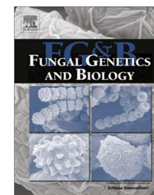
ARTICLE IN PRESS

Fungal Genetics and Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Fungal Genetics and Biology

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



Carbohydrate-related enzymes of important *Phytophthora* plant pathogens

Henk Brouwer [a,1], Pedro M. Coutinho [b], Bernard Henrissat [b,c], Ronald P. de Vries [a,d,*]

[a] CBS-KNAW, Fungal Biodiversity Centre, Uppsalalaan 8, Utrecht 3584 CT, The Netherlands
[b] Architecture et Fonction des Macromolecules Biologiques, UMR7257, CNRS, Univ. Aix-Marseille I & II, 163 Avenue de Luminy, 13288 Marseille, France
[c] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Fungal Molecular Physiology, Utrecht University, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 7 April 2014
Revised 15 August 2014
Accepted 25 August 2014
Available online xxxx

Keywords:
Phytophthora
Pythium
Carbohydrate-related-enzymes
Growth profiling
Carbon source utilization

ABSTRACT

Carbohydrate-Active enZymes (CAZymes) form particularly interesting targets to study in plant pathogens. Despite the fact that many CAZymes are pathogenicity factors, oomycete CAZymes have received significantly less attention than effectors in the literature. Here we present an analysis of the CAZymes present in the *Phytophthora infestans*, *Ph. ramorum*, *Ph. sojae* and *Pythium ultimum* genomes compared to growth of these species on a range of different carbon sources. Growth on these carbon sources indicates that the size of enzyme families involved in degradation of cell-wall related substrates like cellulose, xylan and pectin is not always a good predictor of growth on these substrates. While a capacity to degrade xylan and cellulose exists the products are not fully saccharified and used as a carbon source. The *Phytophthora* genomes encode larger CAZyme sets when compared to *Py. ultimum*, and encode putative cutinases, GH12 xyloglucanases and GH10 xylanases that are missing in the *Py. ultimum* genome. *Phytophthora* spp. also encode a larger number of enzyme families and genes involved in pectin degradation. No loss or gain of complete enzyme families was found between the *Phytophthora* genomes, but there are some marked differences in the size of some enzyme families.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Only very few genera of plant pathogens are as well-known to the general public as *Phytophthora*. *Phytophthora infestans* is the subject of both history and biology classes as the cause of the destructive potato late blight that caused mass starvation and emigration in Ireland in the 1840s (Erwin and Ribeiro, 1996; Ristaino, 2002). The economic and ecological significance of the genus however extends far beyond the impact of its type species *Ph. infestans*. The genus consists of over a hundred currently recognized species (Kroon et al., 2012). International plant trade has spread many *Phytophthora* species beyond their natural area of origin. Some of these invasive species, like *Ph. ramorum* in Europe and North America, and *Ph. cinnamomi* in Australia cause great damage to the ecosystems they invade (Hansen, 2008).

The study of the genus *Phytophthora* has entered a new era with the publication of full genome sequences for *Ph. ramorum, Ph. sojae*, (Tyler et al., 2006) *Ph. infestans* (Haas et al., 2009) and more recently for *Ph. capsici* (Lamour et al., 2012), and *Ph. lateralis* (Quinn et al., 2013). In addition, genomic sequences of related genera have also become available including necrotrophic plant pathogens belonging to the genus *Pythium*: *Py. ultimum* var. *ultimum* (Lévesque et al., 2010), *Py. ultimum* var. *sporangiiferum*, *Py. aphanidermatum*, *Py. arrhenomanes*, *Py. irregulare*, *Py. iwayamai*, and *Py. vexans* (Adhikari et al., 2013) and the obligate biotrophic oomycete pathogens *Hyaloperonospora arabidopsis*, *Albugo laibachii*, and *A. candida* (Baxter et al., 2010; Kemen et al., 2011; Links et al., 2011). The genome of the oomycete fish pathogen *Saprolegnia parasitica* has also been published (Jiang et al., 2013). Robideau et al. (2011) recently published a phylogeny of the different genera in the oomycetes.

