	NR_145167.1	98.1
EC229	Ascomycetes	Helotiales	*Phialocephala*	NR_119482.1	97.7

Table 3. ***Description and BLAST results of bacterial endophytes isolated from Scarlet Knight cranberry roots.***

Strain	Phylum	Order	Genus	Closest NCBI ID	% Identity
EB200	Actinobacteria	Micrococcales	*Cryobacterium*	NR_117386.1	98.1
EB201	Proteobacteria	Rhizobiales	*Methylobacterium*	NR_074244.1	99.9
EB202	Firmicutes	Bacillales	*Bacillus*	NR_040792.1	98.5
EB203	Actinobacteria	Streptomycetales	*Streptomyces*	NR_112359.1	99.8
EB204	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.8
EB205	Proteobacteria	Rhizobiales	*Tardiphaga*	NR_117178.1	99.0
EB206	Actinobacteria	Micrococcales	*Kocuria*	NR_026451.1	99.5
EB207	Actinobacteria	Propionibacteriales	*Nocardioides*	NR_044185.1	96.4
EB208	Proteobacteria	Burkholderiales	*Paraburkholderia*	NR_145902.1	98.8
EB209	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_170438.1	99.8
EB210	Firmicutes	Bacillales	*Cohnella*	NR_148291.1	98.9
EB211	Firmicutes	Bacillales	*Paenibacillus*	NR_117366.1	99.1
EB212	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.2
EB213	Firmicutes	Bacillales	*Bacillus*	NR_075005.2	99.9
EB214	Firmicutes	Bacillales	*Bacillus*	NR_075005.2	99.9
EB215	Proteobacteria	Burkholderiales	*Herbaspirillum*	NR_043582.1	99.9
EB216	Firmicutes	Bacillales	*Cohnella*	NR_148291.1	98.9
EB217	Firmicutes	Bacillales	*Cohnella*	NR_148291.1	98.9
EB218	Firmicutes	Bacillales	*Paenibacillus*	NR_117366.1	99.1
EB219	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.2
EB220	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.2
EB221	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.2
EB222	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.8
EB223	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.8
EB224	Proteobacteria	Pseudomonadales	*Pseudomonas*	NR_126220.1	99.8

3.2 Biocontrol assays

Three bacterial (*Pseudomonas sp.* EB212, *Bacillus sp.* EB213 and *Bacillus sp.* EB214) and three fungal endophytes (*Hyaloscypha sp.* EC200, *Pezicula* sp. EC205 and *Phialocephala sp.* EC208) were selected to test their biocontrol ability when confronted with six cranberry pathogens (Table 4). Bacterial endosymbionts showed a broad inhibition spectrum by suppressing the growth of five tested pathogens. The *Bacillus* strains EB213 and EB14 were the most efficient biocontrol bacteria; they showed a high inhibition index ranging between 65-73% and mainly affected the pathogen *Diaporthe sp.* IS7. In comparison, the mycobionts reduced the growth of three plant pathogens (Fig. 3). EC205 was highly efficient in suppressing *Godronia cassandrae* EC82 (65.6%) and *Alternaria alternata* IS2 (61.1%). In contrast, the fungus EC200 showed modest suppression against pathogens, and EC208 showed no effect on any tested pathogens. Remarkably, the phytopathogen *Penicillium sp.* IS8 was unaffected by all examined endophytes.

Table 4. ***Plant pathogens used in the confrontation against the fungal and bacterial endophytes.***

Plant pathogen	Disease	Tissue	Plant	Reference
Alternaria alternata IS2	fruit rot (black rot)	berries	cranberry, blueberry	(Stretch, 1989)
Peniophora sp. IS5	canker	root, stem	trees, shrubs	(Dick and Dick, 2009)
Diaporthe sp. IS7	twig blight, stem cankers and fruit rot (dieback)	leaves and berries	cranberry	(Michalecka et al., 2017)
Penicillium sp. IS8	fruit rot	berries	cranberry	(Caruso and Sylvia, 2014)
Colletotrichum sp. EC77	fruit rot (bitter rot)	berries	cranberry	(Conti et al., 2022)
Godronia cassandrae EC82	fruit rot (late rot) and twig blight	berries	cranberry	(Conti et al., 2022)

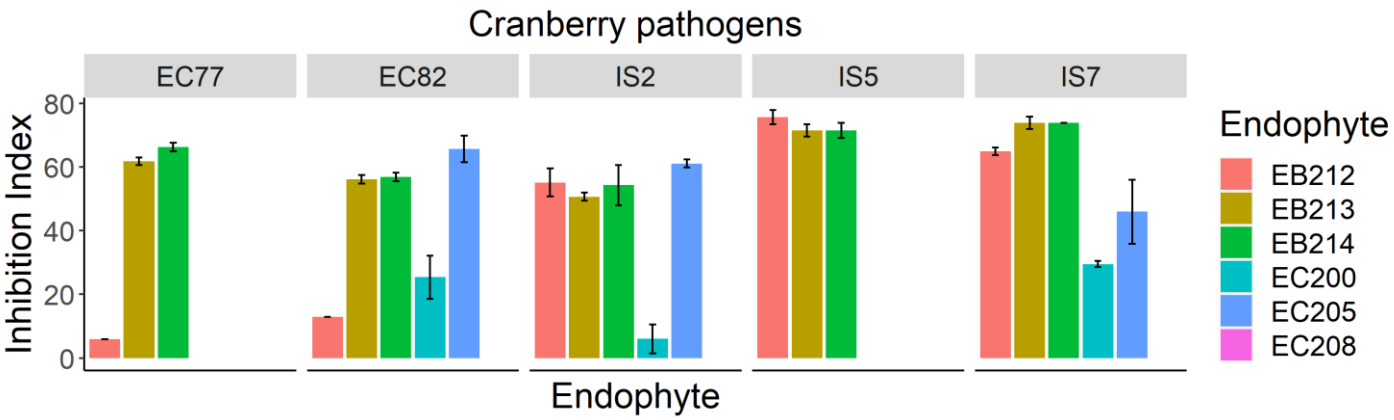

Figure 3. ***Endophyte biocontrol against cranberry plant pathogens.***
*Culture confrontation of endophytes and pathogens was performed on agar plates. Colours codes for endophytes are as follows. Pseudomonas sp. EB212. (coral), Bacillus sp. EB213 (green olive), Bacillus sp. EB214 (green), Hyaloscypha sp. EC200 (light blue), Pezicula sp. EC205 (dark blue) and Phialocephala sp. EC208 (pink). Bars represent the mean and standard deviation. The inhibition index I was calculated 30 days after inoculation by using the formula I =(R1-R2)/R1*100, where R1 is the pathogen radial growth in control plates, and R2 is the pathogen radial growth in the respective screening experiment. Four replicates were performed. The non-parametric test Kruskal-Wallis was used to compare the performance of endophytes (see Methods).*

3.3 Plant-growth promotion assays

The bacterium *Bacillus* sp. EB213, and the fungi *Hyaloscypha* sp. EC200, *Pezicula* sp. EC205, and *Phialocephala sp.* EC208 were selected for testing plant growth promotion of cranberry seedlings. Due to technical difficulties, data from EC205 could not be collected (Figs. 4-5). EB213 insignificantly increased the size and biomass of Scarlet Knight stems (Figs. 4B-C), but did not affect root growth of either Scarlet Knight or Stevens seedlings (Fig. 4A). EC200 and EC208 did not affect the growth of either cultivar seedlings (Figs. 4 and 5). Again, due to technical problems, only stem-size data could be collected for EC208.

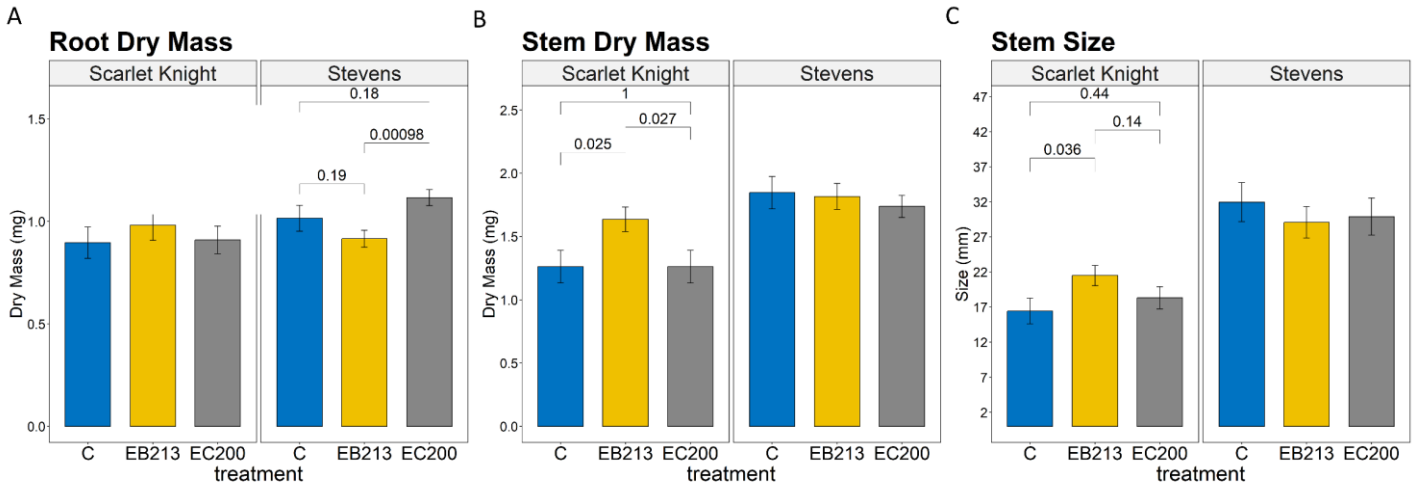

Figure 4. ***Plant growth promotion of Bacillus sp. EB213 and Hyaloscypha sp. EC200.***
Scarlet Knight and Stevens cranberry cultivars seedlings were inoculated with either sterile distilled water (control (C), blue), Bacillus sp. EB213 (yellow) or Hyaloscypha sp. EC200 (grey). All the measurements were performed 40 days after inoculation. Four replicates were performed. The non-parametric test Kruskal-Wallis was used to compare the effects of the treatments. Bar plots represent the mean and the standard deviation. Multiple comparisons were performed with Dunn's test, and P values were added at the top of each panel when significant differences were observed. The Y-axis is the root dry weight in mm ***(A)****, the dry stem weight in mg* ***(B)****, and the stem size in mm* ***(C)****.*

