The synergistic actions of hydrolytic genes in coexpression networks reveal the potential of *Trichoderma harzianum* for cellulose degradation

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Abstract

Background: Bioprospecting key genes and proteins related to plant biomass degradation is an attractive approach for the identification of target genes for biotechnological purposes,

especially genes with potential applications in the biorefinery industry that can enhance second-generation ethanol production technology. *Trichoderma harzianum* is a potential candidate for cellulolytic enzyme production. Herein, the transcriptome, exoproteome, enzymatic activities of extracts, and coexpression networks of the *T. harzianum* strain CBMAI-0179 under biomass degradation conditions were examined.

Results: We used RNA-Seq to identify differentially expressed genes (DEGs) and carbohydrate-active enzyme (CAZyme) genes related to plant biomass degradation and compared them with genes of strains from congeneric species (*T. harzianum* IOC-3844 and *T. atroviride* CBMAI-0020). *T. harzianum* CBMAI-0179 harbors species- and treatment-specific CAZyme genes, transporters and transcription factors. Additionally, we detected important proteins related to biomass degradation, including β-glucosidases, endoglucanases, cellobiohydrolases, lytic polysaccharide monooxygenases (LPMOs), endo-1,4-β-xylanases and β-mannanases, in the exoproteome under cellulose growth conditions. Coexpression networks were constructed to explore the relationships among the genes with corresponding secreted proteins that act synergistically for cellulose degradation. An enriched cluster with degradative enzymes was described, and the subnetwork of CAZymes showed linear correlations among secreted proteins (AA9, GH6, GH10, GH11 and CBM1) and differentially expressed CAZyme genes (GH45, GH7, AA7 and GH1).

Conclusions: Our results provide valuable information for future studies on the genetic regulation of plant cell wall-degrading enzymes. This knowledge can be exploited for the improvement of enzymatic reactions to degrade plant biomass, which is useful for bioethanol production.

Keywords: *Trichoderma harzianum*, Cellulose, RNA-Seq, CAZymes, Exoproteome, Coexpression networks

Background

The expanding worldwide demand for renewable and sustainable energy sources has increased the interest in alternative energy sources, and the production of second-generation biofuels seems to be the most viable option to confront these issues [1, 2]. Lignocellulosic biomass is the most abundant renewable organic carbon resource on earth, consisting of three major polymers, cellulose, hemicellulose, and lignin [2]. However, due to its recalcitrant characteristics that prevent enzyme access, degrading this complex matrix is still a major challenge [3]. For the complete hydrolysis of lignocellulose, a variety of enzymes acting in synergy are required, and much research has focused on this topic in recent decades [4]. Interactions between different enzymes have been investigated to identify optimal combinations and ratios of enzymes for efficient biomass degradation, which are highly dependent on the properties of the lignocellulosic substrates and the surface structure of cellulose microfibrils [4, 5]. Due to their abundance in nature, microorganisms are considered natural producers of enzymes, and many of them, including members of both bacteria and fungi, have evolved to digest lignocellulose [6, 7]. The search for microorganisms that are able to efficiently degrade lignocellulosic biomass is pivotal for the establishment of the sustainable production of bioethanol [8].

Filamentous fungi, including the genera *Trichoderma*, *Aspergillus*, *Penicillium* and *Neurospora*, produce extracellular proteins that act synergistically to degrade plant cell walls and are widely used in the enzymatic industry [9]. Species in the filamentous ascomycete genus *Trichoderma* are among the most commonly isolated saprotrophic fungi [10] and are important from a biotechnological perspective [7]. *Trichoderma* species are widely used in agriculture as biocontrol agents due their ability to antagonize plant-pathogenic fungi and in industry as producers of plant cell wall-degrading enzymes [11-14]. In addition, *Trichoderma*

species are easily isolated from soil and decomposing organic matter [15]. Within the *Trichoderma* genus, *T. reesei* is the most intensively studied species [16]. *T. reesei* is a well-known producer of cellulase and hemicellulase, and due to the high effectiveness of the synergistic cellulases in this species, it is widely employed in industry, and technologies for its use and handling are based on seventy years of experience [8, 16-19]. However, studies on *T. harzianum* strains have shown their potential to produce a set of enzymes that can degrade lignocellulosic biomass [20-23]; therefore, *T. harzianum* strains are being investigated as potentially valuable sources of industrial cellulases [6].

The identification of carbohydrate-active enzymes (CAZymes) that act synergistically under biodegradation conditions [4] has the potential to improve the enzymatic hydrolysis process by optimizing and reducing bioethanol costs. The CAZy database (www.cazy.org) classifies CAZymes into six major groups: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAs), and carbohydrate-binding modules (CBMs) [24]. CAZymes are extensively used for the genetic classification of important hydrolytic enzymes [22, 25].

The conversion of cellulose to glucose involves the synergistic action of three principal groups of enzymes: endo-β-1,4-glucanases (EC 3.2.1.4), β-glucosidases (EC 3.2.1.21), and cellobiohydrolases (EC 3.2.1.91/176) [20, 26]. For hemicellulose hydrolysis, several enzymes are needed, such as endo-1,4-β-xylanases (EC 3.2.1.8), β-xylosidases (EC 3.2.1.37), β-mannanases (EC 3.2.1.78), arabinofuranosidases (EC 3.2.1.55), and acetylxylan esterases (EC 3.1.1.72) [26, 27]. In addition, a number of auxiliary enzymes are involved in this process, such as lytic polysaccharide monooxygenases (LPMOs), cellulose-induced protein 1 and 2 (CIP1 and CIP2) and swollenin, which can increase the hydrolytic performance of enzymatic cocktails used in industry for bioethanol production [6, 28-30].

As genetic variation occurs within species [31, 32], understanding and exploring the genetic mechanisms of different *T. harzianum* strains can provide valuable information for industrial applications. In the present study, we analyzed the enzymatic activity, transcriptome and exoproteome of *T. harzianum* CBMAI-0179 and compared them with those of other *Trichoderma* strains (*T. harzianum* IOC-3844 and *T. atroviride* CBMAI-0020). RNA-Seq analysis was performed to construct coexpression networks, providing novel information about the potential of this *T. harzianum* strain for biotechnological applications. Our findings provide insights into the genes/proteins that act synergistically in plant biomass conversion and can be exploited to improve enzymatic hydrolysis and thereby increase the efficiency of the saccharification of lignocellulosic substrates for bioethanol production.

Results

Transcriptome analysis of Trichoderma *spp. under cellulose growth conditions*

The present study represents the first deep genetic analysis of Th0179, describing and comparing the transcriptome by RNA-Seq under two different growth conditions, cellulose and glucose, to identify the genes involved in plant biomass degradation. Reads were mapped against reference genomes of *T. harzianum* and *T. atroviride*, generating 96.3, 111.8 and 133.3 million paired-end reads for Th0179, Th3844 and Ta0020, respectively. To establish the degrees of similarity and difference in gene expression among strains and between treatments, a principal component analysis (PCA) was performed using the *T. harzianum* T6776 genome as a reference. The PCA results showed clustered groups with higher similarity between treatments than among strains. The transcriptomes of Th0179 and Th3844

were more similar to each other than to the transcriptome of Ta0020 (Fig. 1a), showing that it is possible to capture differences among the three strains.

Venn diagrams of the genes exhibiting expression levels greater than zero were constructed based on the similarities among the genes from all strains, thus showing the species-specific genes expressed under both conditions (Additional file 4: Fig. S3). Through the transcriptome analysis of the strains, we identified 11,250 genes exhibiting expression levels greater than zero under cellulose growth conditions and 11,235 genes exhibiting expression levels greater than zero under glucose growth conditions. The number of genes shared by Th0179 and Th3844 was higher under both conditions than that shared by either Th0179 or Th3844 and Ta0020. Th0179 exhibited the highest number of unique genes, with 374 and 168 unique genes under the cellulose and glucose growth conditions, respectively. Among these unique genes under cellulose growth conditions, we found major facilitator superfamily (MFS) transporters (THAR02_00234, THAR02_00911, THAR02_03251, THAR02_03935, THAR02_07021, THAR02_07705 and THAR02_07942), an ATP-binding cassette (ABC) transporter (THAR02_09958), a drug resistance protein (THAR02_04837), a fungal specific transcription factor (TF) (THAR02_07743), and a C2H2 TF (THAR02_11070). Among the genes that were upregulated in cellulose conditions relative to glucose conditions, 219 were identified for Th0179, 281 were identified for Th3844, and 718 were identified for Ta0020 (Fig. 1b).