Carbohydrate-Active enZymes (CAZymes, www.cazy.org, (Cantarel et al., 2009; Lombard et al., 2014) form a particularly interesting target for study in plant pathogens. Similar to other organisms CAZymes play an important role in the metabolism of oomycete plant pathogens for example to synthesize and modify structural components of the cell wall and storage glucans (Bartnicki-Garcia, 1968; Wang and Bartnicki-Garcia, 1974). Of greater interest however are the CAZymes that interact with the

* Corresponding author at: CBS-KNAW, Fungal Biodiversity Centre, Uppsalalaan 8, Utrecht 3584 CT, The Netherlands.
E-mail address: r.devries@cbs.knaw.nl (R.P. de Vries).
[1] Current address: CTGB, Stadsbrink 5, Wageningen 6707AA, The Netherlands.

http://dx.doi.org/10.1016/j.fgb.2014.08.011
1087-1845/© 2014 Elsevier Inc. All rights reserved.

Please cite this article in press as: Brouwer, H., et al. Carbohydrate-related enzymes of important *Phytophthora* plant pathogens. Fungal Genet. Biol. (2014), http://dx.doi.org/10.1016/j.fgb.2014.08.011

plant host. Plant pathogens deploy an arsenal of enzymes for this purpose (Cantu et al., 2008; de Vries and Visser, 2001). Aside from interactions at a molecular level, plants are defended from pathogens by a physical barrier, the plant cell wall. This barrier can only be partly overcome by mechanical action i.e., formation of appressoria (Grenville-Briggs et al., 2008). CAZymes are needed to break down, or weaken the cell wall to facilitate successful infection of the host. Finally, the pathogen uses the host as a nutrient source and uses CAZymes to digest plant storage compounds and other polysaccharides (Lévesque et al., 2010; de Vries and Visser, 2001).

Plant cell walls consists for a large part of carbohydrates. These include cellulose, hemicellulose and pectin (McNeil et al., 1984). Cellulose is simple in structure and consists of long chains of β-1,4-linked D-glucose residues. Bundled together these polymers form microfibrils that can be linked together by hemicelluloses. Hemicelluloses are subdivided in several classes according to the main sugar in the backbone, and include xylan, xyloglucan, mannan, and galactomannan. Hemicelluloses often have many side groups and are more complex in structure when compared to cellulose. Pectin is a highly variable heteropolysaccharide and is most prominent in the middle lamella and primary cell wall (de Vries and Visser, 2001; Caffall and Mohnen, 2009).

CAZymes are divided into several classes: Glycoside Hydrolases (GH), Glycosyltransferases (GT), Polysaccharide Lyases (PL), Carbohydrate Esterases (CE) and Auxiliary Activities (AA). Within these broad classes, CAZymes can be further categorized in sequence-based families (eg. GH30, CE7) and sometimes subfamilies, eg GH5_1, GH5_2). CAZymes can have a modular architecture: in addition to one or more CAZyme modules from the categories above, they can have signal peptides, GPI anchors or other modules that are involved in targeting and/or retention of the enzyme at locations inside or outside the cell. An interesting category of accessory modules are the Carbohydrate-Binding Modules (CBM) which do not have any catalytic action, but promote the binding of the enzyme to a range of substrates.

CAZymes have been intensively studied in ascomycete species that are used in industrial processes, like *Aspergillus* spp. (Culleton et al., 2013), *Trichoderma reesei* (Schmoll and Kubicek, 2003) and *Myceliophthora thermophila* (Berka et al., 2011).

In contrast, in oomycetes CAZymes have received significantly less attention. The recent publication of oomycete genomes (Tyler et al., 2006; Haas et al., 2009; Lévesque et al., 2010) provides us with an opportunity to study the differences in CAZyme repertoires between related species from taxa outside of the ascomycetes and their consequences. Oomycetes form a particulary interesting group because of their distant relationship to fungi. While oomycetes were originally considered part of the fungal kingdom, they are in fact a separate class of organisms and are currently classified under the Stramenopiles in the "super kingdom" Chromalveolata. Similarities in morphology and other characteristics are the result of convergent evolution (Cavalier-Smith and Chao, 2006; Beakes et al., 2012). As a consequence, most oomycete CAZymes are only distantly related to biochemically characterized enzymes. Reliability of functional annotation depends on the distance to enzymes that have been functionally characterized. In addition, for enzyme families with broad or variable substrate specificity, high sequence-similarity may not be proof of a similar function. Functional annotation of CAZymes can therefore be challenging for non-specialists, in particular for organisms such as the oomycetes.

