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Plant biomass degrading ability of the coprophilic ascomycete fungus *Podospora anserina*

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ABSTRACT

The degradation of plant biomass is a major challenge towards the production of bio-based compounds and materials. As key lignocellulolytic enzyme producers, filamentous fungi represent a promising reservoir to tackle this challenge. Among them, the coprophilous ascomycete *Podospora anserina* has been used as a model organism to study various biological mechanisms because its genetics are well understood and controlled. In 2008, the sequencing of its genome revealed a great diversity of enzymes targeting plant carbohydrates and lignin. Since then, a large array of lignocellulose-acting enzymes has been characterized and genetic analyses have enabled the understanding of *P. anserina* metabolism and development on plant biomass. Overall, these research efforts shed light on *P. anserina* strategy to unlock recalcitrant lignocellulose deconstruction.

Contents

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1. Introduction

Enzymatic conversion of lignocellulosic biomass (i.e. plant material) to bio-products is of great interest for the development of sustainable biorefineries. Plant biomass constitutes the most abundant biological repository of carbon on Earth. It is a complex structure comprising polysaccharides (cellulose, hemicellulose, pectin) and lignin, forming a tight interconnected network. This heterogeneous composition makes it recalcitrant to biotic degradation, which in turn hampers the development of bioprocesses for industrial purposes (Himmel et al., 2007). Because of its complex structure, a number of enzymes with diverse complementary activities are required to perform efficient conversion of plant cell wall to platform molecules or high value compounds, and considerable research efforts have aimed at developing such efficient enzyme systems from microbes.

Filamentous fungi thrive in lignocellulose-rich environments because they are potent biomass degraders thanks to their dedicated enzymatic machinery (Sigoillot et al., 2012) and their ability to penetrate forcibly the biomass. Among them, the filamentous ascomycete fungus *Podospora anserina* has been studied for decades as a model species and numerous studies focused on biological questions such as ageing (Osiewacz et al., 2013), prion mechanisms (Baxa et al., 2004) or fungal reproduction (Silar, 2014; Debuchy et al., 2010). Indeed, genetic analysis is efficient with *P. anserina* thanks to its one-week haplophasic life cycle (Silar, 2013a,b). Moreover, this fungus is easily transformed by exogenous DNA and its genes can be inactivated in one step by replacement with resistance markers (Silar, 2013a,b). One of the outcomes of the genome sequence project of this species (Espagne et al., 2008) was the discovery of a large array of enzymes potentially involved in both cellulose and lignin breakdown, making this fungus a model of choice to better understand the enzymatic deconstruction of plant biomass.

In its natural environment, *P. anserina* is a cosmopolitan coprophilous fungus which frequently develops in the droppings of grass herbivores. As a very late ascomycete degrader that fructifies after the succession of most other coprophilous organisms, *P. anserina* is thought to specifically use the more recalcitrant fraction of lignocellulose (Richardson, 2002). In line with this assumption, this fungus can fructify abundantly using lignocellulosic biomass as sole carbon source (Fig. 1). In addition, it was demonstrated that *P. anserina* differentiates dedicated hyphae to penetrate the biomass, facilitating its digestion (Brun et al., 2009). Development of these hyphae is under the control of signalling pathways that also regulate the transcription of lignocellulolytic genes, connecting differentiation and enzyme production (Bidard et al., 2012).

In this article, we review genomic and post-genomic studies, as well as biochemical and genetic analyses focusing on the exploration of *P. anserina* metabolism and development on biomass.

2. *P. anserina* enzymatic machinery for recalcitrant biomass degradation

Coprophilous fungi hold promise as an enzyme source due to their atypical habitat, as they grow on herbivore dung (Richardson, 2002). The plant biomass that has been digested in the animal digestive track of herbivores is still rich in nutrients. In addition, it is from different origins depending upon the animal diets and ranges from monocotyledons and dicotyledons to ferns and mosses. It has therefore highly variable structures and coprophilous fungus must cope with such variability. The first coprophilous fungi commonly found in dung are saprotrophic zygomycetes that assimilate the most easily utilizable biomass components like hemicelluloses and pectins. For instance, the coprophilous zygomycete *Pilobolus* is often the earliest fungus identified in horse dung (Pointelli et al., 1981; Richardson, 2002). Ascomycetes such as *Chaetomium*, *Sordaria*, and *Podospora* then digest more complex components, and recalcitrant lignin is finally fully degraded by basidiomycetes (e.g., *Coprinopsis cinerea* (Stajich et al., 2010)). As a late grower on the recalcitrant fraction of biomass, *P. anserina* potentially displays a specific and efficient cellulose, hemicellulose and lignin conversion machinery.