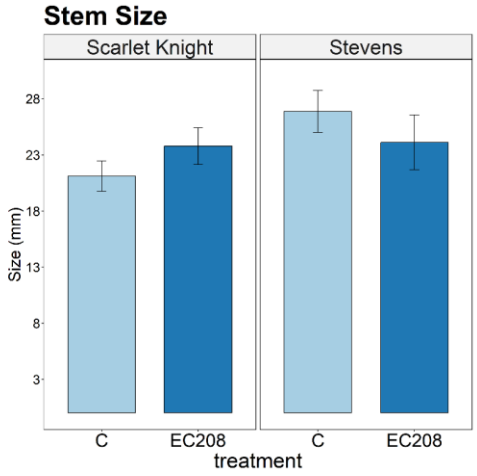

Figure 5. ***Plant growth promotion of Phialocephala sp. EC208****.*
Scarlet Knight and Stevens cranberry cultivars seedlings were inoculated with either sterile distilled water (control (C), light blue) or Phialocephala sp. EC208 (dark blue). The stem mass was calculated for roots and stems after 40 days of inoculation. Four replicates were performed. Treatments were analyzed using ANOVA followed by Tukey's tests (P <0.05) and no significant effects were observed. The bar plots represent the mean and the standard deviation.

3.4 Fungal colonization of cranberry seedlings

The fungi *Hyaloscypha sp.* EC200, *Pezicula sp.* EC205 and *Phialocephala sp.* EC208 were inoculated in cranberry seedlings to assess if they colonize the root cells of their host plant. Microscopic inspection revealed that all three fungi form intracellular structures. EC200 formed thin and dense hyphal coils (Fig. 6), whereas EC205 and EC208 presented two distinct morphologies: darkly pigmented septate hyphae with few twined regions (Figs. 7A and 8A) and microsclerotia-resembling structures (Figs. 7B and 8B).

The three fungi also formed a dense mycelial structure on the root surface (images not shown). However, no clear evidence was found for hyphae extending from within the root cells to the exterior nor from one root cell to another. Thus, it appears that the intracellular fungal structures are confined to individual root cells.

Figure 6. ***Colonization of Hyaloscypha sp. EC200***.

The images were taken one month after inoculating Stevens roots with endophytes. A 40x objective was used. The image shows several root cells apparently colonized with coils of fungal mycelia. The fungal structures appear to be intracellular and confined to single root cells.

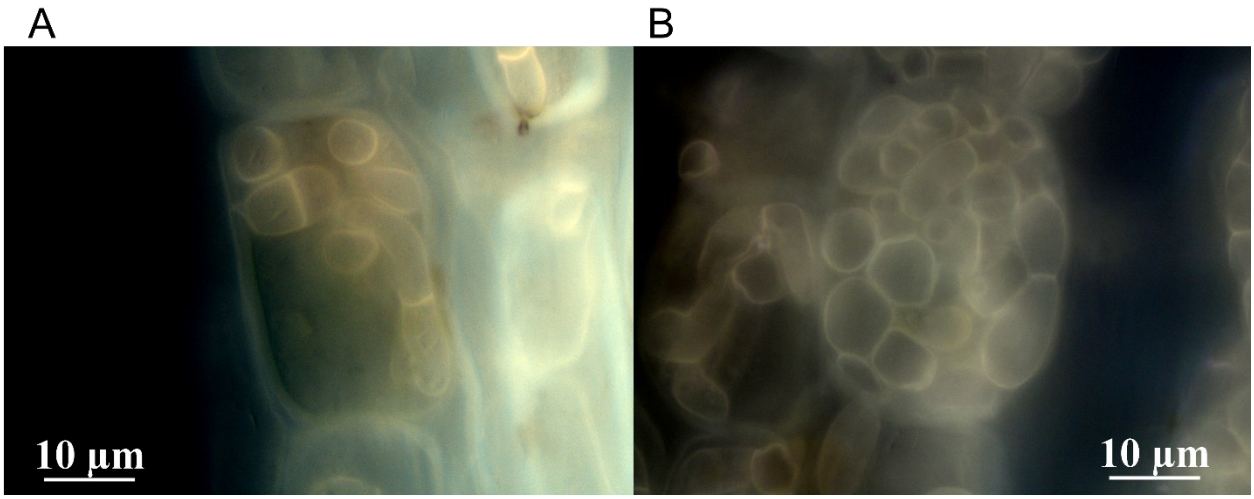

Figure 7. ***Colonization of Pezicula sp. EC205.***

The images were taken one month after inoculating Stevens roots, using a 100x objective. A) A root cell probably in an early stage of fungal colonization. Note the variety of hyphal shapes. B) Fungal structures densely packed in a root cell and resembling microsclerotia.

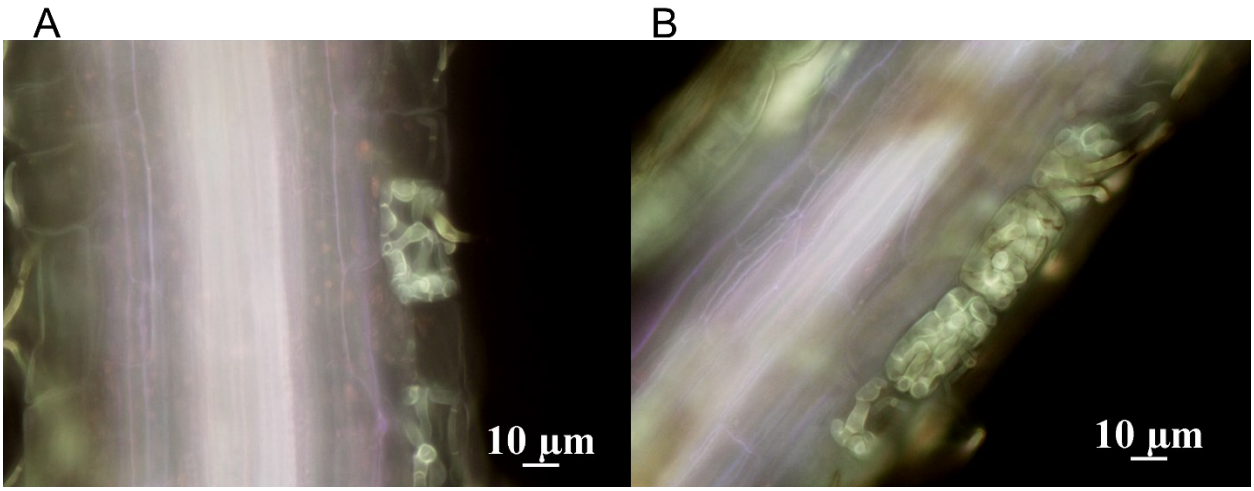

Figure 8. ***Colonization of Phialocephala sp. EC208***.

The images were taken one month after inoculating Stevens roots, using a 20x objective. A) shows in the center to the right fungal mycelia within a root cell. B) shows four root cells colonized by fungal mycelia, which are probably combined with round structures resembling microsclerotia.

3.5 Genomics

3.5.1 Nuclear genome assembly and annotations

The genomes of EC200, EC205, and EC208 were sequenced using Illumina Mi-seq and assembled using an in-house pipeline that incorporates the SPAdes assembler. Structural and functional annotation was performed via the in-house developed EUKKANNOT pipeline using RNA data as support for predicted gene models. The average coverage was 50X, 35X, and 30X for EC200, EC205 and EC208, respectively. Genomic features and assembly statistics are compiled in Table 5.

With assemblies between 55 and 78 Mbp and a G+C content between 42 and 43%, the nuclear genome size and nucleotide bias of the three fungi are within the expected range for Leotiomycetes (Johnston et al., 2019; Knapp et al., 2018; Miyauchi et al., 2020). The number of encoded genes correlates with the genome size. The BUSCO analysis using the Helotiales dataset showed that the three genomes are nearly complete (Table 5).