CAZyme identification and distribution in* Trichoderma *spp.

For CAZymes important in the degradation of biomass, the set of CAZymes was identified by mapping all of the proteins of *T. harzianum* T6776 and *T. atroviride* IMI 206040 against the CAZy database using the BLASTp search tool. Based on the filtering criteria, a total of 631 proteins were retained as CAZyme genes for *T. harzianum*, which

corresponds to 5.5% of the total 11,498 proteins predicted for this organism [33], and 640 proteins were retained for *T. atroviride*, which corresponds to 5.4% of the total 11,816 proteins predicted for this organism [34].

Considering the DEGs under cellulose growth conditions and using the established CAZy database, we found 35, 78 and 31 differentially expressed CAZyme genes in our data (Fig. 1b) for Th0179, Th3844 and Ta0020, respectively. We identified the main CAZyme classes (AA, GH, GT, CE and CBM) and their contents for all strains (Fig. 1c). The Th3844 strain presented the highest number of classified genes from the AA family, a high number of CBMs and twice the number of identified GHs found in the two other strains. Strain Ta0020 presented a higher number of genes from the GT family than Th0179, which exhibited a higher number of classified genes from the CBM family than Ta0020. The differences regarding the specific CAZyme families for each strain are shown in Fig. 2.

The GH group was the most represented class of enzymes present in all evaluated strains. GHs are key enzymes for carbohydrate hydrolysis and include enzymes capable of degrading cellulose [35-37], with many able to cleave glycosidic bonds between glucose molecules. Another important family involved in degradation of the plant cell wall, the AA family, was also identified in all strains [38]. The AA class currently harbors 9 families of ligninolytic enzymes and 6 families of lytic polysaccharide mono-oxygenases that may not act on carbohydrates. However, because lignin is invariably and intimately associated with carbohydrates in the plant cell wall, these lignolytic enzymes cooperate with classical polysaccharide depolymerases, named auxiliary enzymes [24, 38], that differentially influence degradative activity among species.

Cellulose and hemicellulose degradative enzymes in* Trichoderma *spp.

In plant biomass degradation, a variety of enzymes working synergistically are required for complete hydrolysis [4]. Under cellulose growth conditions, we found upregulated families among the CAZymes responsible for cellulose degradation, including endoglucanases (GH5, GH12, GH45 and CBM1), β-glucosidases (GH1 and GH3) and cellobiohydrolases (GH6 and GH7), that play important roles in cellulose degradation. In addition, among the CAZymes responsible for hemicellulose degradation, we identified endo-1,4-β-xylanases (GH10, GH11 and CBM1), arabinofuranosidases (GH54, GH62 and CBM42) and acetylxylan esterases (CE5 and CBM1). Additionally, we found the copper enzymes LPMOs, classified as AAs in the family AA9 [39]; these are considered a breakthrough in the enzymatic degradation of cellulose because they oxidatively cleave glycosidic linkages that render substrates more susceptible to hydrolysis by conventional cellulases [28]. The expression levels in TPM of the main cellulase and hemicellulase families based on their principal enzyme activity present in Th0179, Th3844 and Ta0020 were evaluated using the RNA-Seq data (Fig. 3).

All of the GHs related to cellulose degradation were found in Th3844, including 2 AA9 members with a CBM1 module for cellulose binding. All GHs except GH12, including AA9/CBM1, were detected in Th0179. Only one gene belonging to the GH5 family (TRIATDRAFT_81867) with cellulase activity (EC 3.2.1.4) was observed in Ta0020 (48.12 TPM). The most highly expressed genes were cellobiohydrolases from the GH6/CBM1 (THAR02_04414 – 299.66 TPM for Th0179 and 986.25 TPM for Th3844) and GH7/CBM1 families (THAR02_08897 – 369.92 TPM for Th0179; THAR02_03357 and THAR02_08897 – 1876.89 TPM for Th3844) (Fig. 3a).

In addition, 5 families related to hemicellulose degradation were identified in Th3844, in which endo-1,4-β-xylanases (EC 3.2.1.8) from the GH11 family had the greatest expression levels (THAR02_02147, THAR02_05896, THAR02_08630 and THAR02_08858

– 1013.62 TPM). Endo-1,4-β-xylanase (EC 3.2.1.8) from the GH10 family was found in both *T. harzianum* strains (THAR02_03271 – 47.58 TPM for Th0179 and 741.35 TPM for Th3844). Acetylxylan esterases (EC 3.1.1.72) from the CE5/CBM1 family were found only in Th3844 (THAR02_01449 and THAR02_07663 – 227.56 TPM). We identified only one α-L-arabinofuranosidase (EC 3.2.1.55) from the GH54/CBM42 family (TRIATDRAFT_81098 – 18.07 TPM) in Ta0020, whereas for Th3844, three α-L-arabinofuranosidases from the GH54/CBM42 and GH62 families were identified (Fig. 3b). The Ta0020 strain showed the lowest number of genes and the lowest expression levels of the CAZyme families related to cellulose and hemicellulose degradation. The classification of the CAZyme genes along with their enzyme activities, fold change values, e-values, EC numbers and expression values (TPM) for Th0179 under cellulose fermentative conditions are described in Table 1. The corresponding information for the Th3844 and Ta0020 strains can be found in Additional file 5: Table S2.

[Insert Table 1 here]

Functional annotation of* T. harzianum *CBMAI-0179 in the presence of cellulose

The first functional annotation of the expressed sequences under cellulose fermentative conditions for Th0179 was performed based on GO terms (Fig. 4). A total of 7,718 sequences were annotated, which corresponds to 67.1% of the total 11,498 proteins predicted for this organism (*T. harzianum* T6776) [33]. Under the molecular function category, catalytic activity was the main annotated function, with 3,350 identified sequences. Other functions that were enriched under this category were hydrolase activity, nucleotide binding and TF activity, with 1,111, 899 and 277 sequences, respectively. For the biological process category, the main functions were metabolic process, with 3,607 sequences, and cellular process, with 2,575 sequences. In addition, within the biological process category,

574 sequences were identified as associated with transmembrane transport, and 66 sequences were identified as associated with regulation of catalytic activity. Compared with Th3844, Th0179 presented 163 more sequences related to catalytic activity, 53 more sequences related to hydrolase activity, and 15 more sequences associated with TF activity but 10 fewer sequences related to transmembrane transport, suggesting that these two strains have developed different functional regulation. Ta0020 presented fewer sequences related to any of the above functions than Th3844 and Th0179 except for TF activity, for which Ta0020 had 53 more sequences than Th0179.

Exoproteome and RNA-Seq data correlation of* T. harzianum *CBMAI-0179

Once the transcriptome was characterized, we analyzed the secreted proteins identified in the transcriptome profile of *T. harzianum* CBMAI-0179. A total of 64 proteins, which had been secreted and were present in the culture medium after 96 h of fermentation, were detected in the extracts. Of those, 32 proteins were present in the cellulose aqueous extract (Table 2), 12 were present only in the glucose aqueous extract, and 20 were present under both conditions (Additional file 6: Table S3). Among the 32 secreted proteins detected using cellulose as the carbon source, the main CAZyme families observed exclusively in this supernatant were among of the most important families for cellulose and hemicellulose degradation, such as β-glucosidases (EC 3.2.1.21), endo-β-1,4-glucanases (EC 3.2.1.4), LPMOs (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), endo-1,4-β-xylanases (EC 3.2.1.8), and β-mannanases (EC 3.2.1.78). Two genes from the GH3 (THAR02_00656 and THAR02_00890) and GH5/CBM1 (THAR02_04405 and THAR02_09719) families and one gene from the AA9/CBM1 (THAR02_02134) and GH6/CBM1 (THAR02_04414) families corresponded to the main group of secreted cellulases, whereas one gene from the GH10 (THAR02_03271) family and two genes from the GH11 (THAR02_02147 and

THAR02_05896) family corresponded to the main group of hemicellulases detected in the supernatant. In addition, a member of the GH5 cellulase family with β-mannanase activity (THAR02_03851) was identified. In addition to these secreted proteins, 8 other proteins were classified as uncharacterized proteins. We also identified hemicellulose-degrading enzymes in both extracts, such as α-L-arabinofuranosidase B (EC 3.2.1.55) and xylan 1,4-β-xylosidase (EC 3.2.1.37).

