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journal homepage: www.elsevier.com/locate/funbio

# A genomic comparison of putative pathogenicity-related gene families in five members of the Ophiostomatales with different lifestyles

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ARTICLE INFO

*Article history:*
Received 2 July 2016
Received in revised form
10 December 2016
Accepted 14 December 2016
Available online 27 December 2016
*Corresponding Editor:*
Alga Zuccaro

*Keywords:*
Bark beetle
Bluestain fungi
*Ips typographus*

ABSTRACT

Ophiostomatoid fungi are vectored by their bark-beetle associates and colonize different host tree species. To survive and proliferate in the host, they have evolved mechanisms for detoxification and elimination of host defence compounds, efficient nutrient sequestration, and, in pathogenic species, virulence towards plants. Here, we assembled a draft genome of the spruce pathogen *Ophiostoma bicolor*. For our comparative and phylogenetic analyses, we mined the genomes of closely related species (*Ophiostoma piceae*, *Ophiostoma ulmi*, *Ophiostoma novo-ulmi*, and *Grosmannia clavigera*). Our aim was to acquire a genomic and evolutionary perspective of gene families important in host colonization. Genome comparisons showed that both the nuclear and mitochondrial genomes in our assembly were largely complete. Our *O. bicolor* 25.3 Mbp draft genome had 10 018 predicted genes, 6041 proteins with gene ontology (GO) annotation, 269 carbohydrate-active enzymes (CAZymes), 559 peptidases and inhibitors, and 1373 genes likely involved in pathogen-host interactions. Phylogenetic analyses of selected protein families revealed core sets of cytochrome P450 genes, ABC transporters and backbone genes involved in secondary metabolite (SM) biosynthesis (polyketide synthases (PKS) and non-ribosomal synthases), and species-specific gene losses and duplications. Phylogenetic analyses of protein families of interest provided insight into evolutionary adaptations to host biochemistry in ophiostomatoid fungi.



## Introduction

Symbioses between ophiostomatoid fungi, their tree hosts, and bark beetles are complex associations that differ in species composition, temporal dynamics, and the life-style of the fungal symbiont (Klepzig *et al.* 2004; Giordano *et al.* 2013). Fungi, vectored by beetles, have evolved adaptations to tolerate and eliminate host tree chemical defence

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http://dx.doi.org/10.1016/j.funbio.2016.12.002
1878-6146/

compounds. This results in weakened tree defences and detoxification of the hostile micro-environment, which in turn facilitates tree colonization by both the fungus and the beetle. Some associations of phytopathogenic fungi and bark beetles result in outbreaks that cause significant ecosystem damage and substantial economic losses to the forest products industry (Raffa *et al.* 2008; Seidl *et al.* 2014).

Successful fungal phytopathogenicity results from processes that include tolerance and inactivation of host defences and successful nutrient sequestration from the host. In this context, annotated genomic data and curated gene inventories are invaluable resources for inferring metabolic and pathogenic capabilities of a given ophiostomatoid fungus (DiGuistini *et al.* 2011; Wang *et al.* 2012). In addition, comparative analyses with complete or draft genomic data of related fungal species that have similar lifestyles but occupy different ecological niches can also identify candidate genes and pathways that are important in pathogenicity and virulence (Haridas *et al.* 2013).

Two main mechanisms by which ophiostomatoid fungi can overcome tree defences are enzymatic detoxification of harmful chemicals and their elimination from the cell *via* the action of transporter enzymes. Conifers synthesize terpenoids and phenolic compounds (Keeling & Bohlmann 2006; Li *et al.* 2012; Kolosova & Bohlmann 2012), while in elm trees (*Ulmus* sp.), the major defence compounds are lignin, phenolics, suberin, and sesquiterpenoid quinones with antifungal properties (Aoun *et al.* 2009). The bluestain fungus *Grosmannia clavigera*, a lodgepole pine pathogen, can eliminate toxic pine monoterpenes by an effective efflux system through a substrate-specific ABC transporter, and also by converting them *via* the action of Bayer-Villiger monooxygenases and possibly cytochrome P450 enzymes (Wang *et al.* 2012, 2014). Similarly, spruce phenolic compounds with antifungal properties, such as stilbenes, can be metabolized by another ophiostomatoid fungus *Endoconidiophora polonica* (Hammerbacher *et al.* 2014; Wadke *et al.* 2016). These various fungi can then utilize the products of these metabolic reactions for growth. In addition to these mechanisms of detoxification and elimination of host defence chemicals, fungi often synthesize secondary metabolite (SM)s, such as mycotoxins, to weaken and allow colonization of the host. Most fungal SMs are the products of either non-ribosomal peptide synthases (NRPS) or polyketide synthases (PKS), the backbone genes of secondary metabolism gene clusters. While mycotoxin production has not been very well studied in ophiostomatoid fungi, gene clusters and respective backbone genes have been identified in genomes of a few species (Lah *et al.* 2012; Haridas *et al.* 2013; Comeau *et al.* 2015).