Table 5. ***Statistics of the assembled genomes of the fungi Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208.***

BUSCO represents the percentage of complete BUSCO models using the Helotiales dataset. N= 5177

Features	*Hyaloscypha* sp. EC200	*Pezicula* sp. EC205	*Phialocephala* sp. EC208
Genome size	55.1 Mbp	66.2 Mbp	77.7 Mbp
Coverage	50.8X	34.8X	30.5X
Nr. of contigs	1,475	1,986	456
N50 size	241,8 kbp	334,5 kbp	458,9 kbp
G+C content	43.22%	42.22%	42.78%
No. of genes	17,923	20,112	23,843
Busco completeness	98.8%	95.3%	98.9%

3.5.2 Putative secreted proteins

Secreted proteins of endophytes are typically involved in diverse activities such as soil organic matter decomposition, nutrient uptake and inter and intraspecies interactions. The predicted secreted proteins from the genomes of EC200, EC205, and EC208 were compared with the functional annotation (EUKKANOT, see Section 2.8). About 8% of the predicted extracellular

proteins were annotated as endoplasmic or membrane proteins and therefore excluded from the final results. In addition, we predicted secreted carbohydrate-active enzymes (CAZymes), proteases, and effectors, and inferred biosynthetic gene clusters (BGCs). Figure 9 summarizes the results for EC200, EC2005 and EC208. Published inferred proteomes of Helotiales comprise 9,600 to 23,000 proteins, of which about 4 to 8% are predicted to be extracellular (Knapp et al., 2018; Martino et al., 2018; Miyauchi et al., 2020). Therefore, the proteomes and secretomes of EC200, EC205 and EC208 are within the expected range for Helotiales fungi.

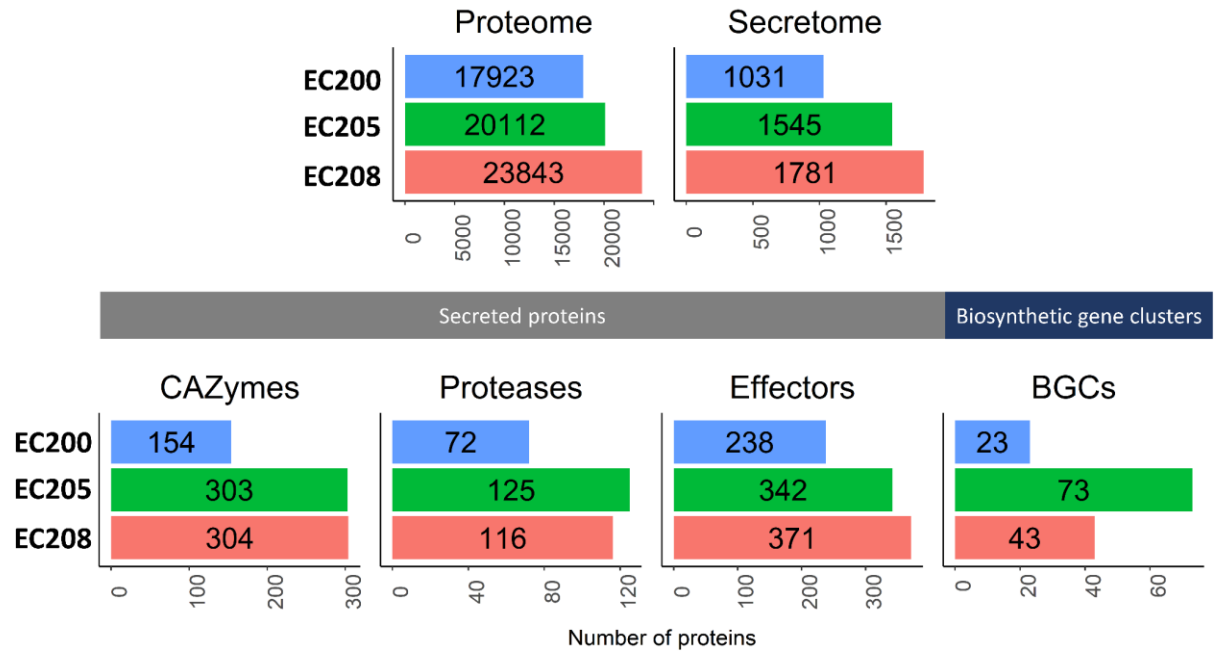

Figure 9. ***Genomic features of the fungi Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208***.
At the top is shown the size of the proteome and secretome for each fungus. At the bottom is shown the number of secreted CAZymes, proteases and effectors, as well as the number of predicted Biosynthetic Gene Clusters (BGCs).

3.5.2.1 CAZymes

Cazymes are versatile proteins with roles in nutrient mobilization from organic matter, microbial inhibition, and root colonization. The genomes of EC200, EC205, and EC208 contain between 154 and 304 predicted CAZymes (Fig. 9). Previous studies predicted similar numbers of secreted CAZymes for a *Hyaloscypha hepaticicola* strain and *Phialocephala scopiformis* (Martino et al., 2018; Miyauchi et al., 2020). The types of enzymes in our three fungal isolates are similar,

including mainly glycosyl hydrolases, auxiliary enzymes and carbohydrate esterases. The only exception is that EC200 lacks polysaccharide lyases, whereas EC205 and EC208 encode several of these enzymes (Fig. 10A).

We also predicted enzymes able to degrade glyco-components of plant and fungal cell walls (PCWDE and FCWDE, Figs. 10B-C). EC200, EC205 and EC208 encode between ~70 and 140 PCWDEs and 27 to 48 FCWDEs that together have similar substrate preferences and that primarily target hemicellulose and glucan from vegetal and fungal sources. EC208 is the only one of the three fungi having CAZymes that degrade galactosaminogalactan.

3.5.2.2 Proteases

The inferred secreted proteases were classified according to their catalytic domain (Fig. 11). With only 72 members, EC200 has much fewer such enzymes compared to EC205 and EC208. Still, the three mycobionts encode a similar number of genes coding for metallo-, aspartic, glutamic and threonine proteases, while cysteine proteases were only found in EC205. Secreted serine proteases make up about 75% of their peptidase complement, which is mainly due to the expansion of the subfamilies S09X and S53 (Appendix 2). Although previous studies of Helotiales including species of *Hyaloscypha* and *Phialocephala* reported a much smaller number of secreted proteases (25-87), the predominance of serine proteases appears to be common to Helotiales (Martino et al., 2018; Miyauchi et al., 2020).

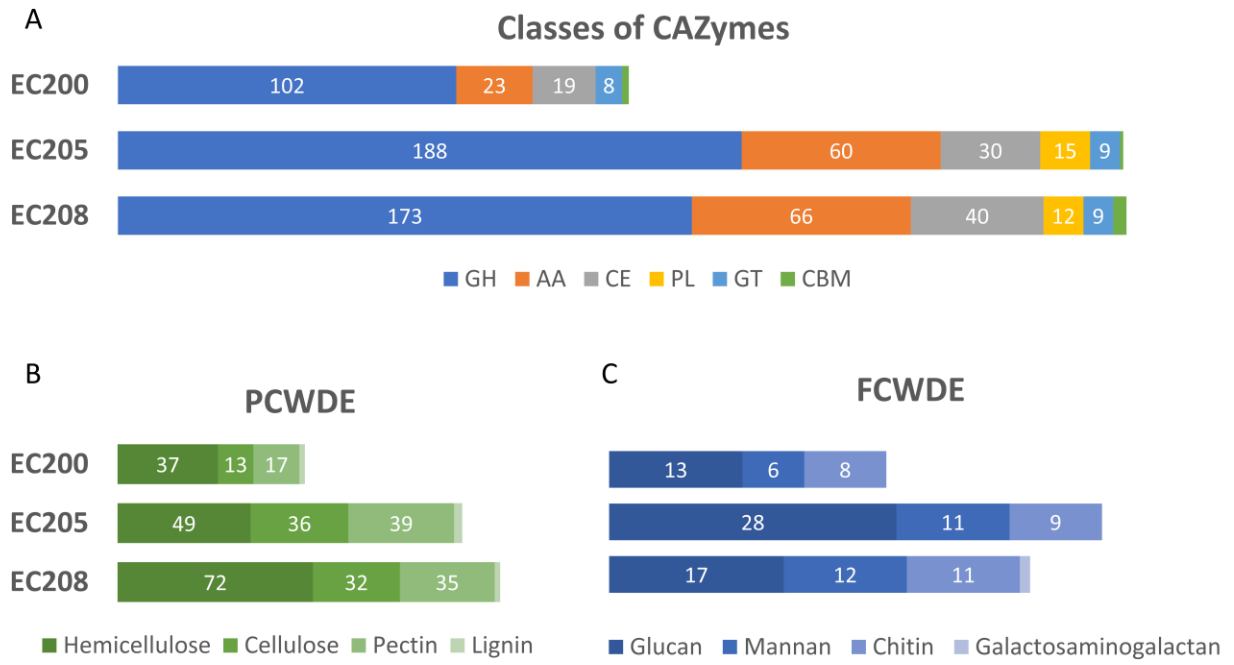

Figure 10. ***Predicted secreted Carbohydrate-active enzymes (CAZymes) of Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208.***
A) *CAZymes classes: Glycosyl Hydrolases (GH), Auxiliary Activities (AA), Carbohydrate Esterases (CE), Polysaccharide Lyases (PLs), Glycosyl Transferases (GTs) and Carbohydrate-Binding Modules (CBM).* ***B)*** *Plant cell wall degrading enzymes (PCWDE) active on cellulose, hemicellulose, pectin, xylan and lignin.* ***C)*** *Fungal cell-wall degrading enzymes (FCWDE) active on chitin, glucan, mannan and galactosaminogalactan. Labels for categories with less than ten proteins are not shown. CAZymes were predicted using dbCAN2, which incorporates three annotation approaches: (i) HMMER search against the dbCAN HMM (hidden Markov model) database; (ii) DIAMOND search against the CAZy pre-annotated CAZyme sequence database and (iii) Hotpep search against the conserved CAZyme short peptide database. Proteins predicted by at least two approaches were selected. Note that dbCAN2 predictions do not use profile-HMM-specific cut-off values, and therefore produce non-negligeable numbers of false negatives and false positives. However, this is the only currently available automated CAZyme-prediction tool.*

