CAZYME REPERTOIRE AND SUGAR METABOLIC PATHWAY OF *Kretzschmaria zonata*

Mariana R. A. Carmo[1], Túlio Morgan[1], Lucas F. Almeida[1*], Rafael F. Alfenas[2] & Gabriela P. Maitan-Alfenas[1]

[1]*Departament of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, Brazil.*
[2]*Departament of Phytopathology, Federal University of Viçosa, Viçosa, Brazil.*
** Corresponding author's email address: lucas.filipe@ufv.br*

ABSTRACT

Phytopathogenic fungi are capable of degrading plant cell walls, secreting several carbohydrate-active enzymes (CAZymes). Polysaccharides degradation allows host infection fungi growth. The large number of fungi found in the most diverse environments contributes to great genetic variability, reflecting a wide diversity of produced enzymes. We evaluated the diversity of CAZymes secreted by *Kretzschmaria zonata* and its sugar metabolic pathway. The repertoire of genes encoding plant cell wall degrading enzymes was broad and showed to be more similar to *Aspergillus niger*, which is a Eurotiomycetes, than to the fungi *Trichoderma reesei* and *Neurospora crassa,* that are Sordariomycetes. When compared with phylogenetically close fungi, the CAZymes profile was similar in number and diversity of enzymes, however when compared to other phytopathogenic fungi the difference was more obvious, showing similarity with the fungus *Cryphonectria parasitica* but great discrepancy with *Bretziella fagacearum* and *Ophiostoma novo-ulmi*. For all the fungi selected for this study, at least one copy of genes-encoding enzymes that catalyze the main reactions of the metabolic pathways were identified. *K. zonata*, possesses a complete metabolic network, except for an absent gene in the galacturonic acid metabolic pathway, and it showed to have a more extensive set of metabolic genes than *T. reesei*.

Keywords: Plant cell wall. Enzymes. Phytopathogenic fungi. Sugar metabolism.

1 INTRODUCTION

The plant cell wall is a rigid barrier composed of a complex network of polysaccharides and other compounds, such as lignin providing mechanical strength and defense against microbial infection[1]. In nature, enzymes from plant pathogenic fungi have shown the ability to degrade the plant cell wall. This process involves an arsenal of cell wall-degrading enzymes (CWDE) that are usually secreted by these fungi, and there is a close relationship between the fungal pathogenicity or virulence and its ability to secrete CWDE[2]. Cell wall degradation occurs to allow the pathogen to penetrate the host, cause the infection, and obtain essential nutrients for its survivalthrough a variety of sugar catabolic pathways[2;3]. Overall, phytopathogenic and non-phytopathogenic fungi show different modes of action in the plant cell wall decomposition, since they show significant differences in the number and diversity of secreted CWDE that are directed by the genetic background.

During the degradation of the plant cell wall process, the complex polysaccharides in this structure that cannot be taken into the cell in this polymeric format are degraded into various monosaccharides used by the fungus metabolism to obtain energy. The monomers are taken up by cells and converted by a variety of sugar catabolic pathways into the compounds needed by the fungal cells for their growth[3;4]. Thus, the cell wall degradation is closely connected to the metabolic pathways of the fungus. Therefore, the investigation and identification of the main enzymes involved in sugar metabolic networks are very important for a better understanding of the enzymatic profile, as well as its growth under specific cultivation conditions and the fungal behavior on its natural habitat, which can contribute to improving their use for industrial applications[5;3].

Many enzymes are required for complete plant cell wall degradation, most of which are classified in the Carbohydrate-Active enzymes (CAZy) database[6].CAZymes can be related to the breakdown, biosynthesis, or modification of glycoconjugates, oligo- and polysaccharides. The interaction between a phytopathogenic fungus and the host occurs mainly by the action of the CAZymes in the plant cell wall; therefore, the identification and characterization of these enzymes on a molecular level is of great importance[7;8;9].

In this study, we report the profile of CAZyme-coding genes and the sugar metabolic network of the phytopathogen fungus *Kretzschmaria zonata*. The information shown here provides knowledge of the outstanding ability of *K. zonata* to decompose plant cell wall polysaccharides, aiming to increase the efficiency of existing industrial processes to generate sustainable chemicals from renewable materials.