[Insert Table 2 here]

Correlating the exoproteome data with the transcriptome data under cellulose growth conditions, we observed the expression levels of genes that play important roles in plant biomass degradation. CAZyme genes showing high TPM values in cellulose included one cellulose 1,4-β-cellobiosidase (nonreducing end) (EC 3.2.1.91) from the GH6/CBM1 family (299.66 TPM), one cellulase (EC 3.2.1.4) from the AA9/CBM1 family (138.62 TPM) and one endo-1,4-β-xylanase (EC 3.2.1.8) from the GH11/CBM1 family (119.56 TPM). Two uncharacterized proteins (THAR02_02133 and THAR02_08479) with the CBM1 module of cellulose binding also showed increased expression levels under the cellulose condition. In contrast, the THAR02_00656 gene, which displays β-glucosidase (EC 3.2.1.21) activity and is from the GH3 family, had the lowest expression level (5.08 TPM) among the CAZyme genes related to biomass degradation, indicating that genes with low expression levels are also important for functional secreted proteins [40].

Coexpression networks for* T. harzianum *CBMAI-0179

The coexpression network was assembled for Th0179 and included all genes obtained from the mapping results using the *T. harzianum* T6776 genome as a reference under both conditions, and the DEGs and CAZyme genes were highlighted in the network (Fig. 5a). This network, constructed based on the expression level data (TPM), was composed of a total of

11,104 nodes with 153,893 edges. We detected 219 genes corresponding to the DEGs under cellulose growth conditions and 367 genes corresponding to the nodes under glucose growth conditions. The respective CAZyme genes from both conditions were also identified in this network, with 35 CAZymes under cellulose growth conditions and 28 CAZymes under glucose growth conditions. The DEGs tended to cluster in the top (DEGs in glucose) and bottom (DEGs in cellulose) parts of the network, reflecting the different regulation of degradative activities according to substrate.

A subnetwork was generated based only on the secreted proteins that were present in the cellulose aqueous extract and that had corresponding genes in the coexpression network (Fig. 5b). This subnetwork exclusively represented the genes and their closest related genes that are correlated to the proteins secreted. It was composed of 713 nodes and 6,124 edges, including the 32 genes that encode the secreted proteins. We also identified 39 DEGs under cellulose growth conditions, 8 DEGs under glucose growth conditions, 6 CAZyme genes related to cellulose degradation and 1 CAZyme gene under glucose growth conditions in this subnetwork. Among the CAZyme genes under cellulose growth conditions, the GH1 family (THAR02_02251 and THAR02_05432) with β-glucosidase activity, the GH7/CBM1 family (THAR02_08897) with cellulose 1,4-β-cellobiosidase activity, and the GH45/CBM1 family (THAR02_02979) with cellulase activity were found in this subnetwork. Despite the different functions of the related genes, it is predicted that these genes participate in the genetic regulation of the detected CAZymes/proteins and are important to the regulation of the hydrolytic system.

The cluster analysis classified 11,101 genes in 84 clusters (Additional file 7: Table S4). We identified an enriched cluster composed of 196 nodes and 2,125 edges (Fig. 5c and Additional file 7: Table S4) containing the greatest number of CAZyme genes among the clusters. Of the 12 CAZyme genes detected, 7 corresponded to the secreted proteins detected

in the cellulose aqueous extract, and 5 were differentially expressed. In addition, we identified 20 DEGs under cellulose growth conditions and 119 uncharacterized proteins in this cluster, showing a strong correlation with the CAZyme genes, which indicates that the unknown genes are important to the degradation process. The CAZyme genes from the cluster analysis were selected to generate a new subnetwork with their corresponding edges (Fig. 5d), showing linear correlations among secreted proteins (AA9, GH6, GH10, GH11 and CBM1) and differentially expressed CAZyme genes (GH45, GH7, AA7 and GH1).

Discussion

Several studies, including transcriptomic and proteomic studies, have been performed using filamentous fungi to bioprospect efficient catalysts for the development and improvement of enzymatic cocktails to degrade plant biomass. Some fungi, including *T. reesei*, *A. niger*, *A. nidulans* and *N. crassa* [9, 16, 17, 41-43], have been used for this purpose; however, the search for new efficient strains is ongoing. Understanding the molecular mechanisms by which filamentous fungi degrade plant biomass can improve the saccharification process, a very important step in the production of second-generation ethanol [42, 44]. The identification of new species and powerful enzymes can enhance the technologies in the biofuel industry.

Trichoderma spp. are capable of degrading both fungal and plant cell wall materials [45]. In this study, we chose to investigate species of the genus *Trichoderma* because this genus is a common soil and wood-degrading fungi distributed worldwide [46-48], is easily isolated from decomposing organic matter and soil [49], and harbors great potential to produce enzymes that degrade plant biomass [10, 12, 22]. We selected two strains of *T. harzianum* and one strain of *T. atroviride* to capture differences among strains. Different biotechnological approaches were used in bioprospecting new and efficient enzymes for

possible applications in the enzymatic hydrolysis process. We performed transcriptome, exoproteome and coexpression network analyses of a *T. harzianum* strain that has potential for plant biomass degradation under cellulose growth conditions to gain insights into the genes and proteins produced that are associated with cellulose hydrolysis. The analyses of genetic expression together with the identified secreted proteins under biomass degradation conditions allowed us to construct coexpression networks to investigate the relationships among the genes. Furthermore, we compared the expression levels with *Trichoderma* spp. that have been previously studied under the same conditions [10].

Through RNA-Seq, we identified the DEGs in all strains (Fig. 1b). Although Ta0020 presented the highest number of identified DEGs, this strain is less efficient than other *Trichoderma* strains at degrading plant biomass. *T. atroviride* is mostly used as a biocontrol agent and is among the best mycoparasitic fungus used in agriculture [34, 50, 51]. Although the *T. atroviride* strain produced 31 differentially expressed CAZymes, it seemed to produce less efficient enzymes with lower expression levels for plant biomass degradation than *T. harzianum*, since it was observed to harbor only one gene with cellulase activity from the GH5 family (TRIATDRAFT_81867) and only one gene with hemicellulase activity from the GH54/CBM42 family (TRIATDRAFT_81098) (Fig. 3). In this analysis, each strain presented a different set of genes with different expression levels, which can be attributed to strain differences in the regulatory mechanisms of hydrolysis. Additionally, the carbon source, which plays an important role in the production of enzymes, promotes the expression of different sets of genes as the fungus seeks to adapt to new environments [10]. The *T. harzianum* strains showed high numbers of CAZyme genes with enhanced specificity for biomass degradation.

Among the main CAZyme classes detected in all strains, GHs were well represented, with 21 genes in Th0179 and twice that in Th3844 (Fig. 1c). GHs compose an extremely

important class in several metabolic routes in fungi, including genes involved in cellulose and chitin degradation [12, 36]. Here, we found the main CAZyme families responsible for cellulose degradation, such as GH1, GH3, GH5, GH6, GH7, GH12, GH45 and an LPMO from the AA9 family [12, 28]. The CAZyme families responsible for hemicellulose degradation were as follows: GH10, GH11, GH54, GH62 and CE5 [12, 30, 52-54]. Within the GH class, an important family expected in our data was GH18, which has chitinase activity; this family was expected since members of the genus *Trichoderma* (such as *T. harzianum* and *T. atroviride*) are capable of mycoparasitism and because this class is directly related to the biological control of these species [17, 35, 55].