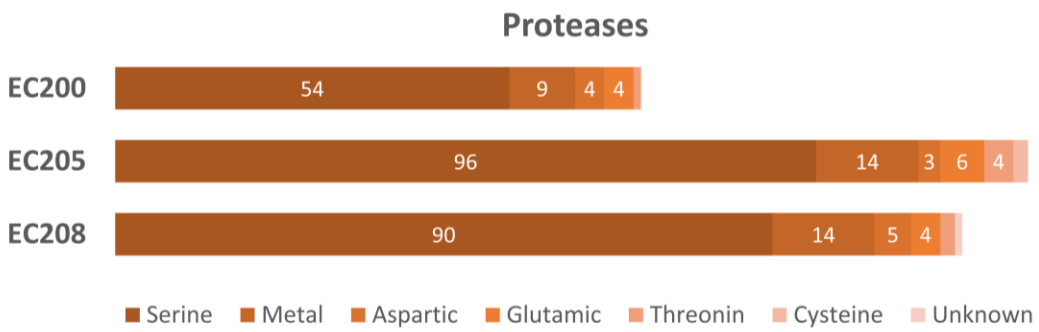

Figure 11. ***Predicted secreted proteases of Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208.***
The horizontal bars represent the total repertoire of proteases and the classified according to their catalytic domain. Proteases were predicted by blasting the secretome against the MEROPS database using the Hotpep-protease software v1.0. The best hit was retained, and proteins with an e-value <0.00001 were considered proteases. Labels for categories with less than four proteins are not shown.

3.5.2.3 Effector proteins

Effector proteins are central to establishing and maintaining endosymbiosis. Fungal effectors remodel the cell wall to evade plant immunity or regulate plant defensive pathways in the host's cytoplasm. A previous study reported that Helotiales fungi encode between 150 and 650 candidate effectors (Miyauchi et al., 2020). Here, we predicted that EC200, EC205, and EC208 encode as many as 238, 342 and 371 candidate effectors, respectively (Fig. 9). Of these, about 80% were predicted to be targeted to the apoplast (Fig. 12). The most frequently conserved domains among the known proteins include Abhydrolase, chitin binding, WSC and LysM domains (Appendix 3). About 68% of the candidate effectors were annotated as hypothetical or uncharacterized proteins.

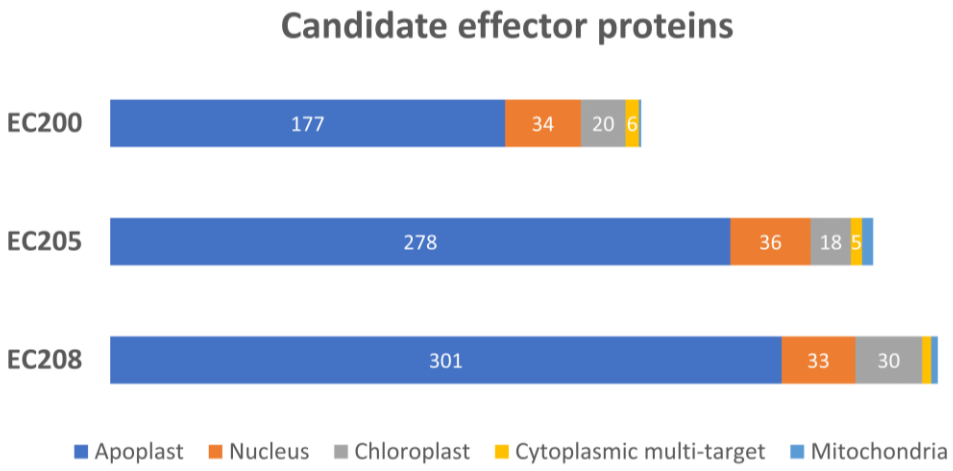

Figure 12. ***Candidate effectors of Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208.***
The horizontal bars represent the totality of effector proteins predicted to be targeted to the apoplast, nucleus, chloroplast, mitochondria, or multiple targets in the cytoplasm. Labels for categories with less than five proteins are not shown. Candidate effectors were inferred by analyzing the secretome with EffectorP3, deepredeff, and FunEffector_pred. Only proteins predicted by at least two tools were considered candidate effectors. Afterwards, effectors were submitted to the LOCALIZER v1.0.4 tool to infer their possible target in the plant cytoplasm.

3.5.3 Biosynthetic Gene Clusters (BGCs)

Secondary metabolites are synthesized by various enzymes that are typically encoded by genes arranged in biosynthetic gene clusters (BGCs). We observed that EC205 harbours the largest biosynthetic machinery (Figs. 9 and 13). Among the genes involved in secondary metabolite synthesis, type 1 polyketide synthases (T1PKS) are the most frequent ones among the three fungi, followed by terpene synthases and non-ribosomal peptide synthetases (NRPS) (Fig. 13). Hybrid BGCs, such as T1PKS-NRPS, were also found (Appendix 4). From the 139 BGCs found across the three genomes, only twelve have been reported in other microorganisms. The compounds they synthesize are listed in Table 6. Nearly 90 BGCs are reported here for the first time (Appendix 4).

Homology inference demonstrated that most BGCs are strain-specific (Appendix 5). Among the shared BGCs are a T1PKS-synthesizing melanin, a terpene synthase associated with squalestatin, and an unknown T3PKS.

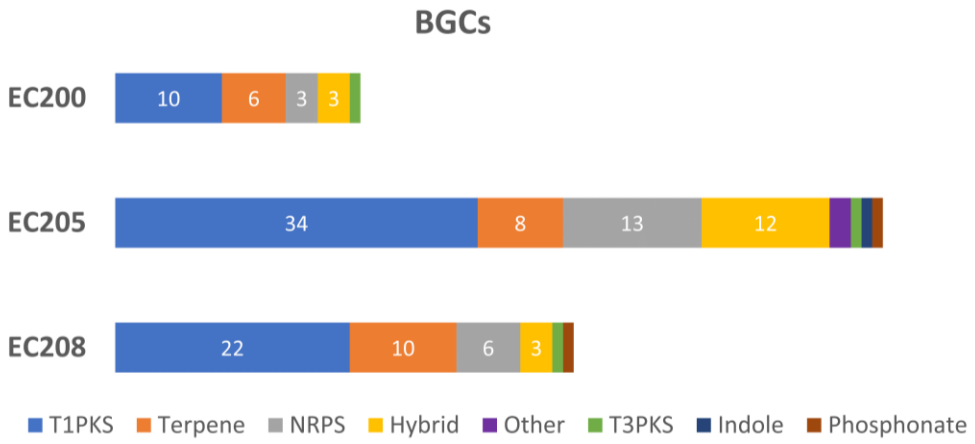

Figure 13. ***Predicted BGCs in the genomes of Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp. EC208.***
The analysis was performed using the Antismash pipeline v.6. Bar plots represent the number of different BGCs; Labels for classes with less than three members are not shown.

Table 6. ***Known BGCs found in the genomes of Hyaloscypha sp. EC200, Pezicula sp. EC205 and Phialocephala sp.EC208.***

		Number of BGCs		
Type of BGC	**Product**	**EC200**	**EC205**	**EC208**
T1PKS	1,3,6,8-tetrahydroxynaphthalene (the backbone of melanin)	2	3	2
T1PKS	Naphthopyrone	1	-	-
Clavaric acid	Clavaric acid	1	-	-
T1PKS	Pyranonigrin E	-	1	-
NRPS	AbT1	-	-	1
NRPS	Alternapyrone	-	-	1

3.6 Transcriptomics

3.6.1 GO terms and KEGG pathways enrichment

To gain insight into gene-expression changes of the mycobionts when in contact with their host plant, we sequenced the transcriptomes of EC200, EC205, and EC208, cultured in the presence and absence of cranberry extract. Enrichment analyses of GO terms and KEGG pathways were performed for the genes differentially expressed in cultures with and without the extract. EC205 and EC208 revealed considerably impacted gene expression, but not so for EC200 (Figs. 14-15).

In the presence of cranberry extract, GO terms and KEGG pathways associated with the metabolism of nitrogen, carbon and lipids were reduced in EC205 and EC208 (Figs. 14A and 15A). A similar effect was observed for the GO categories ribosome, the plasma membrane, the synthesis of hydrolases and secondary metabolites, the secretory system, and the electron transport chain (Figs. 14A and 15A). Likewise, GO terms for mitochondrion and mitochondrial matrix were downregulated in EC208 (Fig. 14B), and GO terms for the respirasome (Fig. 14B) and the oxidative phosphorylation pathway were reduced in EC205 (Fig. 15B).