2 MATERIAL & METHODS

CAZyme identification was generated by the Carbohydrate-Active enzymes database team according to their annotation pipeline[10]. Numbers of genes per CAZy family for the selected fungi were obtained from Mycocosm[11] and manually assigned to the substrate they act on, as previously described [12].

Orthologous SMGs were identified using OrthoFinder[13]. The protein sequences of the fungal species were downloaded from the JGI MycoCosm Portal[11] as input. The OrthoFinder method provides a fast, accurate and comprehensive platform to infer the complete set of orthologs between selected species based on the phylogenetic information from the ortho-group tree. OrthoFinder was performed using default parameters with DIAMOND[14] for sequence similarity searches and Dendro-BLAST for the tree inference of orthogroups. The identified orthologs were then porjected on the previously generated models of *A. niger* and *T. reesei* [3;5], to generate metabolic models for each species.

For the growth profile, the strain *Kretzschamria zonata* was grown on minimal medium (MM), on 1.5 % (w/v) agar plates with one of the eight monosaccharides (D-glucose, D-fructose, D-galactose, D-mannose, D-xylose, L-arabinose, D-rhamnose and D-glucuronic acid) or one of the six polysaccharides (xylan, galactomannan, starch, cellulose, inulin and pectin). Growth was performed at 28 °C. Plates containing only minimal medium, without carbon source, were used as controls. Growth was scored based on colony diameter and mycelium density. Growth was performed in duplicate, and no variation was observed between the duplicates for any tested carbon source.

3 RESULTS & DISCUSSION

The comparison between *Kretzschmaria zonata* and other fungi was performed, and these fungi were divided into three different groups.The lifestyle of fungi was also considered for comparison. Group I contained phylogenetically close species with different lifestyle: *Kretschmaria deusta* IL1129, *Kretschmaria deusta* CBS 826.72, *Xylaria cf. heliscus* FL0509, and *Hypoxylon submonticulosum* NC0708. Group II contained only phylogenetically distant phytopathogenic fungi, such as *Ophiostoma novo-ulmi* subsp. *novo-ulmi* H327, *Cryphonectria parasitica* EP155 v2.0 and *Bretziella fagacearum* C519 v1.0. Finally, group III contained fungi known as good producers of CWDE, *A. niger* and *T. reesei* QM6a that are saprobic. The fungus *N. crassa* was also used for comparison because it is a well-characterized species among filamentous fungi and also a member of the Sordariomycetes, although taxonomically distant from *K. zonata*. All of these fungi belong to the Ascomycota phylum.

Figure 1 shows a comparison based in substrates of the CAZyme distribution considering the main families involved in the CWDE of the phytopathogenic fungus *K. zonata* GFP 132 with the previously selected fungi divided into three groups. It is observed that in group I, very similar profiles were obtained in relation to the main CAZyme families involved in the cellulose, xylan, xyloglucan, and pectin degradation, and the number and diversity of enzymes. This similarity suggests that high gene conservation was maintained in taxonomically close fungi. Despite this, some fungi in this group have different lifestyles, some are pathogenic and others are saprobic, and the profile was similar, suggesting that a different lifestyle among phylogenetically close fungi does not change the CAZyme content. In group II, phytopathogenic fungi that are phylogenetically distant from *K. zonata* a large variation in the CAZyme profile was observed, suggesting that a similar lifestyle of taxonomically distant organisms is not accompanied by a similar CAZyme genome content. Regarding group III, *Kretzschmaria zonata* showed interesting differences when compared with *A. niger*, *N. crassa* and *T. reesei*. The *K. zonata* obtained a greater number of CAZyme-encoding genes than *T. reesei* and *N. crassa*, which belong to the same class as *K. zonata*, and more closely resembling the profile presented by *A. niger*, which belongs to a different class than other fungi. *K. zonata* also stood out in this group in the number of genes encoding auxiliary enzymes that act in cellulose degradation and in the diversity of putative enzymes that act in the xylan depolymerization. In this context, the study demonstrates that the CAZy profile of the fungus *K. zonata* belonging to the *Xylariaceae* family can be an interesting source of enzymes for biotechnological application.

