Fungal Genetics and Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Fungal Genetics and Biology

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

Transcriptome and exoproteome analysis of utilization of plant-derived biomass by *Myceliophthora thermophila*

Magdalena Anna Kolbusz [a,b,c,*], Marcos di Falco [a], Nadeeza Ishmael [a], Sandrine Marqueteau [a], Marie-Claude Moisan [a], Cassio da Silva Baptista [a,1], Justin Powlowski [a,c], Adrian Tsang [a,b]

[a] Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke Street West, Montréal, Québec H4B 1R6, Canada
[b] Department of Biology, Concordia University, 7141 Sherbrooke Street West, Montréal, Québec H4B 1R6, Canada
[c] Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke Street West, Montréal, Québec H4B 1R6, Canada

ARTICLE INFO

Article history:
Received 15 March 2014
Revised 16 May 2014
Accepted 17 May 2014
Available online xxxx

Keywords:
Myceliophthora thermophila
Biomass degradation
Carbohydrate-active enzymes
RNA-Seq
Mass spectrometry

ABSTRACT

Myceliophthora thermophila is a thermophilic fungus whose genome encodes a wide range of carbohydrate-active enzymes (CAZymes) involved in plant biomass degradation. Such enzymes have potential applications in turning different kinds of lignocellulosic feedstock into sugar precursors for biofuels and chemicals. The present study examined and compared the transcriptomes and exoproteomes of *M. thermophila* during cultivation on different types of complex biomass to gain insight into how its secreted enzymatic machinery varies with different sources of lignocellulose. In the transcriptome analysis three monocot (barley, oat, triticale) and three dicot (alfalfa, canola, flax) plants were used whereas in the proteome analysis additional substrates, i.e. wood and corn stover pulps, were included. A core set of 59 genes encoding CAZymes was up-regulated in response to both monocot and dicot straws, including nine polysaccharide monooxygenases and GH10, but not GH11, xylanases. Genes encoding additional xylanolytic enzymes were up-regulated during growth on monocot straws, while genes encoding additional pectinolytic enzymes were up-regulated in response to dicot biomass. Exoproteome analysis was generally consistent with the conclusions drawn from transcriptome analysis, but additional CAZymes that accumulated to high levels were identified. Despite the wide variety of biomass sources tested some CAZy family members were not expressed under any condition. The results of this study provide a comprehensive view from both transcriptome and exoproteome levels, of how *M. thermophila* responds to a wide range of biomass sources using its genomic resources.

1. Introduction

Plant cell wall, composed of lignocellulosic material, represents an abundant renewable resource and is a raw material used in many branches of industry such as paper, food, animal feed and biofuel production (Minic and Jouanin, 2006; van den Brink and de Vries, 2011). Processing of these materials for industrial applications can be realized by enzymes that are produced by lignocellulolytic microorganisms. As lignocellulose is composed of the structural polymers cellulose, hemicellulose, pectin, and lignin, its efficient deconstruction is a complex process requiring the synergistic action of multiple enzymes. Enzymes that break down plant cell wall can be categorized by reaction type into the following classes: glycoside hydrolases (GH), polysaccharide lyases (PL), carbohydrate esterases (CE) and auxiliary activities (AA). Based on structural similarity, each of these broad activities can be divided into carbohydrate-active enzyme (CAZyme) families

Abbreviations: GH, glycoside hydrolase; CE, carbohydrate esterase; PL, polysaccharide lyase; AA, auxiliary activity family; CAZymes, carbohydrate-active enzymes; HWKP, hardwood Kraft pulp; HWMP, hardwood mechanical pulp; SWKP, softwood Kraft pulp; SWMP, softwood mechanical pulp; PCS, pretreated corn stover; JGI, Joint Genome Institute; ACN, acetonitrile; FA, formic acid; MS, mass spectrometer.

* Corresponding author. Address: Centre for Structural and Functional Genomics, Concordia University, L-GE-120.11, 7141 Sherbrooke Street West, Montréal, Québec H4B 1R6, Canada.