Only a few differences in expression changes were observed between EC205 and EC208. For example, in EC205, GO terms related to DNA repair and cellular stress response were upregulated. Similarly, the mitotic cell cycle, the fungal cell wall biogenesis and the signal transduction mechanisms were triggered only in EC205 (Fig. 14C). In contrast, copper and iron homeostasis was downregulated in EC208 (Fig. 14C).

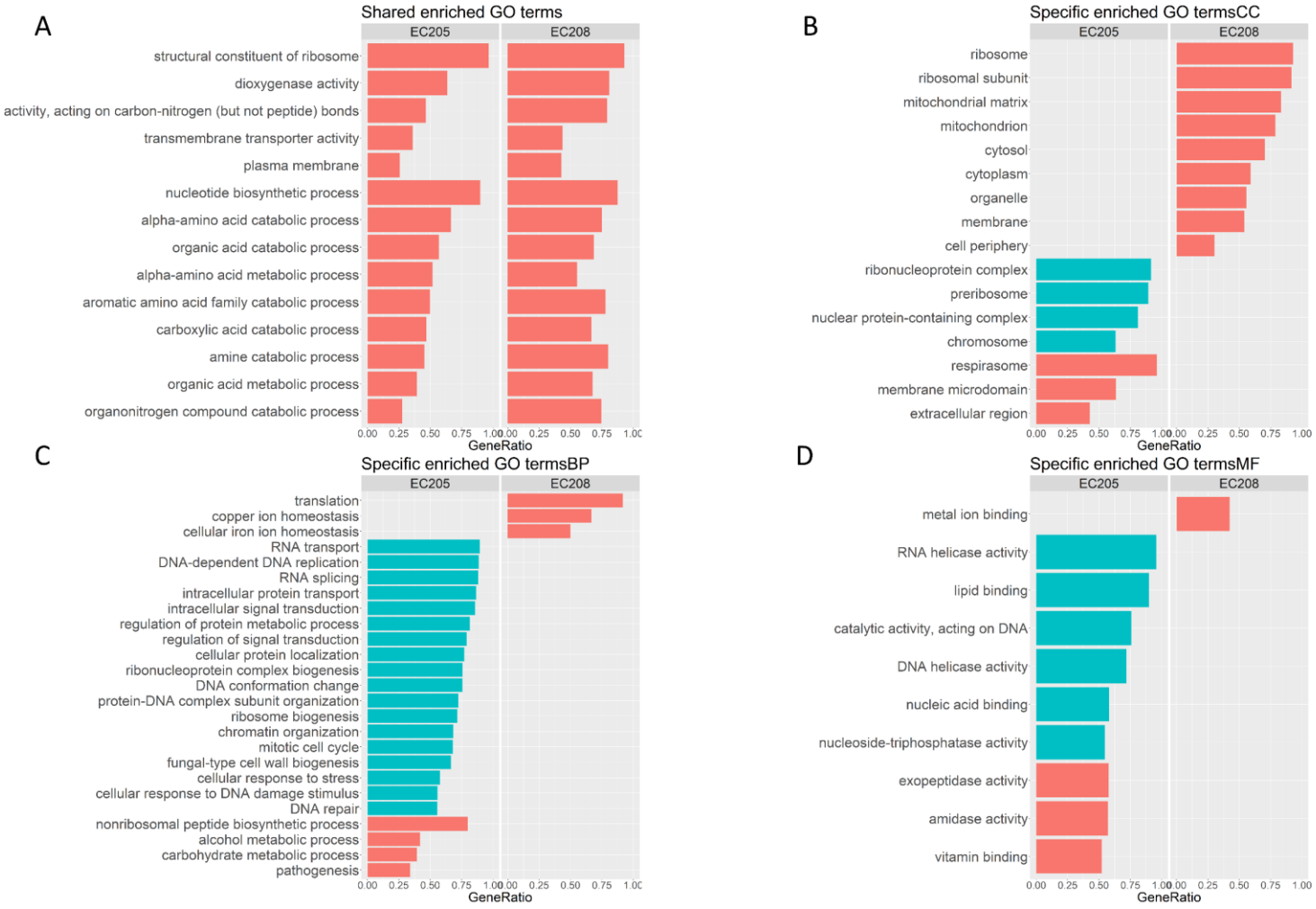

Figure 14. ***Enriched GO terms.***

The X axis indicates the gene ratio which was calculated by dividing the number of genes that contributed to the enrichment of a given GO term by the total of genes annotated for that GO term in each fungus. The Y axis indicates the description of the enriched GO terms. Orange bars represent downregulated GO terms while blue bars represent up regulated terms. ***A)*** *The commonly enriched GO terms in the two fungi Pezicula sp. EC205 and Phialocephala sp. EC208.* ***B)*** *The enriched GO terms in the category of cellular component that were enriched only in one fungus.* ***C)*** *The biologic GO terms that where specifically enriched in either EC205 or EC208.* ***D)*** *The molecular function GO terms that were enriched either in EC205 or in EC208.*

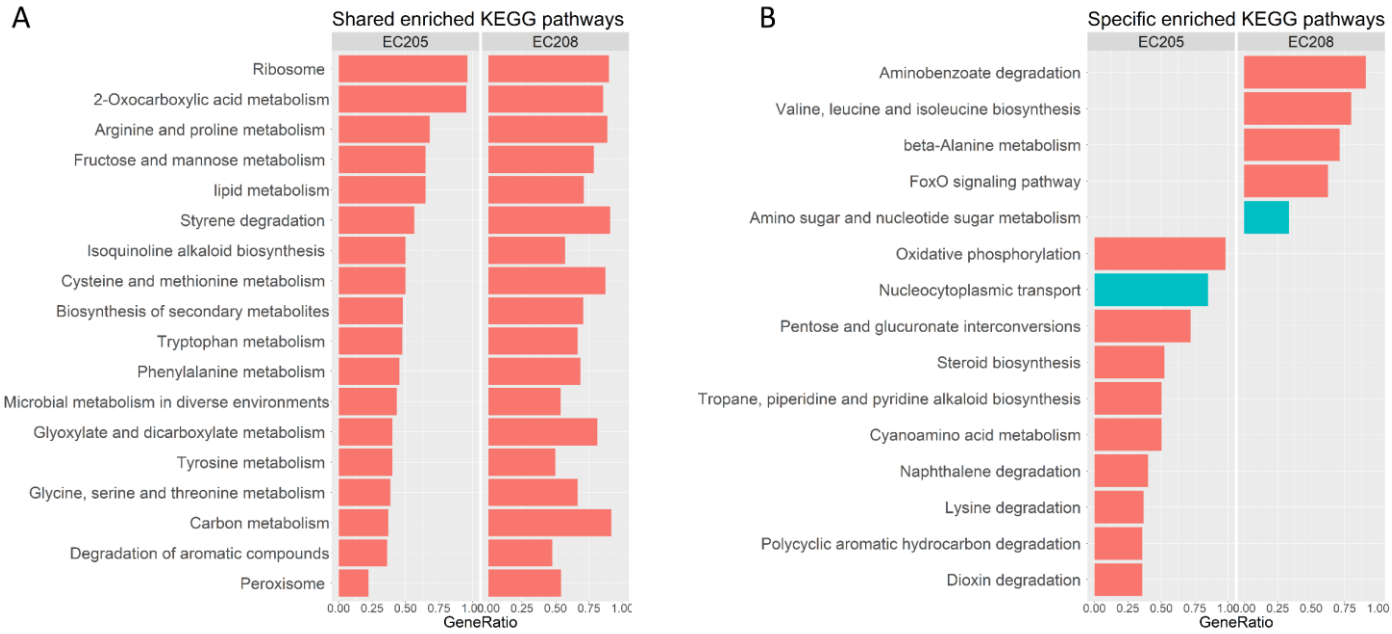

Figure 15. ***KEGG pathways enrichment.***

The X-axis indicates the gene ratio which was calculated by dividing the number of genes that contributed to the enrichment of a given pathway by the total of genes annotated for that pathway in each fungus. The Y-axis indicates the description of the enriched pathway. Orange bars represent downregulated pathways while blue bars represent upregulated ***A)*** *The commonly enriched KEGG pathways in the two fungi Pezicula sp. EC205 and Phialocephala sp. EC208.* ***B)*** *The pathways enriched only in one fungus.*

Chapter 4 – Discussion, conclusions, and future work

4.1 Helotiales and Pseudomonales dominate the roots of cranberry

Here we present the first study that explores the diversity of endophytes in the roots of organically grown cranberry plants. This work is also one of the few investigating both bacterial and fungal rhizobionts of Ericaceae simultaneously. In total, we isolated and identified 30 fungal and 25 bacterial isolates (Tables 2 and 3). The fungal endosymbionts were dominated by the Helotiales genera *Phialocephala*, *Hyaloscypha* (more precisely, the *Hyaloscypha* aggregate previously known as *Rhizoscyphus* aggregate; Fehrer et al., 2019) and *Pezicula* (synonym *Cryptosporiopsis;* Walker et al., 2011). Among bacterial isolates, members of Pseudomonales were the most frequent endophytes (Figs. 1 and 2).