Although tree-colonizing fungi can utilize degradation products of host defence compounds, their main source of nutrients are complex molecules such as polysaccharides and proteins that are degraded by extracellular carbohydrate-active enzymes (CAZymes) and proteases (DiGuistini *et al.* 2011; Haridas *et al.* 2013). Pine triglycerides, which can be degraded by extracellular lipases, also serve as a carbon source (Gao *et al.* 1994; DiGuistini *et al.* 2011). Initial conversion of these compounds is likely achieved by substrate-specific enzymes, after which the products are channelled into primary metabolism pathways, such as the main fatty acid beta-oxidation pathway (DiGuistini *et al.* 2011; Wang *et al.* 2014).

In this study, we sequenced and assembled a draft genome of the Norway spruce (*Picea abies*) fungal pathogen *Ophiostoma bicolor*, vectored by the European Spruce Bark Beetle *Ips typographus* (Kirisits 2004). This fungus is abundant in both non-epidemic conditions and during bark-beetle outbreaks (Marini *et al.* 2012; Giordano *et al.* 2013). In comparison to the aggressive pathogen *E. polonica*, the early colonist of sapwood following a bark-beetle attack, *Ophiostoma bicolor* is moderately pathogenic (Sallé *et al.* 2005; Giordano *et al.* 2013; Repe *et al.* 2015; Linnakoski *et al.* 2016).

We compared its gene inventory with that of four recently sequenced genomes of fungi from the order Ophistomatales (Sordariomycetes) (de Beer & Wingfield 2013) that differ in their host tree preference and tolerance of tree-specific defence chemicals, level of pathogenicity, as well as colonization dynamics. These include *Ophiostoma piceae* (Haridas *et al.* 2013), a lodgepole pine saprophyte, and two elm tree pathogens *Ophiostoma ulmi* (Khoshraftar *et al.* 2013) and *Ophiostoma novo-ulmi* (Forgetta *et al.* 2013; Comeau *et al.* 2015). The latter two, vectored by two genera of scotylid beetles, have caused the Dutch elm disease (DED) pandemics in both North America and Europe during the last century (Comeau *et al.* 2015). We also included an aggressive pathogen of lodgepole pine from a sister genus, *G. clavigera* (DiGuistini *et al.* 2011). This species of fungus and its insect vector, the Mountain Pine Beetle (MPB) *Dendroctonus ponderosae*, are responsible for killing millions of hectares of lodgepole pine forests in western North America (Kurz *et al.* 2008).

Our objective was to obtain a genomic and evolutionary perspective for genes implicated in infection and nutrient sequestration across the currently available genomes of five fungi from the order Ophiostomatales. We phylogenetically analysed selected protein families that are possibly important in pathogenicity and in utilization and tolerance of host tree defences. The former include backbone genes of SM biosynthetic clusters, while the latter include ABC transporter family enzymes and cytochromes P450.

## Materials and methods

### *Strain and growth conditions*

The *Ophiostoma bicolor* strain ZLVG358 used in this study was collected from secondary *Picea abies* stands in Kočevje (Slovenia), and deposited in the culture collection of the Laboratory for Forest Protection (ZLVG) at the Slovenian Forestry Institute, Ljubljana, Slovenia (Repe *et al.* 2013). Mycelium was cultured at room temperature on MEA (1.6 % Oxoid malt extract agar and 1.5 % technical agar No. 3, pH 5–6) plates, overlaid with cellophane. DNA was extracted following a published protocol (DiGuistini *et al.* 2011).

RNA was extracted from mycelia grown under control and terpene treatment conditions as described previously (DiGuistini *et al.* 2011; Wang *et al.* 2012). Briefly, two strips of filter paper were placed inside the lid of a glass plate and 200 ml of a mixture of monoterpenes was added onto the filter paper. The mixture was composed of alpha-pinene (33 %), (–)-beta-