In comparing the two *T. harzianum* strains (Th0179 and Th3844), we observed that Th0179 presented fewer CAZyme genes related to cellulose degradation than Th3844, with lower expression levels (Fig. 3a). However, in measuring the enzymatic activity from the culture supernatants after 96 h of growth, we found that both strains had similar cellulase activity profiles during growth on cellulose (Additional file 1: Fig. S1), suggesting greater potential of Th0179 to degrade cellulose. The detected enzymatic activity is related to proteins secreted into the medium by the cells, and only the most stable proteins are detected in this environment [10]. It is interesting to observe which proteins found in the exoproteome of Th0179 may respond to this increased cellulase activity. A similar profile was observed in *T. reesei*, the most studied fungus within this genus and an important industrial producer of cellulolytic enzymes [13]: few CAZyme genes have been detected in its machinery, but it has high cellulase activity [10, 19, 56].

The identification of LPMOs as a group of enzymes that accelerate the breakdown of carbohydrate polymers, such as cellulose, by oxidative cleavage has been a breakthrough in lignocellulose conversion research. The supplementation of AA9 enzymes in commercial

cocktails improves the hydrolysis of lignocellulose; they assist cellulases in attacking crystalline substrate areas, resulting in rapid and relatively complete surface degradation [19].

CAZyme genes related to cellulose degradation, including endo-β-1,4-glucanases, β-glucosidases, and cellobiohydrolases, and CAZyme genes related to hemicellulose degradation, such as endo-1,4-β-xylanases, were identified in the Th0179 transcriptome under cellulose growth conditions (Table 1). In addition, we verified important secreted CAZymes that play important roles in biomass degradation (Table 2), such as β-glucosidases (A0A0F9XRC5 and A0A0F9XQT4), an LPMO (A0A0F9XMI8), a cellulose 1,4-β-cellobiosidase (nonreducing end) (A0A0G0AEM7), cellulases (A0A0F9XG06 and A0A0F9WYH5), endo-1,4-β-xylanases (A0A0F9Y0Y9, A0A0H3UCP8, and A0A0F9XXA4), and an endo-1,4-β-mannosidase (A0A0G0AGG8). One β-glucosidase from the GH3 family (THAR02_00656) was found in the exoproteome, which had the lowest expression level among the CAZymes related to biomass degradation; the same pattern was observed for *T. harzianum* IOC-3844 [10]. All of these enzymes are well known and can be used to improve enzymatic cocktails that can function in the degradation of specific substrates [57], such as the Brazilian biomass sugarcane bagasse. For instance, it is known that β-glucosidases are the rate-limiting enzymes in the degradation of cellulose [58]. These enzymes play a critical role among enzymes in enzymatic cocktails for biomass degradation. Therefore, improving the activities of such enzymes can enhance the efficiency of commercial enzymatic cocktails for bioethanol production [3].

Horta et al. [10] analyzed the exoproteome of Th3844 and Ta0020 under equivalent conditions, selecting a set of 80 proteins for a complete classification and analysis of expression levels based on their transcriptomes. Comparison of the Th0179 and Th3844 exoproteomes indicated that both produced many of the main CAZymes listed above; however, the THAR02_05896 gene, which encodes an endo-1,4-β-xylanase protein, and the

THAR02_03851 gene, which encodes a protein with endo-1,4-β-mannosidase activity, were not detected in the Th3844 secretome. In the present study, when these exoproteomes were compared, we noticed that most of the proteins were differentially expressed in cellulose only for Th0179, with an emphasis on the AA9/CBM1 family (THAR02_02134), showing a 2.65-fold change (138.62 TPM). Among the strains, Ta0020 exhibited the lowest number of secreted proteins related to cellulose and hemicellulose degradation. Thus, the exoproteome analysis identified key enzymes that may be fundamental for cellulose hydrolysis and that act synergistically for efficient plant biomass degradation.

The organization of the transcriptomic data into coexpression networks using graph theory allowed the construction of gene interaction networks that were represented by nodes connected to edges [59]. Nodes represent the genes, and the edges represent the connections among these genes. Correlations are determined based on the expression level of the genes pair by pair, indicating that genes spatially closer to one another are more highly correlated than the genes that are farther apart. The coexpression subnetwork (Fig. 5b) revealed complex, specific relationships between CAZyme genes and genes involved in the production and secretion of the detected proteins and is helpful for understanding the functions and regulation of genes. Networks such as this one can be used as a platform to search for target genes or proteins in future studies to comprehend the synergistic relationships between genes and their regulation, which is very useful information for understanding the saccharification process. We identified an enriched cluster with strong correlations among genes, CAZyme genes, secreted proteins and unknown genes in the cluster analysis.

Furthermore, through the functional annotation analysis of Th0179, we identified TFs and transporters, which should be investigated in further studies to better understand the mechanisms by which these genes are regulated. Most sugar transporters have yet to be characterized [1] but play important roles in taking up mono- or disaccharides into fungal

cells after biomass degradation [1, 60]. Fungal gene expression is controlled at the transcriptional level [61], and its regulation affects the composition of enzyme mixtures; accordingly, it is studied in several species because of its potential applications [62]. This further emphasizes the importance of deleting and/or overexpressing TFs that regulate specific genes directly involved in plant biomass degradation [61].

In summary, the analyses of the enzymatic activity of cellulase, the transcriptome, the exoproteome and the coexpression networks revealed important enzymes that *T. harzianum* CBMAI-0179 uses to hydrolyze cellulose and that most likely act synergistically to depolymerize polysaccharides. The results suggest great potential of this strain to degrade cellulose and can contribute to the optimization of enzymatic cocktails for bioethanol production.

Conclusions

Bioprospecting new catalytic enzymes and improving technologies for the efficient enzymatic conversion of plant biomass are required for advancing biofuel production. *T. harzianum* CBMAI-0179 is a novel potential candidate producer of plant cell wall polysaccharide-degrading enzymes that can be biotechnologically exploited for plant biomass degradation. The cellulase activity profile indicated high efficiency and high potential of this strain for cellulose degradation. A set of highly expressed CAZymes and proteins that are species- and treatment-specific was observed in both the transcriptome and exoproteome analyses. The coexpression network revealed coexpressed genes and CAZymes that act synergistically to hydrolyze cellulose. In addition, the cluster analysis revealed genes with strong correlations that are necessary for saccharification. Combined, these tools provide a powerful approach for protein discovery and the selection of target genes through the heterologous expression of proteins. In future studies, these tools can aid the selection of new

species and the optimization of the production of powerful enzymes for use in enzymatic cocktails for second-generation bioethanol production.

Methods

Fungal strains, fermentation and enzymatic activities

The species originated from the Brazilian Collection of Environment and Industry Microorganisms (CBMAI), located on CPQBA/UNICAMP, in Campinas, Brazil. *T. harzianum* CBMAI-0179 (Th0179), *T. harzianum* IOC-3844 (Th3844) and *T. atroviride* CBMAI-0020 (Ta0020) strains were grown as described in a previous work on solid medium to produce sufficient spores for the fermentation process, which was performed in biological triplicates using crystalline cellulose (Celuflok, São Paulo, Brazil; degree of crystallinity, 0.72 g/g; composition, 0.857 g/g cellulose and 0.146 g/g hemicellulose) or glucose as the carbon source [10]. Glucose was used as a control in all experimental conditions. Supernatants were collected to measure enzymatic activity and determine the exoproteome profile.

Xylanase and β-glucosidase activities were determined using the methods described by Bailey and Poutanen [63] and Zhang et al. [64], respectively. Cellulase activity was determined using the filter paper activity (FPA) test according to Ghose [65]. Protein levels were measured based on the Bradford [66] method. The enzymatic activities and protein contents in culture supernatants were determined in a previous work and are shown in Additional file 1: Fig. S1.

RNA extraction

Mycelial samples from cellulose and glucose conditions were extracted from Th0179, Th3844 and Ta0020 after 96 h of fermentation, stored at -80 °C, ground in liquid nitrogen using a mortar and pestle, and used for RNA extraction using the LiCl RNA extraction protocol according to the method reported by Oliveira et al. [67].

Data sources

The nucleotide and protein sequences of *T. harzianum* T6776 (PRJNA252551) and *T. atroviride* IMI 206040 (PRJNA19867) used as reference for transcriptome assembly were downloaded from the NCBI database (www.ncbi.nlm.nig.gov).