Our results provide a glimpse at the diversity of root endophytes in organic cranberry, but the small sampling size was relatively small (four plants). Further analyses are needed to determine whether the microbial profile presented here is found as well when sampling is expanded, and across fields and seasons. Since many microbes cannot be easily isolated or grown axenically, the results obtained by isolation techniques underestimate the microbial richness sheltered inside plants. Therefore, combining traditional isolation and culture-independent molecular methods should provide a more realistic picture of root endo-microbiota (Yang et al., 2018).

Interestingly, studies performed by others using both classic isolation techniques and culture-independent approaches came to a similar conclusion as we, notably that Ericaceae commonly associate with the genera *Hyaloscypha*, *Pezicula*, and *Phialocephala* (Bougoure and Cairney, 2005; Obase and Matsuda, 2014; Walker et al., 2011; Zhang et al., 2009, 2016). These genera seem to co-occur in ericaceous plants more frequently than expected by chance, suggesting that their colonization may be facilitated by the host (Gorzelak et al., 2012). The presence of these genera has also been observed in wild cranberry-related plants like *Vaccinium uliginosum* (bog bilberry) and *Vaccinium membranaceum* (huckleberry) (Gorzelak et al., 2012; Yang et al., 2018). Similarly, the genus *Pseudomonas* has been reported as the most abundant isolated bacteria from the roots of

Vaccinium corymbosum L. (blueberry) and different cultivars of cranberry (Elazreg, 2020; Ortiz-Galeana et al., 2018; Salhi et al., 2022)

Pseudomonas sp. and the other identified bacterial genera, including *Bacillus* sp., *Streptomyces* sp. and *Herbaspirillum* sp. (Table 3), have been reported to improve plant fitness (Monteiro et al., 2012; Olanrewaju and Babalola, 2019; Qessaoui et al., 2019; Sansinenea, 2019). Similarly, the frequently isolated fungi *Hyaloscypha* sp., *Pezicula* sp., and *Phialocephala* sp. have been observed to establish mutualistic associations with various plants (Kosola et al., 2007; Lin et al., 2011; Song et al., 2021; Surono and Narisawa, 2017). These findings strongly suggest that bacterial and fungal endophytes are responsible for the vigor of the sampled plants. However, among our fungal collection, we also identified a few potentially pathogenic genera such as *Penicillium* and *Paraphoma* (Figure 1 and Table 2) (Cao and Li, 2022; Caruso and Sylvia, 2014), indicating that mutualist and antagonist microbes can cohabitate a plant simultaneously.

We found a significant difference in the fungal community when comparing organic field results with conventional farms. For instance, while organic plants seem to contain a small compendium of potential pathogenic fungi, the conventionally farmed plants were dominated by reported pathogens such as *Penicillium* sp., *Colletotrichum* sp., and *Diaporthe* sp. (Salhi et al., 2022). Furthermore, in conventionally farmed plants, the otherwise common ericoid fungi were almost undetectable. Apparently, the application of chemical fungicides in conventional agriculture acts detrimentally on ericoid fungi in particular. Thus, under an organic agriculture scheme, ericoid fungi should have better chances to proliferate, as they commonly do in wild plants (Gorzelak et al., 2012; Yang et al., 2018). When ericoid fungi are present to compete with latent pathogens for space and resources will probably result in a reduction of the pathogen population.

4.2 Dominant endophytes inhibit cranberry fruit rot pathogens

Phytopathogenic fungi colonize belowground and aerial tissue of cranberry plants. Fungi damaging the berries are economically most significant in intensive agriculture. Some studies estimate that cranberry fruit rot causes about 33% losses of the production worldwide (Caruso and Sylvia, 2014; Conti et al., 2021, 2019; Dick and Dick, 2009; Michalecka et al., 2017; Stretch,

1989). Cranberry fruit rot is commonly associated with various fungi (Conti et al., 2022, 2021, 2019), but *G. cassandrae* seems to be the major culprit on Quebec's conventional and organic farms (Conti et al., 2022).

Our results indicate that bacterial and fungal endophytes *Pseudomonas* sp. EB212, *Bacillus* sp. (EB213 and EB214), *Hyaloscypha* sp. EC200 and *Pezicula* sp. EC205 inhibit altogether the growth of five cranberry pathogens, including *G. cassandra*e EC82 (Fig. 3).

It would be worthwhile to assess these isolates for their ability to reduce fruit rot. The corresponding pathogens are typically present in the soil, leaves, and flowers prior to invading the berries (Sabaratnam et al., 2014; Tadych et al., 2015; Waller et al., 2020). The above pathogen-suppressing isolates readily grow in soil. Furthermore, *Pseudomonas* and *Bacillus* are able to colonize aerial plant tissue, and *Hyaloscypha* and *Phialocephala* were detected in the leaves and flowers of blueberry plants (Daghino et al., 2022). Therefore, we posit that cranberry endophytes have the capacity to suppress fruit rot agents in the soil or aerial tissue long before these pathogens infect the berries.

4.3 Secondary metabolites in plant-endophyte interactions

Endophytes are a prolific source of bioactive compounds with industrial, pharmaceutical, and agricultural applications. Secondary metabolites are primarily investigated for their antimicrobial capabilities. Our genomic analyses revealed that the genomes of fungi EC200, EC205 and EC208 combined encoded nearly 140 biosynthetic gene clusters (mostly PKS, terpenes and NRPS). Of only 10% of these BGCs, the compound they product could be inferred. According to our genomic results, melanin is the sole known secondary metabolite all three fungi can synthesize. Melanin, in particular the DHN form, is a secondary metabolite produced by most Ascomycotina (Bell and Wheeler, 2003; Berthelot et al., 2020; Motoyama, 2020). Fungal melanin has been proposed to be favourable for plant development by serving as an extracellular redox buffer to alleviate environmental stress (Santos et al., 2021; Zhan et al., 2011).

4.4 Plant growth-promotion of *Bacillus* sp. EB213 is host-genotype dependent

The genus *Bacillus* is well known for its plant growth promotion ability, with many species reported to solubilize nutrients and produce phytohormones favouring the development of their host (Aloo et al., 2019; Sansinenea, 2019). We show that EB213 increased the stem biomass of cranberry seedlings of the cultivar Scarlet Knight (Fig. 4), but not so of Stevens.

It has been documented earlier that distinct plant genotypes respond differently to endophytic colonization. For example, some cultivars of rice and the grass *Brachypodium distachyon* presented different growth results when inoculated with either *Herbaspirillum seropedicae* or *Burkholderia kururiensis* (do Amaral et al., 2016; Vargas et al., 2012).

4.5 The hydrolytic profile of fungal endophytes suggests their dual nature as endosymbionts and saprotrophs

Our genomic analyses revealed that the endophytes EC200, EC205 and EC208 share an extensive hydrolytic machinery composed of CAZymes, proteases and many cell-wall degrading enzymes (PCWDE and FCWDE) (Figs. 10-11). A similar hydrolytic profile was reported for certain Helotiales including ericoid fungi and saprotrophs (Knapp et al., 2018; Martino et al., 2018; Miyauchi et al., 2020). These findings indicate that cranberry endophytes can adopt a saprotrophic lifestyle.

Endophytes with a saprotrophic profile could represent an ecologic advantage for ericaceous plants, members of which grow in infertile and acidic soils where decaying matter accumulates. In such edaphic conditions, nutrients like nitrogen and phosphorous are primarily bound to organic macromolecules in vegetal and fungal necro mass and, therefore, inaccessible for plants (Cairney and Meharg, 2003; Wei et al., 2022). The ericoid fungi *Hyaloscypha*, *Pezicula* and *Phialocephala* can establish intracellular structures in the root cells and at the same time extend hyphae to the rhizosphere, forming a plant-soil interface. Extraradical hyphae could secrete hydrolytic enzymes facilitating the depolymerization of decaying matter, a mechanism enabling the mobilization of organic nutrients to support plant nutrition in peaty soils (Kerley and Read, 1995; Perotto et al.,

2012). Moreover, fungal hydrolytic enzymes could also provide access to the root endosphere for a more efficient nutrient exchange (Jumpponen et al., 1998; Martino et al., 2018).

Previous studies evidenced the efficiency of *Hyaloscypha, Pezicula* and *Phialocephala* in decomposing organic matter. Several secreted enzymes have been observed to break down cellulose, pectin, chitin and proteins (Kerley and Read, 1995; Lin et al., 2011; Surono and Narisawa, 2017). Moreover, on organic-rich media, the fungi improved the plant nutrition of different seedlings of ericaceous plants (Kosola et al., 2007; Lin et al., 2011). Likewise, genes coding for proteases and CAZymes have been observed to be highly induced in *Hyaloscypha* and *Phialocephala* in symbiosis with blueberry and Norway spruce seedlings (Martino et al., 2018; Reininger and Schlegel, 2016). These findings suggest that hydrolytic enzymes of the endophytes EC200, EC205 and EC208 could be instrumental in improving cranberry plant nutrition and colonizing the root endosphere.

4.6 Effectors

Previously, effector proteins were believed to be a distinct feature of phytopathogenic fungi as these proteins assist microbes in evading the plant immune system and favour colonization (Stone et al., 2022). It was recently discovered that plant colonization by mutualist endophytes was also enabled by effectors (Dubey et al., 2020; Rafiqi et al., 2013; Romero-Contreras et al., 2019; Zeng et al., 2020). Our genomic survey revealed that EC200, EC205 and EC208 encode several candidate effectors (Figs. 9 and 12). These findings suggest that effector proteins are spread across plant-associated fungi irrespective of their lifestyle (Kristianingsih and MacLean, 2021; Sperschneider and Dodds, 2022; C. Wang et al., 2020).