2.1. Comparative genomics and post-genomics reveal P. anserina strengths to convert lignocellulose

The sequencing of *P. anserina* genome was carried out in 2008 (Espagne et al., 2008). Annotation of genes encoding carbohydrate active enzymes (CAZymes; www.CAZy.org, Lombard et al., 2014) revealed a large diversity of plant cell wall-targeting activities and one of the highest numbers of Carbohydrate Binding Modules (CBMs) among fungal genomes available at the time. Comparison of closely related fungi such as *Chaetomium globosum* and *Neurospora crassa* and with the industrial workhorse *Trichoderma reesei* confirmed that *P. anserina* encodes a complete machinery for plant cell wall conversion including some auxiliary activity (AA) enzymes potentially targeting lignin (Fig. 2). Enzymes targeting cellulose, i.e., cellobiohydrolases (GH6, GH7) and endoglucanases (GH5, GH12, GH45) are abundant in *P. anserina*. Its genome contains more than 100 CBMs, among which 28 CBM1, specific of cellulose recognition. As a comparison, the cellulose degrader *T. reesei* holds only 36 CBMs. It is generally acknowledged that the presence of CBMs improves the activity of CAZymes by targeting the substrate and binding the catalytic domain to it (for a review see Várnai et al., 2014). Together with *C. globosum*, *P. anserina* displays an impressive set of Lytic Polysaccharide Monooxygenases (LPMOs) with 33 members of the family AA9. This multiplicity of genes raises the question of the functional relevance of LPMOs in fungi, i.e. functional redundancy or functional diversification or fine-tuned regulation of alternative genes and/or adaptations to the degradation of the substrates. Hemicellulolytic enzymes are also abundant with GH10 and GH11 xylanases, GH5 and GH26 mannanases, GH51 and GH62 arabinofuranosidases, and carbohydrate esterases of family CE1 mostly. In contrast to Aspergilli species, only a few pectin-targeting enzymes are found in *P. anserina* (e.g. one member of the GH78 and no member of families GH28 and GH88) (Coutinho et al., 2009). Accordingly, *P.*

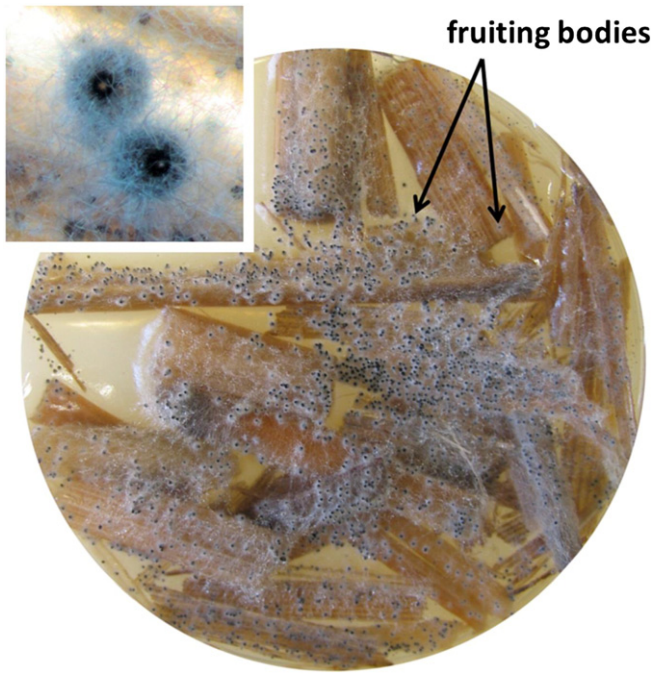

Fig. 1. *P. anserina* fruiting bodies on miscanthus as sole carbon source. Miscanthus supplemented with nitrogen and oligo-elements was inoculated with heterokaryotic self-fertile *P. anserina*. Ten days after inoculation, numerous spore-bearing fruiting bodies are indicative of the ability of the fungus to efficiently scavenge energy from miscanthus.