Library construction and RNA-Seq

RNA samples were quantified using a NanoDrop 8000 (Thermo Scientific, Wilmington, DE, USA). The libraries were constructed using 1 µg of each RNA sample obtained from the mycelial samples and the TruSeq RNA Sample Preparation Kit v2 [68] (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The expected target sizes were confirmed using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and the DNA 1000 Kit, and the libraries were quantified by qPCR using the KAPA library quantification Kit for Illumina platforms (Kapa Biosystems, Wilmington, MA, USA). The average insertion size was 260 bp. A total of 18 biological triplicate samples were multiplexed with different adapters and organized in different lanes of the flow cell for high-throughput sequencing. The sequencing was carried out on the HiSeq 2500 platform (Illumina, San Diego, CA, USA) according to the manufacturer's specifications for paired-end reads of 150 bp.

Transcriptome assembly and mapping

After sequencing was completed, the data were transferred to a local high-performance computing server at the Center for Molecular Biology and Genetic Engineering (CBMEG, University of Campinas, Campinas, Brazil). FastQC v0.11.5 [69] was used to visually assess the quality of the sequencing reads. Removal of the remaining adapter sequences and quality trimming with a sliding window of size 4, minimum quality of 15, and length filtering (minimal length of 36 bp) was performed with Trimmomatic v0.36 [70].

The RNA-Seq data were analyzed using CLC Genomics Workbench software (v6.5.2; CLC bio, Finlandsgade, Denmark) [71]. The reads were mapped against the reference genomes of *T. harzianum* T6776 [33] and *T. atroviride* IMI 206040 [34] using the following parameters: minimum length fraction = 0.5; minimum similarity fraction = 0.8; and maximum number of hits for a read = 10. For the paired settings, the parameters were minimum distance = 150 and maximum distance = 300, including the broken pairs counting scheme. The gene expression values were expressed in reads per kilobase of exon model per million mapped reads (RPKM), and the normalized value for each sample was calculated in transcripts per million (TPM) [72]. To statistically analyze the differentially expressed genes (DEGs), the following parameters were used: fold change greater than or equal to 1.5 or lower than or equal to -1.5 and p-value lower than 0.05.

Gene comparisons between species

Venn diagrams were constructed to compare the genes with TPM expression values greater than zero under both conditions from all species using http://bioinformatics.psb.ugent.be/webtools/Venn/.

Transcriptome annotation and CAZyme determination

Sequences were functionally annotated according to the Gene Ontology (GO) terms [73] with Blast2Go v4.1.9 [74] using BLASTx-fast and a cutoff e-value of 10^{-6}. Information derived from the CAZy database [24] was downloaded (www.cazy.org) to locally build a CAZy database (2017). The protein sequences of *T. harzianum* T6776 and *T. atroviride* IMI 206040 were used as queries in basic local alignment search tool (BLAST) searches against the locally built CAZy BLAST database. BLAST matches showing an e-value less than 10^{-11}, identity greater than 30% and queries covering greater than 70% of the sequence length were selected and classified according to the CAZyme catalytic group as GHs, CBMs, GTs, CEs, AAs or PLs and their respective CAZyme families.

CAZymes were also annotated according to Enzyme Commission (EC) number [75] through BRENDA (Braunschweig Enzyme Database) [76] (www.brenda-enzymes.org), using BLASTp with an e-value cutoff of 10^{-10}, identity greater than 30% and queries covering greater than 60% of the sequence length.

Coexpression networks

The coexpression networks for Th0179 were assembled from the mapped RNA-Seq data against the genome of *T. harzianum* T6776 using biological triplicates. The network was assembled by calculating Pearson's correlation coefficient for each pair of genes. Genes showing null values for most of the replicates under different experimental conditions were excluded to decrease noise and to remove residuals from the analysis. The highest reciprocal rank (HRR) method proposed by Mutwil et al. [77] was used to empirically filter the edges, retaining edges with an HRR less than or equal to 30. Thus, only edges representing the strongest correlations were selected. Cytoscape software v3.6.0 [78] was used for data analysis and network construction. The cluster analysis procedure was performed with the Heuristic Cluster Chiseling Algorithm (HCCA) [77].

Exoproteome analysis

The analysis of the exoproteome of Th0179 under both fermentative conditions was performed via liquid chromatography tandem mass spectrometry (LC-MS/MS) using the data-independent method of acquisition MS^E as described by Horta et al. [10] The LC-MS/MS data were processed using ProteinLynx Global Server (PLGS) v3.0.1 software (Waters, Milford, MA, USA), and the proteins in the processed files (.pkl) were identified by comparison to the *Trichoderma* sequence database available in the UniProt Knowledgebase (UniProtKB; https://www.uniprot.org/uniprot/). BLASTp searches of the fasta sequences of the identified proteins were performed against the *T. harzianum* T6776 genome to identify the secreted proteins and compare them to the transcriptome.

RT-qPCR analysis

To validate the expression profiles of the transcriptome analysis, reverse transcription-quantitative PCR (RT-qPCR) was performed for selected genes. The RNeasy Mini Kit (Qiagen) was used for RNA extraction, and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer's instructions. DEGs with a high number of reads and TPM were selected and are shown in Additional file 2: Table S1, together with the primers and annealing temperatures. Primers were synthesized using the Primer3Plus web interface [79] with a fusion temperature between 58 and 60 °C and amplicon sizes between 120 and 200 bp.

Quantification of gene expression was performed by continuously monitoring SYBR Green fluorescence. The reactions were performed in triplicate in a total volume of 6.22 μL. Each reaction contained 3.12 μL of SYBR Green Supermix (Bio-Rad, USA), 1.0 μL of forward and reverse primers and 2.1 μL of diluted cDNA. The reactions were assembled in

384-well plates. PCR amplification-based expression profiling of the selected genes was performed using specific endogenous controls for each strain, which are described in Additional file 2: Table S4. RT-qPCR was conducted with the CFX384 Touch Real-Time PCR Detection System (Bio-Rad). The real-time PCR program was as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 15 sec at 95 °C and 60 sec at 60 °C. Gene expression was calculated via the delta-delta cycle threshold method [80]. The obtained RT-qPCR results were compared with the RNA-Seq results from the generated assemblies. The selected genes exhibited the same expression profiles between the RT-qPCR and RNA-Seq analyses (Additional file 3: Fig. S2).

Additional files

Additional file 1: Fig. S1. Enzymatic activity. Enzymatic activities (UI mL^{-1}) of β-glucosidase (a), cellulase (b), and xylanase (c) and protein contents (d) in the culture supernatants of *Trichoderma* spp. measured after 96 h of growth. Each bar represents the mean and standard deviation of biological triplicates.

Additional file 2: Table S1. Sequences of the primers from the endogenous controls and DEGs in RNA-Seq data selected for RT-qPCR.

Additional file 3: Fig. S2. RNA-Seq analysis validation. The obtained RT-qPCR results were compared with the RNA-Seq results for transcriptome analysis validation of *Trichoderma* spp.

Additional file 4: Fig. S3. Venn diagrams. Venn diagrams of the genes identified in *Trichoderma* spp. with expression levels higher than zero under cellulose (a) and glucose (b) growth conditions using the *T. harzianum* T6776 genome as a reference.

Additional file 5: Table S2. Classification of the CAZyme genes under cellulose conditions for *T. harzianum* IOC-3844 and *T. atroviride* CBMAI-0020.

Additional file 6: Table S3. Proteins identified in both the transcriptome and exoproteome of *T. harzianum* CBMAI-0179 under both glucose and cellulose growth conditions.

Additional file 7: Table S4. Genes identified in the cluster analysis from the main coexpression network of *T. harzianum* CBMAI-0179.