We observed that about 60% of the predicted effectors lack known conserved domains. Among the known proteins, the domains abhydrolase, WSC and LysM were the most frequent. WSC and LysM effector proteins were shown to be secreted to the apoplast and remodel the fungal cell-wall binding chitin oligomers, which avoids eliciting the plant immune system (Kombrink and Thomma, 2013; Sperschneider et al., 2016; Wawra et al., 2016). Mutualistic microorganisms such as *Piriformospora indica* and *Rhizophagus irregularis* are reported to exploit these proteins to facilitate the colonization of *Medicago truncatula* and *Hordeum vulgare* (barley). These findings

suggest that the WSC and LysM homologs of EC200, EC205, and EC208 assist in host-plant colonization (Zeng et al., 2020; Zuccaro et al., 2011).

4.7 Fungal endophytes invade cells of cranberry seedlings but show no effect on plant growth

Our experiments with cranberry seedlings indicate that the fungi EC200, EC205, and EC208 can colonize the root endosphere and grow hypha alongside the roots. The three fungi showed intracellular structures that are commonly observed in Ericaceae (Bruzone et al., 2017; Fehrer et al., 2019; Yang et al., 2018), but in the plant-growth promotion assay, no effect on the development of cranberry seedlings was observed (Fig. 4). The reason for the apparent lack of effect might be that in our experiments, the seedlings were not supplemented with organic matter, and therefore, not suited for testing the endophytes' efficiency in improving plant nutrition, despite the formidable degradative gene complement the three fungi dispose of (Figs. 10-11). Therefore, we speculate that under field conditions, the fungal endophytes are able to decompose the organic sources, liberate nutrients, and thus improve plant nutrition. One example is the ericoid fungus *Lachnum* sp. that increased the dry mass of cranberry cuttings cultured in medium complemented with peat substrate (Salhi et al., 2022).

4.8 Impact of cranberry extract on fungal gene expression

To study cranberry-endophyte interactions indirectly, we analyzed the transcriptome of cultures grown in the presence and absence of a cranberry extract. Simulating the presence of the plant host by adding plant extract to the endophyte culture is often described in the literature. Yet, it appears that this methodology was inefficient in simulating a successful symbiosis with cranberry plants. The reason for this could be that the medium employed was not comparable to the natural conditions. In particular, the presence of berry material in the cranberry extract may have increased the levels of flavonoids such as the red pigments anthocyanin and proanthocyanidin. These flavonoids can be detrimental to fungi as shown for *Botrytis cinerea*, *Candida* spp., and *Cryptococcus neoformans* (Mendoza et al., 2013; Patel et al., 2011). Specifically, flavonoids may cause the loss of cell wall integrity and elicit cell reprogramming (Ishida et al., 2006), a phenomenon that is in agreement with the gene expression profile of EC205, which showed enrichment of GO terms related to cell wall biogenesis, cellular response to stress, DNA damage,

and chromatin organization, when the fungus was cultivated in the presence of cranberry extract (Fig. 14C). A strategy to simulate more realistically the presence of the host plant involves the cultivation of endophytes in a medium, in which the roots of a cranberry seedling are suspended, as realized in our most recent study (Thimmappa et al., 2023).

4.9 Conclusions and prospective future work

We found that ericoid fungi were abundant in plants farmed under organic conditions, contrary to conventionally farmed plants, which instead were dominated by potential pathogens. The different microbial profiles from organically and conventionally cultivated cranberry plants support the hypothesis that agricultural practices shape the endophytic community in a decisive fashion.

Moreover, our results provide hits about how fungal endophytes may improve cranberry fitness. EC200, EC205 and EC208 encode a large arsenal of hydrolytic enzymes that could facilitate the decomposition of organic matter to render nutrients accessible to plants in particular in cranberry fields where nutrients are primarily trapped in decaying matter. Likewise, such enzymes may accelerate the colonization of the root endosphere and thus increase the transfer of soil nutrients to the host.

Cranberry endophytes may also improve plant fitness indirectly by suppressing antagonistic microbes. Our results show that select bacterial and fungal endophytes are able to suppress the growth of cranberry pathogens. Because our isolates inhibited *G. cassandrae*, the major cranberry fruit rot agent in Quebec, they are prime candidates to fight fruit rot in cranberry fields.

The here described isolation and identification of cranberry-root endophytes and decoding of their genomes are the first steps toward elucidating cranberry-endophyte interactions at the molecular level. Our work lays the groundwork for future investigation in the genetic regulation of fungal endophytes while engaging in endosymbiosis. In addition, our work provides the foundation for proteomic and metabolomic analyses to shed light on the vast biosynthetic machinery of the fungal endophytes examined here. A final line of future research could focus on the genetic underpinning that allows mycobionts to improve plant nutrition conditions and reduce fruit rot under field

conditions. Advances in this direction would open the door to employing cranberry endophytes as bio-fertilizers and bio-fungicides, which should accelerate the transition from conventional to organic agriculture, thus reducing the environmental and health risks arising through the use of agrochemicals.

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Appendices

1. Composition of the minimum mineral growth medium for plants

Medium composition	Concentration
$MgSO_4 \cdot 7H_2O$	730 mg/L
KNO_3	80 mg/L
KCl	65 mg/L
$KH_2PO_4 \cdot 3H_2O$	4.8 mg/L
Ca(NO3)2·4H2O	288 mg/L
KI	0.75 mg/L
NaFe(III) EDTA sodium salt	8 mg/L
$MnSO_4 \cdot H_2O$	4.66 mg/L
$ZnSO_4 \cdot 7H_2O$	2.65 mg/L
H_3BO_3	1.5 mg/L
$CuSO_4 \cdot 5H2O$	0.13 mg/L
$Na_2MoO_4 \cdot 2H_2O$	2.4 ug/L
Sucrose	100 mg/L
Gelzan	4 g/L
Gamborg's Vitamin Solution (Gamborg et al., 1968)	1X

2. Proteases subfamilies

Subfamilies of proteases			
Subfamily	**EC200**	**EC205**	**EC208**
A01A	4	3	5
C45		1	
C56		1	
G01	4	6	4
M12B	2	1	2
M14A	1	3	2
M16A	1		
M16C		1	
M20A	1	1	2
M28A	1	3	2
M28E	2	2	4
M28X	1	2	1
M35		1	1
S01A		1	
S08A	2	9	4
S09B			1
S09C		1	
S09X	20	44	41
S10	6	13	14
S12	1	4	2
S28	3	2	4
S33	2	6	4
S53	20	16	20
T03	1	4	2
U74			1
TOTAL	72	125	116

3. Top 10 conserved domains in the effectomes of EC200, EC205 and EC208

EC200	
Domain	**Frequency**
WSC	5
Abhydrolase	3
ChtBD1	3
Cupredoxin	3
Glyco_hydro_28	3
Pectinesterase	3
PemB	3
Pgu1	3
PL-6	3
PLN02432	3

EC205	
Domain	**Frequency**
Abhydrolase	10
LysM	6
Pgu1	6
PL-6	6
CVNH	5
cytochrome_P450	5
CFEM	4
Fasciclin	4
Glyco_hydro_28	4
p450	4

EC208	
Domain	**Frequency**
Abhydrolase	8
PL-6	7
WSC	6
CFEM	5
Cutinase	4
Glyco_hydro_28	4
SGNH_hydrolase	4
Amb_all	3
CBM_1	3
Chitin_bind_1	3

4. BGCs found in EC200, EC205, and EC208

Org	Region	from	to	kind_of_cluster	type	Most similar known cluste	Similarity
EC200	Region 18.1	320755	367480	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	1
EC200	Region 85.1	156387	203011	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	1
EC200	Region 173.1	11368	60629	neighbouring	T1PKS,other	naphthopyrone	1
EC200	Region 240.1	39921	53004	single	terpene	clavaric acid	1
EC200	Region 67.1	107118	128710	single	terpene	squalestatin S1	0.4
EC200	Region 4.1	391886	456028	single	T1PKS	fumagillin / β-trans-bergamot	0.13
EC200	Region 17.1	394217	454598	neighbouring	indole,T1PKS		
EC200	Region 265.1	2969	41550	single	T1PKS		
EC200	Region 37.1	105003	126879	single	terpene		
EC200	Region 62.1	1	42558	single	T1PKS		
EC200	Region 65.1	120691	162203	single	T3PKS		
EC200	Region 77.1	25315	73304	single	T1PKS		
EC200	Region 89.1	83966	105274	single	terpene		
EC200	Region 91.1	49510	97484	single	T1PKS		
EC200	Region 106.1	54021	106146	chemical_hybrid	T1PKS,NRPS		
EC200	Region 110.1	93714	141353	single	T1PKS		
EC200	Region 13.1	180868	202062	single	terpene		
EC200	Region 135.1	18133	39631	single	terpene		
EC200	Region 140.1	62591	109496	single	NRPS		
EC200	Region 154.1	1	45075	single	T1PKS		
EC200	Region 174.1	53704	96837	single	NRPS		
EC200	Region 2.1	448560	503245	single	NRPS		
EC200	Region 96.1	80916	129011	single	T1PKS		