E-mail addresses: magdalena.kolbusz@concordia.ca (M.A. Kolbusz), marcos.difalco@concordia.ca (M. di Falco), nadeeza.ishmael@concordia.ca (N. Ishmael), sandrine.marqueteau@concordia.ca (S. Marqueteau), marie-claude.moisan@concordia.ca (M.-C. Moisan), Cassio.Baptista@nrc-cnrc.gc.ca (C. da Silva Baptista), justin.powlowski@concordia.ca (J. Powlowski), adrian.tsang@concordia.ca (A. Tsang).

[1] Present address: Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montréal, Québec H4P 2R2, Canada.

http://dx.doi.org/10.1016/j.fgb.2014.05.006
1087-1845/

(Cantarel et al., 2009). Suites of enzymes from multiple families function in concert to degrade the different components of lignocellulose.

Lignocellulose degradation in the terrestrial biosphere is mostly performed by filamentous fungi and they are the main source of commercial enzymes for the degradation of plant-derived biomass (Sanchez, 2009). An increasing number of genomes of lignocellulolytic fungi from different habitats is being sequenced to prospect the diversity of enzymes used to deconstruct plant cell wall; for example, *Phanerochaete chrysosporium* (Martinez et al., 2004), *Trichoderma reesei* (Martinez et al., 2008), *Aspergillus niger* (Andersen et al., 2011; Pel et al., 2007), *Thielavia terrestris* and *Myceliophthora thermophila* (Berka et al., 2011), and multiple brown-rot and white-rot basidiomycetes (Floudas et al., 2012). Using the genome sequence as a reference, transcriptome and exoproteome approaches have been used to examine the lignocellulolytic enzymes produced by fungi when cultured in defined polysaccharides and in complex biomass (Berka et al., 2011; de Souza et al., 2011; Eastwood et al., 2011; Martinez et al., 2009; Tsang et al., 2009; Vanden Wymelenberg et al., 2009). These studies show that in response to homogeneous polysaccharides fungi produce enzymes whose activities are best suited for their degradation. For example, *A. niger* produces predominantly pectinases in the presence of pectin and mannanases in the presence of mannan (Tsang et al., 2009). However, different fungi produce different combinations of enzymes when exposed to complex biomass (Berka et al., 2011; Eastwood et al., 2011; Martinez et al., 2009). For example, in the presence of alfalfa straw *T. terrestris* up-regulates cellulase genes while *M. thermophila* up-regulates pectinase genes. In the presence of barley straw, both *T. terrestris* and *M. thermophila* up-regulate genes encoding cellulases and xylanases (Berka et al., 2011).

Thermophilic fungi are attractive to industry because they can potentially be developed into platforms for the production of chemicals and materials at elevated temperatures. Enzymes from thermophilic fungi tend to be more thermostable than enzymes from mesophilic fungi (Margaritis and Merchant, 1986). *M. thermophila*, basionym *Sporotrichum thermophile* (Apinis, 1963), in particular has received substantial attention in the discovery of lignocellulolytic enzymes. Cellulolytic activity from *M. thermophila* has been shown to be many times higher than enzymes from the most active mesophilic fungi (Berka et al., 2011; Bhat and Maheshwari, 1987). This organism has been used to identify novel lignocellulolytic activities; for example, extracellular aldonolactonase and glucuronyl esterase (Beeson et al., 2011; Topakas et al., 2010). A cellulase from *M. thermophila* has been shown to be effective in the liquefaction of agricultural straw (Karnaouri et al., 2014). Laccase from this organism has been used to delignify plant-derived biomass (Rico et al., 2014). Furthermore, *M. thermophila* has been developed into a platform for industrial enzyme production (Visser et al., 2011). Along with *T. terrestris*, *M. thermophila* is the first filamentous fungus with a finished genome (Berka et al., 2011). Sequence analysis revealed that *M. thermophila* possesses a large repertoire of genes encoding lignocellulolytic enzymes. Like other species in the Chaetomiaceae family, *M. thermophila* harbours an expanded family of genes encoding polysaccharide monooxygenases, 23 genes compared to three genes in *T. reesei* (Berka et al., 2011).