List of abbreviations

AA: Auxiliary enzymes; **BLAST:** Basic Local Alignment Search Tool; **bp:** Base pair; **BRENDA:** Braunschweig Enzyme Database; **CAZymes:** Carbohydrate-active enzymes; **CBM:** Carbohydrate-binding module; **cDNA:** Complementary DNA; **CE:** Carbohydrate esterases; **CEL:** Cellulose; **DEGs:** Differentially expressed genes; **EC:** Enzyme commission number; **FPA:** Filter paper activity; **GH:** Glycoside hydrolases; **GLU:** Glucose; **GO:** Gene Ontology; **GT:** Glycosyltransferases; **HCCA:** Heuristic Cluster Chiseling Algorithm; **HRR:** Highest reciprocal rank; **kb:** Kilobases; **LPMO:** Lytic polysaccharides monooxygenase; **MA2:** Malt extract agar, 2% w/w; **Mb:** Megabase; **mL:** Milliliter; **mRNA:** Messenger RNA; **PCA:** Principal component analysis; **PDA:** Potato dextrose agar; **PL:** Polysaccharide lyases; **RNA:** Ribonucleic acid; **RNA-Seq:** RNA sequencing; **RPKM:** Reads per kilobase of exon model per million mapped reads; **RT-qPCR:** Real-time quantitative PCR; **Ta0020:** *Trichoderma atroviride* CBMAI-0020; **TFs**: Transcription factors; **Th0179:** *Trichoderma harzianum* CBMAI-0179; **Th3844:** *Trichoderma harzianum* IOC-3844; **TPM:** Transcripts per million; **UI:** International Unit; **µl:** Microliter

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during this study are included in this published article and Additional files 1, 2, 3, 4, 5, 6 and 7. The reads have been deposited at the NCBI Sequence Read Archive and can be accessed under the BioProject number PRJNA336221.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DAA and APS conceived and designed the study. DAA, MACH, JAFF and NFM performed the data analysis. DAA drafted the manuscript, which was critically revised by MACH, JAFF and APS. All authors read and approved the final manuscript.

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Figure legends

Fig. 1. The transcriptome profiles, gene expression comparison and the main CAZyme classes identified for all strains. PCA of transcriptome mapping according to species and growth conditions (CEL – cellulose and GLU – glucose) using the *T. harzianum* T6776 genome as a reference (a), number of DEGs and differentially expressed CAZyme genes in *T. harzianum* CBMAI-0179 (Th0179), *T. harzianum* IOC-3844 (Th3844), and *T. atroviride* CBMAI-0020 (Ta0020) under cellulose growth conditions (b), the differentially expressed CAZyme classes identified for each strain under cellulose growth conditions (c).

Fig. 2. Distribution of CAZyme families in *Trichoderma* spp. Classification and quantification of CAZyme families in *T. harzianum* CBMAI-0179 (a), *T. harzianum* IOC-3844 (b), and *T. atroviride* CBMAI-0020 (c) under cellulose growth conditions.

Fig. 3. Evaluation of CAZyme family expression in *Trichoderma* spp. via RNA-Seq. Quantification of the expression of the main families related to cellulose (a) and hemicellulose (b) degradation in TPM.

Fig. 4. GO terms of *T. harzianum* CBMAI-0179 under cellulose growth conditions. The sequences were annotated according to the main GO terms: molecular function (a), biological process (b), and cellular component (c).

Fig. 5. Coexpression networks of *T. harzianum* CBMAI-0179. Complete coexpression network (a), the coexpression subnetwork based on the exoproteome data (b), the enriched cluster analysis of the coexpression network (c), the subnetwork of the CAZyme genes and the secreted proteins identified in the cluster analysis (d). Red squares indicate DEGs under cellulose growth conditions, blue squares indicate DEGs under glucose growth conditions, yellow triangles indicate CAZyme genes under cellulose growth conditions, light blue triangles indicate CAZyme genes under glucose growth conditions and purple hexagons indicate the secreted proteins under cellulose growth conditions.

Tables

Table 1. Classification of the CAZyme genes under cellulose growth conditions for *T. harzianum* CBMAI-0179

Gene ID	Protein product	Fold change	e-value	CAZy classification	Enzyme activity	EC number	Cellulose TPM	Glucose TPM
THAR02_00068	KKP07860.1	1.87	1.00E-143	GH18 CBM1	chitinase	3.2.1.14	42.13	22.14
THAR02_00890	KKP07011.1	2.10	4.00E-15	GH3	beta-glucosidase	3.2.1.21	68.64	32.01
THAR02_01434	KKP06476.1	1.75	0	GH16	endo-1,3(4)-beta-glucanase	3.2.1.6	341.65	191.83
THAR02_01911	KKP05955.1	1.64	2.00E-53	GT4	1-acyl-sn-glycerol-3-phosphate acyltransferase	-	94.76	56.86
THAR02_02132	KKP05758.1	2.89	0	GH3	beta-glucosidase	3.2.1.21	16.39	5.56
THAR02_02133	KKP05759.1	2.27	3.00E-160	CBM1	cellulase	3.2.1.4	47.86	20.67
THAR02_02134	KKP05760.1	2.60	0	AA9 CBM1	cellulase	3.2.1.4	138.62	52.35
THAR02_02251	KKP05610.1	2.21	0	GH1	beta-glucosidase	3.2.1.21	88.05	39.02
THAR02_02560	KKP05371.1	1.97	2.00E-29	GH18	uncharacterized protein	-	151.83	75.55
THAR02	KKP04958.1	2.05	3.00E-114	GH45 CBM1	cellulase	3.2.1.4	52.97	25.32

_02979								
THAR02_03008	KKP04907.1	2.02	7.00E-30	GH55	glucan 1,3-beta-glucosidase	3.2.1.58	28.88	14.01
THAR02_03217	KKP04674.1	1.52	8.00E-14	GH17	hypothetical protein THAR02_03217	-	71.68	46.17
THAR02_03271	KKP04658.1	2.25	5.00E-137	GH10	endo-1,4-beta-xylanase	3.2.1.8	47.58	20.75
THAR02_03302	KKP04612.1	1.85	4.00E-95	GH16	glucan endo-1,3-beta-D-glucosidase	3.2.1.39	212.11	112.64
THAR02_04021	KKP03872.1	2.11	2.00E-84	GH72	1,3-beta-glucanosyltransferase	2.4.1.-	178.64	82.96
THAR02_04405	KKP03485.1	2.09	0	GH5 CBM1	cellulase	3.2.1.4	39.92	18.77
THAR02_04414	KKP03494.1	1.52	0	GH6 CBM1	cellulose 1,4-beta-cellobiosidase (nonreducing end)	3.2.1.91	299.66	192.97
THAR02_05432	KKP02477.1	2.64	0	GH1	beta-glucosidase	3.2.1.21	125.51	46.73
THAR02_05677	KKP02215.1	40.48	2.00E-127	AA7	UDP-N-acetylmuramate dehydrogenase	-	20.74	0.50
THAR02_07531	KKP00372.1	2.05	0	GH20	beta-N-acetylhexosaminidase	3.2.1.52	1925.74	920.50
THAR02_07716	KKP00192.1	1.98	4.00E-92	GH64 CBM6	glucanase B	-	271.18	134.28
THAR02_07777	KKP00125.1	1.79	0	GH18 CBM1	chitinase	3.2.1.14	108.01	59.24
THAR02_07958	KKO99924.1	2.60	3.00E-162	GH27	alpha-galactosidase	3.2.1.22	22.70	8.58
THAR02_08897	KKO99004.1	1.73	0	GH7 CBM1	cellulose 1,4-beta-cellobiosidase (reducing end)	3.2.1.176	369.92	209.24
THAR02_10108	KKO97789.1	1.51	4.00E-29	CE9	glucosamine-6-phosphate isomerase	-	3705.87	2411.20
THAR02_10110	KKO97791.1	1.64	0	CE9	N-acetylglucosamine-6-phosphate deacetylase	3.5.1.25	6362.40	3809.76
THAR02_10273	KKO97625.1	1.62	0	GH17	glucan endo-1,3-beta-D-glucosidase	3.2.1.39	95.61	57.93

Table 2. Proteins identified in both the transcriptome and exoproteome of *T. harzianum* CBMAI-0179 grown in cellulose

