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Genomic and transcriptomic analysis of *Laccaria bicolor* CAZome reveals insights into polysaccharides remodelling during symbiosis establishment

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ABSTRACT

Ectomycorrhizal fungi, living in soil forests, are required microorganisms to sustain tree growth and productivity. The establishment of mutualistic interaction with roots to form ectomycorrhiza (ECM) is not well known at the molecular level. In particular, how fungal and plant cell walls are rearranged to establish a fully functional ectomycorrhiza is poorly understood. Nevertheless, it is likely that Carbohydrate Active enZymes (CAZyme) produced by the fungus participate in this process.

Genome-wide transcriptome profiling during ECM development was used to examine how the CAZome of *Laccaria bicolor* is regulated during symbiosis establishment.

CAZymes active on fungal cell wall were upregulated during ECM development in particular after 4 weeks of contact when the hyphae are surrounding the root cells and start to colonize the apoplast. We demonstrated that one expansin-like protein, whose expression is specific to symbiotic tissues, localizes within fungal cell wall.

Whereas *L. bicolor* genome contained a constricted repertoire of CAZymes active on cellulose and hemicellulose, these CAZymes were expressed during the first steps of root cells colonization. *L. bicolor* retained the ability to use homogalacturonan, a pectin-derived substrate, as carbon source. CAZymes likely involved in pectin hydrolysis were mainly expressed at the stage of a fully mature ECM.

All together, our data suggest an active remodelling of fungal cell wall with a possible involvement of expansin during ECM development. By contrast, a soft remodelling of the plant cell wall likely occurs through the loosening of the cellulose microfibrils by AA9 or GH12 CAZymes and middle lamella smooth remodelling through pectin (homogalacturonan) hydrolysis likely by GH28, GH12 CAZymes.

1. Introduction

Ectomycorrhizal interactions (ECM) established between the root systems of perennial plants and hyphae from soil-borne fungi are ubiquitous in boreal and temperate forests. This symbiosis is a mutualistic interaction leading to fair nutrient exchanges. ECM fungi indeed obtain carbon (C) from the plant photosynthate and in return provide plant with some essential nutrients such as

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phosphate, nitrogen and potassium (Casieri et al., 2013). These interactions are crucial for tree health and growth (biomass production) through increasing efficiency of nutrient uptake, modulating plant hormone signalling and finally increasing plant resistance to pests (Smith and Read, 2008). In addition, these interactions play a key role in nutrient recycling (Read and Perez-Moreno, 2003; Smith and Read, 2008).

The role of ECM fungi in soil C cycling may be multiple and likely opposite. They have been first considered as organisms leading to plant C sequestration into soils, as some studies estimate that 10–50% of the C-fixed by photosynthesis is allocated to ECM fungi (Simard and Jones, 2002). Indeed, radiocarbon analysis revealed that recently fixed carbon composed most of the biomass of ECM fungi (Henn and Chapela, 2001; Hobbie et al., 2002; Le Tacon et al., 2013; Treseder et al., 2006). Clemmensen et al. (2013) showed recently that in boreal forest islands, up to 70% of soil carbon was derived from roots and mycelium. They also showed that soils with the highest ECM fungi-derived biomass accumulate more rapidly C than soils containing saprotrophic fungi-derived biomass.

Secondly, in addition to saprotrophic fungi, ECM fungi have been considered as possible decomposers, thus participating to release of CO_2 to the atmosphere. Indeed, living ECM root tips display proteases, N-acetylglucosaminidase, glucuronidase, cellobiohydrolase, b-glucosidase, laccases activities (Burke et al., 2014; Courty et al., 2010), enzymes possibly active against cellulose, chitin and aromatic C compounds, respectively. In addition, several studies suggest that ECM fungi could grow from fungal or animal necromass to obtain C and N likely through high chitinase activity (Buée et al., 2007; Rajala et al., 2012). The role of ECM fungi as decomposers has been suggested to occur during the period of low C supply by the plant (Talbot et al., 2008) and ECM fungi are likely to better target soil nutrient (N and P) rich compounds, usually present in organic forms (Talbot and Treseder, 2010). Indeed, recent analysis showed that the ECM fungus *Paxillus involutus*, when growing on organic matter, is producing hydroxyl radicals through a Fenton system similar to the system used by wood-decomposing fungi to efficiently disrupt organic matter (Rineau et al., 2012). However, this degradative system is triggered by glucose, suggesting that this system is efficient to digest organic matter but that the ECM fungi may not use the released carbon. This reinforces the idea that ECM fungi are degrading organic matter to access N and P rather than C, as the latter is delivered directly by the root cells (Talbot and Treseder, 2010). Whether or not ECM fungi retain saprotrophic growth ability for carbon sources under natural conditions is still under debate. Several studies demonstrated that the litter horizon of boreal and temperate forests is mainly inhabited with saprotrophic fungi, whereas the deeper horizons containing more decomposed litter and humus, are dominated by ECM fungi (Clemmensen et al., 2013; Lindahl et al., 2007). This vertical stratification suggests to the authors that it is unlikely that ECM fungi can grow saprotrophically on this low energetic value material (Baldrian, 2009).