Combined transcriptome and exoproteome approaches have been used to study fungal deconstruction of plant-derived biomass (Berka et al., 2011; Eastwood et al., 2011; Martinez et al., 2009; Vanden Wymelenberg et al., 2009; Vanden Wymelenberg et al., 2010). In these studies, transcriptome analyses were used to evaluate quantitatively the regulation of biomass-degrading enzymes while exoproteome analyses were mainly confined to the identification of extracellular proteins. We have previously used genome-wide transcriptome and exoproteome approaches to examine the utilization of barley and alfalfa straws (Berka et al., 2011). A wide variety of lignocellulosic biomass is expected to be used to fuel future industry. To gain insights into the combinations of enzymes needed to effectively deconstruct lignocellulosic biomass, in this study we have expanded the transcriptome analysis to agricultural straws from canola, flax, oat and triticale. We have examined the exoproteomes of *M. thermophila* grown in these substrates as well as in pretreated corn stover, hardwood and softwood pulps. We attempt to integrate transcriptome data and exoproteome results generated by mass spectrometry-based semi-quantitative proteomics analysis. Since the molecular function of many of the genes that are differentially regulated under these culture conditions are not known or poorly characterized, we focus our analysis on the CAZymes.

2. Materials and methods

2.1. Data source

Genomic sequence and functional annotation information were obtained from *M. thermophila* Annotation version v2.0 (http://www.jgi.doe.gov) and as described (Berka et al., 2011). In some cases, additional analysis was carried out by comparison with characterized lignocellulose-active enzymes catalogued in the *Mycoclap* database (Murphy et al., 2011).

2.2. Cultivation of M. thermophila and sample preparation

Conidial suspensions were prepared by growing *M. thermophila* on yeast-starch agar [YpSs; 0.4% yeast extract, 1.5% soluble starch, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 1.5% agar, pH 7.0] for 5 days at 45 °C in the dark. Spores were collected using 0.02% Tween 80/0.5% saline solution and counted using a hemacytometer.

To culture in liquid suspensions, conidia at 10^6 spores/mL were inoculated in 10× TDM (Roy and Archibald, 1993) containing 2% carbon source and incubated at 45 °C with shaking at 150 rpm. Agricultural straws were gifts from Agriculture and Agri-Food Canada (Saskatoon, Saskatchewan and Lethbridge, Alberta), softwood and hardwood pulps from FPInnovations (Pointe-Claire, Québec), and pretreated corn stover from the National Renewable Energy Laboratory (Golden, Colorado). The straws were ground to 0.5 mm before use. For transcriptome analysis, mycelia were harvested after 16–24 h of growth by filtering through Miracloth (Calbiochem, San Diego, CA, USA), ground in liquid nitrogen, and total RNA was extracted with Trizol (Invitrogen) as described (Semova et al., 2006).

To obtain extracellular proteins for the substrates comparison experiment, mycelia were cultured as described above but grown without shaking to minimize cell lysis and the subsequent release of intracellular proteins into the extracellular medium. Samples were collected at late growth phase, which corresponds to 40 h of growth for all samples with exceptions for PCS and SWMP which were harvested at 46 and 64 h respectively. Mycelia were removed by centrifugation at 2500*g* for 30 min at 4 °C and further clarified by centrifugation at 37,500*g* for 1 h at 4 °C. For the time course experiment, mycelia were grown, starting from conidia, for 20 h in 2% fructose and washed with water through Miracloth. One gram of mycelia was transferred to a 500-ml Erlenmeyer flask containing 50 ml of media containing 1% of carbon source and grown with shaking at 250 rpm. Samples were taken 4 h, 8 h, 24 h and 48 h after transfer for preparation of extracellular proteins.

2.3. Transcriptome analysis

The transcriptome data from *M. thermophila* cultured in glucose, barley straw and alfalfa straw used for comparative analysis was

Please cite this article in press as: Kolbusz, M.A., et al. Transcriptome and exoproteome analysis of utilization of plant-derived biomass by *Myceliophthora thermophila*. Fungal Genet. Biol. (2014), http://dx.doi.org/10.1016/j.fgb.2014.05.006