Org	Region	from	to	kind_of_cluster	type	Most similar known cluste	Similarity
EC205	Region 145.1	65368	107496	single	T1PKS	pyranonigrin E	100
EC205	Region 22.1	6367	52824	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	100
EC205	Region 316.1	1	26156	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	100
EC205	Region 7.1	555560	602325	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	100
EC205	Region 123.1	106829	167603	single	T1PKS	botcinic acid	61
EC205	Region 144.1	58555	118044	single	T1PKS	tricholignan A	50
EC205	Region 43.1	271063	292209	single	terpene	PR-toxin	50
EC205	Region 64.1	162183	207926	single	T1PKS	ascochlorin	50
EC205	Region 102.1	61361	116542	single	NRPS	apicidin	45
EC205	Region 11.1	195284	251200	single	NRPS	paenibacterin	40
EC205	Region 18.1	110926	132774	single	terpene	botrydial	40
EC205	Region 24.2	430945	505020	neighbouring	T1PKS,NRPS	citreoviridin	40
EC205	Region 30.1	253341	274939	single	terpene	squalestatin S1	40
EC205	Region 34.1	388236	449024	single	T1PKS	betaenone A / betaenone B /	37
EC205	Region 155.1	54	112804	single	T1PKS	asperfuranone	36
EC205	Region 142.1	54679	141961	chemical_hybrid	NRPS,T1PKS,in	fujikurin A / fujikurin B / fujiku	33
EC205	Region 228.1	6407	53872	single	T1PKS	neurosporin A	33
EC205	Region 24.1	3006	59238	single	T1PKS	azanigerone A	33
EC205	Region 88.2	117778	168817	single	NRPS	phyllostictine A / phyllostictine	30
EC205	Region 60.1	136024	184662	single	T1PKS	shanorellin	28
EC205	Region 36.2	128961	178073	single	T1PKS	eupenifeldin	27
EC205	Region 133.2	85456	139375	single	NRPS	aspercryptins	26
EC205	Region 26.1	478951	520106	chemical_hybrid	T1PKS,NRPS	oxaleimide C	25
EC205	Region 296.1	49	30229	single	T1PKS	fusarielin H	25
EC205	Region 99.2	111227	158364	single	T1PKS	oxyjavanicin	25
EC205	Region 57.2	167498	264510	neighbouring	NRPS,T1PKS	aspyridone A	22
EC205	Region 65.1	44585	124099	single	T1PKS	compactin	22
EC205	Region 87.1	64251	113944	single	T1PKS	monacolin K	22
EC205	Region 133.1	14193	63329	single	T1PKS	phomoidride	20
EC205	Region 19.1	287639	335425	single	T1PKS	4-epi-15-epi-brefeldin A	20
EC205	Region 179.1	11656	94926	neighbouring	T1PKS,NRPS	cornexistin	19
EC205	Region 58.1	95429	150248	single	T1PKS	zearalenone	18
EC205	Region 76.2	127795	233986	single	NRPS	aspercryptins	13
EC205	Region 13.1	49800	115150	single	NRPS	destruxin A	9
EC205	Region 241.1	14002	57591	single	T1PKS	viridicatumtoxin / previridicatu	9
EC205	Region 74.2	99672	154815	neighbouring	NRPS,T1PKS	pneumocandin B0 / pneumoca	9
EC205	Region 1.1	245613	266772	single	terpene		
EC205	Region 108.1	4131	57844	neighbouring	terpene,T1PKS		
EC205	Region 115.1	27963	116297	neighbouring	T1PKS,NRPS		
EC205	Region 119.1	121521	167192	single	T1PKS		
EC205	Region 121.1	59964	130101	neighbouring	NRPS,fungal-RiPP		
EC205	Region 125.1	78988	126710	single	T1PKS		
EC205	Region 13.2	394748	435892	single	other		
EC205	Region 14.1	569702	591181	single	terpene		
EC205	Region 143.1	114347	136281	single	terpene		
EC205	Region 150.1	26175	73836	single	T1PKS		
EC205	Region 169.1	49583	90455	single	phosphonate		
EC205	Region 177.1	37615	85169	single	T1PKS		
EC205	Region 18.2	403999	451393	single	NRPS		
EC205	Region 188.1	1	37888	chemical_hybrid	T1PKS,NRPS		
EC205	Region 205.1	3434	79688	interleaved	T1PKS,indole		
EC205	Region 22.2	364701	385630	single	terpene		
EC205	Region 264.1	16211	45251	single	T1PKS		
EC205	Region 32.1	48558	90046	single	T3PKS		
EC205	Region 36.1	61313	89528	single	T1PKS		
EC205	Region 4.1	601540	655735	single	NRPS		
EC205	Region 41.1	155434	176631	single	terpene		
EC205	Region 49.1	244661	291024	single	T1PKS		
EC205	Region 5.1	848831	897326	single	T1PKS		
EC205	Region 53.1	284748	331770	single	T1PKS		
EC205	Region 57.1	35955	77217	single	other		
EC205	Region 61.1	152292	207051	single	NRPS		
EC205	Region 68.1	23753	45222	single	indole		
EC205	Region 71.1	197963	244319	single	NRPS		
EC205	Region 74.1	42080	85947	single	T1PKS		
EC205	Region 76.1	50939	116713	single	NRPS		
EC205	Region 80.1	192770	241994	single	T1PKS		
EC205	Region 88.1	1	40304	chemical_hybrid	T1PKS,NRPS		
EC205	Region 89.1	51366	95650	single	NRPS		
EC205	Region 90.1	1	26176	single	betalactone		
EC205	Region 90.2	38194	85341	single	T1PKS		
EC205	Region 96.1	82907	129538	single	T1PKS		
EC205	Region 99.1	1	56402	single	NRPS		

Org	Region	from	to	kind_of_cluster	type	Most similar known cluste	Similarity
EC208	Region 20.1	644228	692548	single	T1PKS	alternapyrone	100
EC208	Region 25.1	43572	87573	single	NRPS	AbT1	100
EC208	Region 12.1	329220	376182	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	100
EC208	Region 85.1	185315	232062	single	T1PKS	1,3,6,8-tetrahydroxynaphthale	100
EC208	Region 56.1	272912	294513	single	terpene	squalestatin S1	40
EC208	Region 126.1	51202	101865	chemical_hybrid	NRPS,T1PKS	leporin B	27
EC208	Region 123.1	93564	141362	single	T1PKS	citrinin	25
EC208	Region 121.1	85254	137578	chemical_hybrid	T1PKS,NRPS	cytochalasin E / cytochalasin H	15
EC208	Region 1.1	1023803	1068873	single	T1PKS		
EC208	Region 1.2	1578511	1599452	single	terpene		
EC208	Region 2.1	30745	51898	single	terpene		
EC208	Region 5.1	11485	60119	single	T1PKS		
EC208	Region 8.1	140496	161713	single	terpene		
EC208	Region 9.1	277318	327137	single	T1PKS		
EC208	Region 13.1	614862	636189	single	terpene		
EC208	Region 15.1	36260	58138	single	terpene		
EC208	Region 18.1	724268	745810	single	terpene		
EC208	Region 21.1	354099	408615	single	NRPS		
EC208	Region 23.1	296624	348717	chemical_hybrid	T1PKS,NRPS		
EC208	Region 25.2	439938	487646	single	T1PKS		
EC208	Region 29.1	216217	257765	single	T3PKS		
EC208	Region 33.1	403173	446796	single	NRPS		
EC208	Region 36.1	742	45745	single	NRPS		
EC208	Region 39.1	166286	214014	single	T1PKS		
EC208	Region 45.1	393903	444165	single	T1PKS		
EC208	Region 51.1	145823	193124	single	T1PKS		
EC208	Region 64.1	12615	56696	single	NRPS		
EC208	Region 71.1	310403	358813	single	T1PKS		
EC208	Region 73.1	225140	270240	single	T1PKS		
EC208	Region 77.1	153997	200621	single	T1PKS		
EC208	Region 87.1	42540	91028	single	T1PKS		
EC208	Region 101.1	217624	254643	single	T1PKS		
EC208	Region 110.1	43388	89714	single	T1PKS		
EC208	Region 118.1	177539	212287	single	T1PKS		
EC208	Region 128.1	13015	34391	single	terpene		
EC208	Region 138.1	16549	38539	single	terpene		
EC208	Region 140.1	11834	58966	single	T1PKS		
EC208	Region 157.1	39592	87415	single	T1PKS		
EC208	Region 162.1	65376	115847	single	T1PKS		
EC208	Region 186.1	1	39938	single	phosphonate		
EC208	Region 197.1	18019	65524	single	T1PKS		
EC208	Region 252.1	1	31861	single	NRPS		
EC208	Region 285.1	1661	16152	single	terpene		

5. BGCs shared by two or three endophytes

Product	Kind	Group	EC200	EC205	EC208
unknown	NRPS	g1	1	1	1
unknown	NRPS	g2		1	1
1,3,6,8-tetrahydroxynaphthalene_100 (melanin)	T1PKS	g3	2	3	2
unknown	T1PKS	g5		1	1
unknown	T1PKS	g6		1	1
unknown	T1PKS	g7	2		1
unknown	T3PKS	g8	1	1	1
unknown	Terpene	g9	1	1	1
unknown	Terpene	g10	1	1	1
unknown	Terpene	g11	1	1	1
unknown	Terpene	g12	1		1