Gene ID	Accession number	Protein name	e-value	CAZy classification	EC number	Cellulose TPM	Glucose TPM
THAR02_00377	G0RX84	Predicted protein	3.00E-15	-	-	1113.19	1128.71
THAR02_00656	A0A0F9XRC5	Beta-glucosidase	0	GH3	3.2.1.21	5.08	2.55
THAR02_00890	A0A0F9XQT4	Beta-glucosidase	0	GH3	3.2.1.21	68.64	32.01
THAR02_01069	G9MX73	Glycoside hydrolase family 64 protein	1.00E-64	GH64	-	17.67	25.95
THAR02_01434	A0A0F9XP75	Uncharacterized protein	0	GH16	3.2.1.6	341.65	191.83
THAR02_01982	A0A0F9Y1F6	Beta-galactosidase	0	GH35	3.2.1.23	10.08	6.85
THAR02_02133	A0A0G0AME2	Uncharacterized protein	0	CBM1	-	47.86	20.67
THAR02_02134	A0A0F9XMI8	Cellulase	0	AA9 CBM1	3.2.1.4	138.62	52.35
THAR02_02147	A0A0F9Y0Y9	Endo-1,4-beta-xylanase	0	GH11 CBM1	3.2.1.8	119.56	86.24
THAR02_02289	A0A0F9Y0G5	Cel74a	0	CBM1	-	22.53	24.27
THAR02_03210	A0A0F9ZXC9	WSC domain-containing protein	0	AA5_1	-	33.57	38.75
THAR02_03271	A0A0F9XXA4	Beta-xylanase	0	GH10	3.2.1.8	47.58	20.75
THAR02_03624	G0RX52	Extracellular metalloproteinase (Fungalysin)	0	CBM50	3.4.24.-	1.02	0.31
THAR02_03851	A0A0G0AGG8	Mannan endo-1,4-β-mannosidase	0	GH5 CBM1	3.2.1.78	67.98	57.54
THAR02_04062	A0A0F9XH17	Uncharacterized protein	2.00E-170	-	-	21.80	18.38
THAR02_04344	G9MY63	Glycoside hydrolase family 71 protein	0	GH71 CBM24	3.2.1.59	3.82	3.87
THAR02_04405	A0A0F9XG06	Cellulase	0	GH5 CBM1	3.2.1.4	39.92	18.77
THAR02_04414	A0A0G0AEM7	Cellulose 1,4-β-cellobiosidase (nonreducing end)	0	GH6 CBM1	3.2.1.91	299.66	192.97
THAR02_04626	G9NK86	Glycoside hydrolase family 92 protein	0	GH92	-	1.62	0.36
THAR02_04782	A0A024HVI0	Chitinase 18-5 (Fragment)	6.00E-80	GH18	-	21.24	16.93
THAR02_05380	A0A0F9XQN9	Uncharacterized protein	3.00E-171	-	-	102.99	81.78
THAR02_05501	G0R911	Glycoside hydrolase family 92	0	GH92	-	3.88	2.03
THAR02_05896	A0A0H3UCP8	Endo-1,4-beta-xylanase	8.00E-156	GH11	3.2.1.8	33.19	27.46
THAR02_06252	A0A0F9XN06	Murein transglycosylase	0	GH71 CBM24	3.2.1.59	81.33	131.74
THAR02_07321	A0A0F9X7S7	Uncharacterized protein	0	-	-	33.34	42.55
THAR02_07975	G0RXE3	Predicted protein	0	-	-	8.39	15.64
THAR02_08235	A0A0F9ZHA7	Chitinase 3	0	GH18	3.2.1.96	62.16	62.00
THAR02_08478	A0A0G0A296	Uncharacterized protein	0	GH30_7	3.2.1.-	6.37	2.67
THAR02_08479	A0A0F9X463	Uncharacterized protein	0	CBM1	-	28.08	15.80
THAR02_09247	A0A0F9ZZN6	Uncharacterized protein	0	CBM43	-	142.27	133.87
THAR02_09257	E2PTX8	Endochitinase 42 (Fragment)	6.00E-36	GH18	-	8.28	8.50

THAR02_09719	A0A0F9WYH5	Cellulase	0	GH5 CBM1	3.2.1.4	18.31	12.14

925

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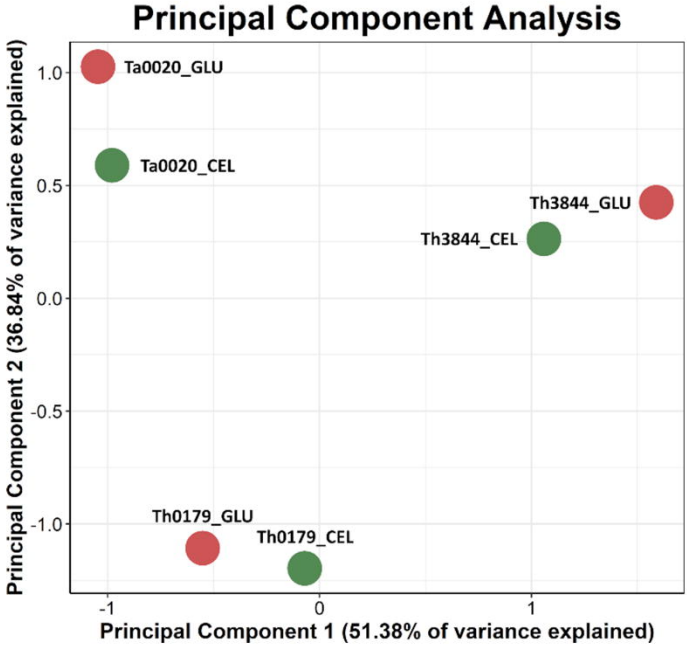
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Principal Component 1 (51.38% of variance explained)

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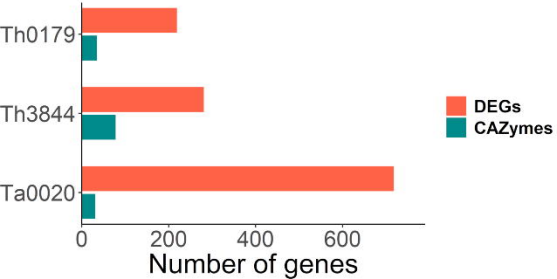
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DEGs
CAZymes