Phylogenomic analysis of saprotrophic and ECM fungi from *Amanita* genus showed that apparition of ECM is associated with losses of cellulase genes playing a key role in saprotrophic growth (Wolfe et al., 2012). Recent comparative genomic analysis within Basidiomycota (containing saprotrophic and ECM fungi) highlight that ECM and brown rot fungi have a reduced or null set of certain plant Carbohydrate-Active enZymes (CAZymes). These losses or contraction are then correlated with the fungal way of life. In particular, Eastwood et al. (2011) highlight that ECM way of life was likely arising from brown-rot lifestyle. A similar correlation between fungal way of life and gene contraction has been shown regarding lignin-degradation enzymatic machinery (Floudas et al., 2012). Reduced numbers of these particular CAZymes, coupled with retention of the ability to degrade soil organic matter through oxidative mechanisms, suggest that ECM fungi are able to participate to the slow C cycle.

At the cellular level, morphogenesis of the ECM fungus-plant symbiotic interaction is well known (Brun et al., 1995; Horan et al., 1988; Kottke and Oberwinkler, 1987; Massicotte et al., 1987). Fungal hyphae form a mantle around lateral roots and penetrate within the apoplast of cortical root cells to form the Hartig net, the zone of nutrients and signalling compounds exchange. This intimate connection between root and fungal cells requires both fungal and plant cell wall remodelling to form the apoplastic symbiotic interface. On the plant side, elongation of epidermal cells and arrest of growth were observed (Peterson and Bonfante, 1994). Internal fungal hyphae are embedded in a matrix composed of polysaccharides, cystein-rich proteins and glycoproteins (Dexheimer and Pargney, 1991). Distribution of several fungal proteins such as hydrophobins, mannoproteins at the cell surface is changing during ECM establishment (Tagu and Martin, 1996). Symbiotic interactions rely on efficient communication between both partners and efficient nutrient exchanges through the symbiotic interface. The plant cell wall is thus the place of the earliest communication event between plants and fungal symbionts. So far, it is not clear whether the fungal symbiont has a role in remodelling the plant cell wall during ECM development. Among the enzymes required for fungal and plant cell remodelling, CAZymes are likely the most important enzymes involved. Indeed, they are responsible for the biosynthesis, degradation and modification of oligo- and polysaccharides as well as of glycoconjugates. They are divided into four classes: Glycoside-Hydrolases (GH), glycosyltransferases (GT), Polysaccharide Lyases (PL) and Carbohydrate Esterases (CE). Recently, several classes of Auxiliary Activities (AA) containing redox enzymes acting together with CAZymes to degrade lignocellulose material were added within the CAZy database (www.cazy.org, [Lombard et al., 2014]). All CAZymes and auxiliary activity enzymes can be attached to carbohydrate-binding modules (CBMs) that can promote enzymatic deconstruction of polysaccharide (Levasseur et al., 2013; Boraston et al., 2004; Hervé et al., 2010).

All together, previous analyses suggest that CAZymes may be crucial enzymes to explain ECM double way of life. Indeed, they may be involved throughout the continuum between saprotrophy and mutualism: from soil C-acquisition, fungal cell wall morphogenesis to plant cell wall remodelling for symbiosis establishment. To address the potential roles of fungal CAZymes within this continuum and their putative role in symbiosis establishment, we took advantage of the availability of the genome of the ECM fungus *Laccaria bicolor* (Basidiomycotina, Agaricomycotina, Agaricales, Hydnangiaceae) (Martin et al., 2008) as well as other genomes within the Agaricomycotina sub-phylum (Floudas et al., 2012). Genome analyses already revealed that, compared to other fungi, *L. bicolor* possesses an enhanced repertoire for protein degradation but a reduced repertoire of CAZymes targeting cellulose and lignin (Martin et al., 2008). In this report, we combined genomic and transcriptomic analyses to provide a comprehensive view of the CAZome of *L. bicolor* and its regulation during symbiosis establishment. We compared the abundance and distribution of CAZyme families in *L. bicolor* to those found in other Basidiomycetes with various lifestyles. We further functionally analyzed, by immunolocalization, one *L. bicolor* protein (expansin-like protein) whose expression is induced during ECM development.

2. Materials and methods

2.1. Detection and modular annotation of putative CAZymes in the genome of Laccaria bicolor

The search for catalytic modules specific to CAZymes (GHs, GTs, PLs and CEs) and their ancillary carbohydrate-binding modules