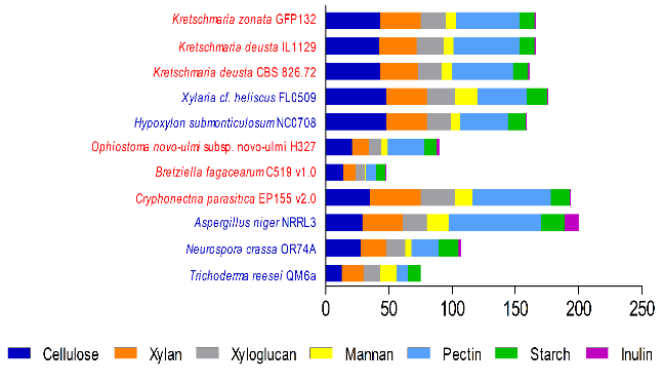

Figure 1 - Distribution of CAZyme genes based on substrate in the genome of the selected fungi based on plant biomass degradation. Phytopathogenic fungi are highlighted in red and saprobes are in blue.

The fungus was able to grow on practically all the tested carbon sources, with very similar growth among the monosaccharides, except for galacturonic acid where no growth was observed. Concerning the polysaccharides, the greatest growth for pectin and celulose corroborates with the number of genes predicted for the fungus, 50 and 43 predicted genes for pectin and cellulose degradation, respectively, being the polysaccharides with the most predicted genes. The low growth in inulin can be explained by the presence of only 1 gene predicted for the degradation of this polysaccharide.

The sugar metabolic model revealed that the fungus *K. zonata* possesses genes for most of the main pathways, except for the galacturonic acid catabolism pathway, which may explain the non-growth on this monosaccharide. *K. zonata* showed the most similar profile to the *A. niger* and when compared to the other selected fungi that were used in the study, it was one of those that showed a more complete metabolic network (Figure 2).

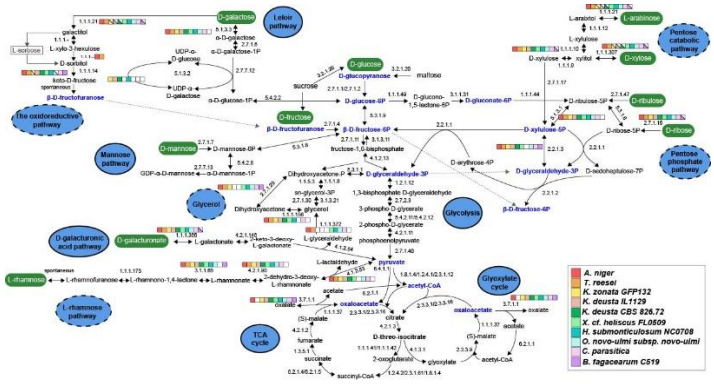

Figure 2 - Sugar metabolic networks of *K. zonata, K. deusta, X. xf. heliscus*, *H. submonticulosum, O. novo-ulmi subsp. novo-ulmi, C. parasítica and B. fagacearum.* The names of the sugar metabolic pathways are shown in blue circles. Dashed lines connect metabolites from different pathways. The genes that were present in two orthogroups and had differences are represented as a half-colored square.

4 CONCLUSION

This study showed the diversity of the CAZyme-coding genes secreted by the fungus *K. zonata* and the sugar metabolic pathway. Its enzymatic profile was more similar to that of the fungus *A. niger*, which belongs to a different class, than the profiles from *T. reesei* and *N. crassa*, which are Sordariomycetes, such as the fungus of interest. This result suggests that *K. zonata* has a more generalistic approach concerning carbon utilization. The fungus also stood out for possessing the genes for all the metabolic pathways of the main sugars, except for galacturonic acid. It also showed more complete pathways than other fungi which are already well studied and used in industrial processes such as *T. reesei*. These results improve knowledge about the enzymatic profile of *K. zonata*, which is rich in CAZymes like the Eurotiomycetes fungi, its mechanisms for degrading plant biomass, and its growth profile, aiming at its enzymes application in industrial processes.

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