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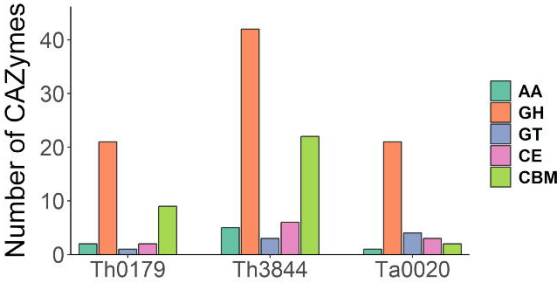
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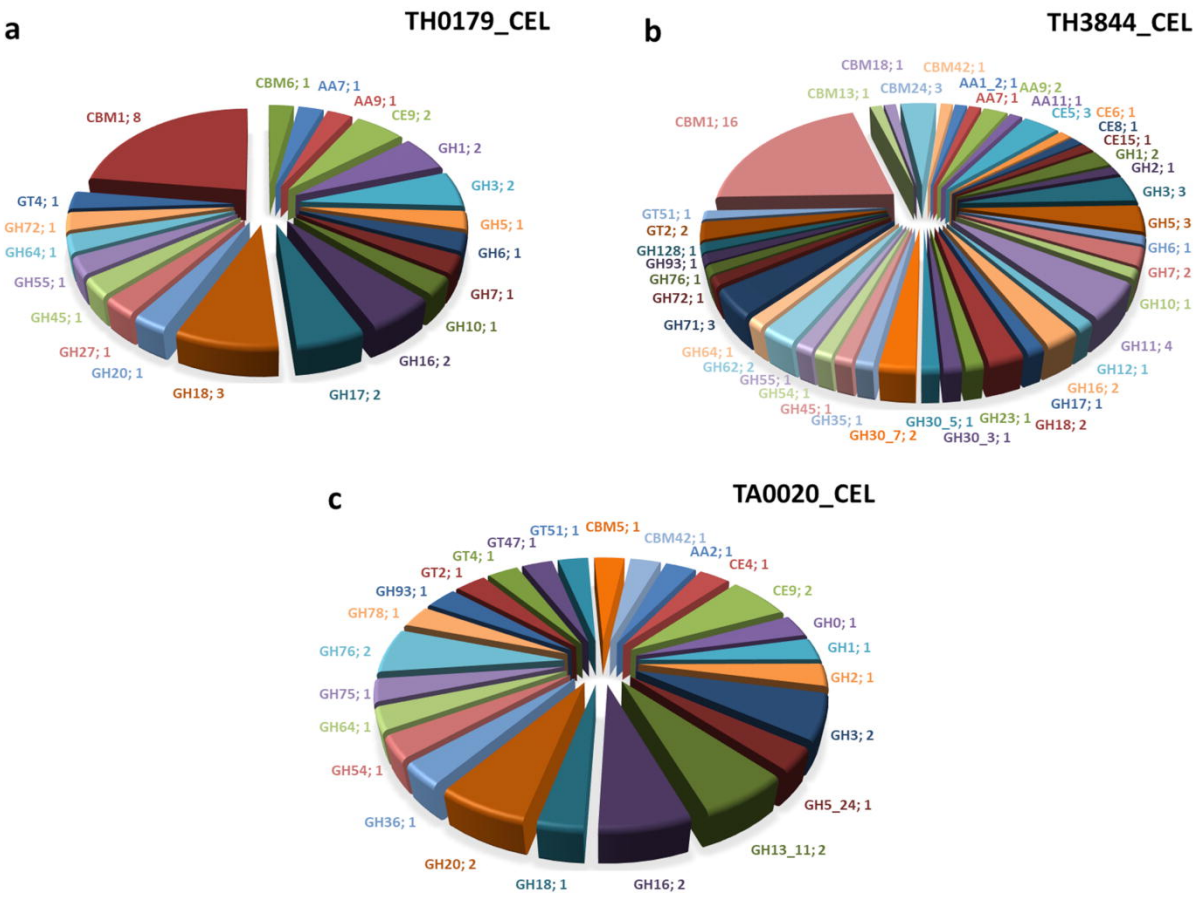
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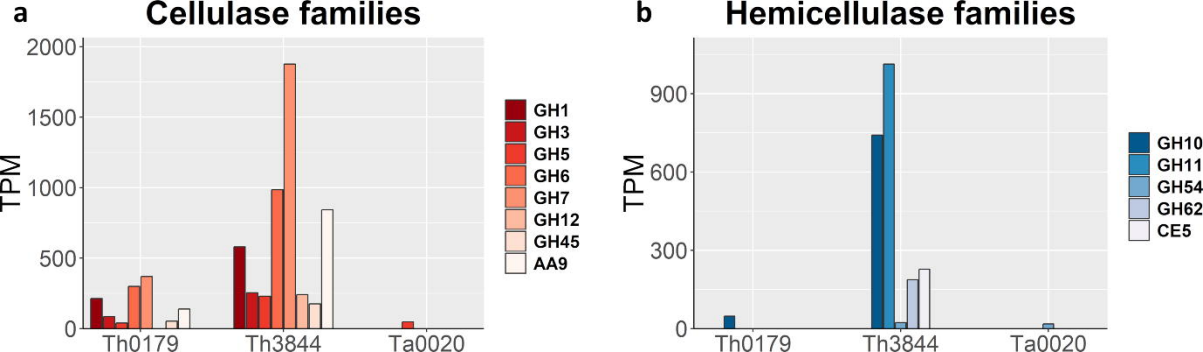
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Molecular Function

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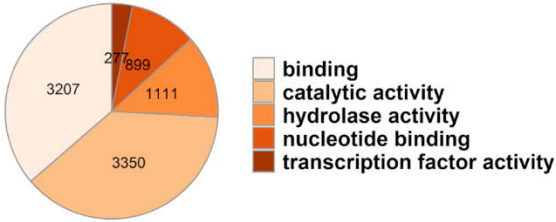

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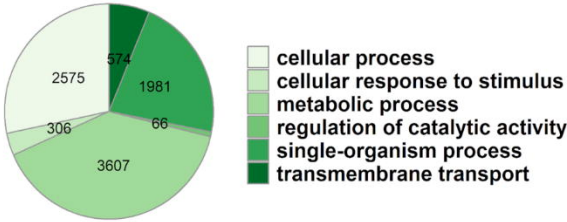

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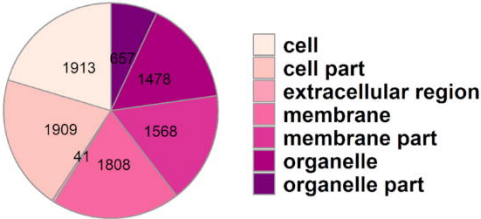

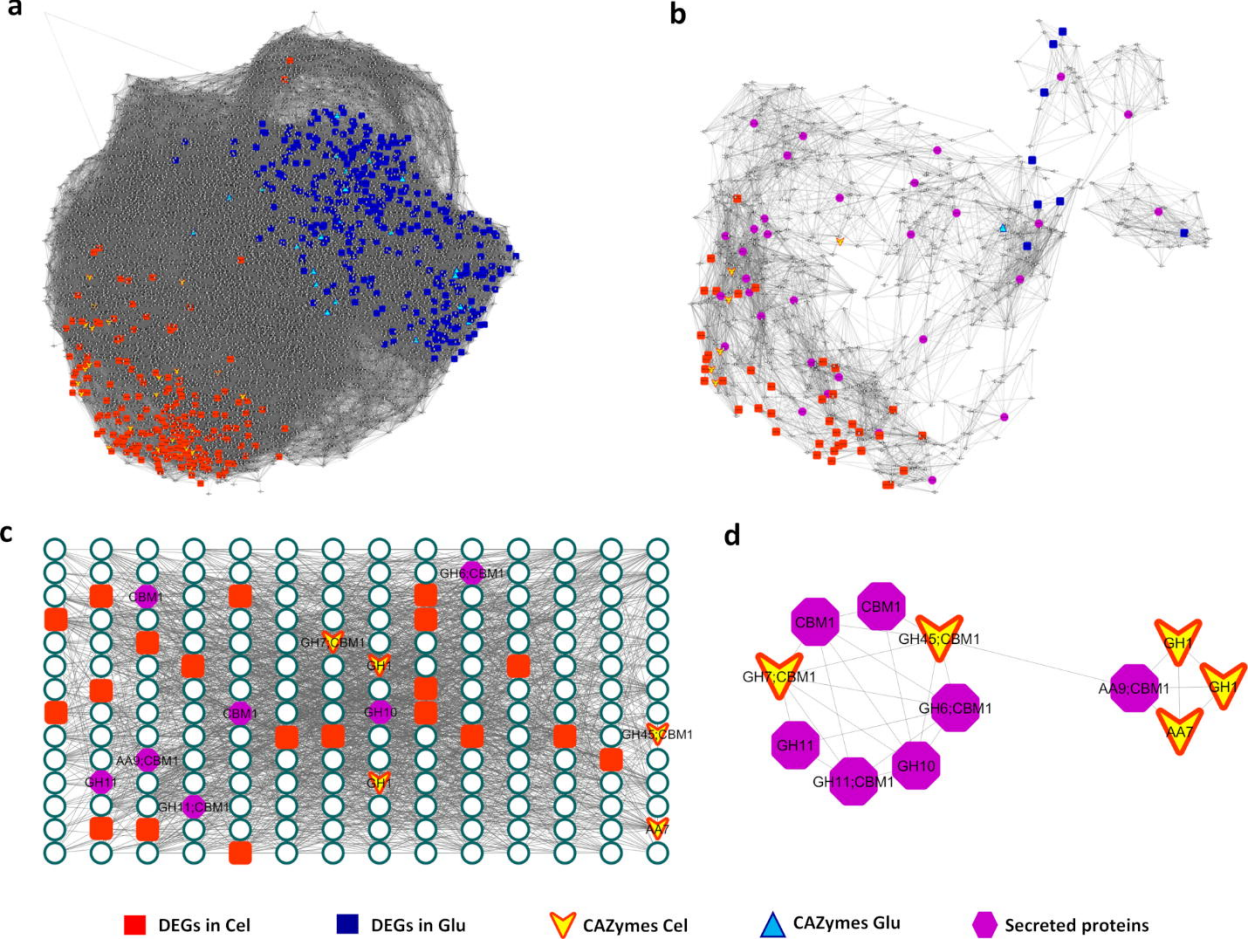
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DEGs in Cel
DEGs in Glu
CAZymes Cel
CAZymes Glu
Secreted proteins