In 2010 the genome of *Py. ultimum* was published (Lévesque et al., 2010), for which we used the CAZy pipeline for annotation of the CAZyme content of the genome (Cantarel et al., 2009; Lombard et al., 2014). The analysis of the CAZyme content of the *Py. ultimum* genome showed some marked differences between *Py. ultimum* and *Phytophthora* genomes. *Py. ultimum* did not contain cutinases, and its xylan degrading capacity was limited to absent. The *Py. ultimum* genome was also significantly smaller than that of *Phytophthora* spp. (Lévesque et al., 2010). Carbohydrate-active enzymes of *Phytophthora* spp. have been analyzed using different annotation pipelines (Ospina-Giraldo et al., 2010a; Zerillo et al., 2013), giving highly variable results. Here, we have analyzed growth profiles for the sequenced strains of *Ph. infestans*, *Ph. ramorum* and *Ph. sojae* and *Py. ultimum* and a number of other representative isolates from these species on a range of different carbon sources. The growth profiles were compared to an analysis of the *Ph. infestans, Ph. ramorum* and *Ph. sojae* and *Py. ultimum* genomes using the CAZy pipeline, allowing for a better interpretation and comparison of the CAZyme content of these genomes.

2. Material and methods

2.1. Strains and culture conditions

Abbreviations for taxa in this document differ from standard abbreviation protocol in that *Pythium* is abbreviated to *Py*., and *Phytophthora* to *Ph*., to make it easier to distinguish between the two taxa. The isolates used in this study are listed in Table 1. The *Ph. infestans* and *Ph. sojae* strains, *Ph. ramorum* strain PR102 and *Py. ultimum* strain DAOM BR144 correspond to the isolates for which the genome has been sequenced (Tyler et al., 2006; Haas et al., 2009; Lévesque et al., 2010). *Ph. ramorum* strain CBS 101553 and *Py ultimum* CBS 398.51 are the ex-type strains for their species, and as such represent well characterized isolates.

Growth on different carbon sources was compared using Minimal Medium (MM), which consisted of (per liter): 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.4 mg $MnSO_4$, 0.4 mg $ZnSO_4$, 1.05 g NH_4Cl, 6.8 ml 1 M $CaCl_2{\cdot}2H_2O$, 2 ml 1 M $MgSO{\cdot}7H_2O$, 4 mg $FeSO_4$, 1 mg thiamine and 1% (w/v) agarose. Thiamine was omitted from medium used to grow *Py. ultimum.*

Carbon sources were added to MM at the following concentrations: 1% (w/v) for cellulose, soluble starch, citrus pectin and birchwood xylan and 25 mM for D-glucose, D-fructose, D-xylose, cellobiose, sucrose and L-arabinose. The pH of the medium was adjusted to 6.0 and the medium was autoclaved at 121 °C for 20 min. $CaCl_2$ and $MgSO_4$ were autoclaved separately from the rest of the medium and monosaccharides, $FeSO_4$ and thiamine were filter sterilized (Whatman 0.2 μm millipore filter, Dassel, Germany).

The strains were initially grown on Potato Carrot Agar (Gams et al., 1998). A small agar plug containing mycelium (1 mm diameter) was transferred from the edge of a vigorously growing 1-day-old colony to the center of the Petri dishes with the different media. The cultures were incubated in the dark at 21 °C. Mycelium density and colony diameter were measured daily for the first 5 days and again after 7 days. Colony morphology pictures were taken, and pH was measured after 7 days. The growth test was performed twice for each strain. Mycelium density was measured by

Table 1
Isolates used in this study.

Species	Strain ID	Alternative strain ID
Py. ultimum	DAOM B144[a]	CBS 805.95
Py. ultimum	CBS 398.51[b]	
Ph. ramorum	CBS 101553[b]	
Ph. ramorum	PR102[c]	CBS 126586
Ph. sojae	P6497[c]	CBS 125701
Ph. infestans	T30-4[d]	CBS 120920

[a] Sequenced strain (Lévesque et al., 2010).
[b] Ex-type strain.
[c] Sequenced strain (Tyler et al., 2006).
[d] Sequenced strain (Haas et al., 2009).

Download English Version:

https://daneshyari.com/en/article/8470839

Download Persian Version:

https://daneshyari.com/article/8470839

Daneshyari.com