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Identification and Expression of Some Plant Cell Wall-Degrading Enzymes Present in Three Ontogenetics Stages of *Thecaphora frezii*, a Peanut (*Arachis hypogaea* L.) Pathogenic Fungus

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Abstract

Peanuts can be affected by the presence of pathogenic microorganisms. The fungus *Thecaphora frezii* (*T. frezii*), which belongs to the taxonomic class Ustilaginomycetes, is the causal agent of the disease known as "peanut smut". The life cycle of this fungus includes three stages, namely teliospores, basidiospores and hyphae. In the hyphae stage, infection occurs in the peanut plant, which requires the involvement of some enzymes secreted by the fungus. These include the Plant Cell Wall-Degrading Enzymes (PCWDEs), which degrade various polysaccharides. This study aimed to identify the presence of transcript for enzymes belonging to the PCWDEs from three stages of *T. frezii*. For this, total RNA was extracted from the three ontogenetic stages of *T. frezii*. These samples were analyzed using an RNA-Seq approach and some transcripts were quantified using Real Time PCR. The analysis of the data provided by the RNA-Seq of the three *T. frezii* stages, it was possible to identify some transcripts that could encode enzymes compatible with polysaccharides degradation that are part of the plant cell wall. In *T. frezii* transcriptome, 40 deduced proteins would be enzymes with functions of PCWDEs were identified. They were divided into 27 glycoside hydrolases; two polysaccharide lyases; three carbohydrate esterases and eight enzymes with auxiliary activities. In addition, the fungal SNF1 gene was identified whose activity could be affected by high glucose level, and indirectly influence

the levels of some PCWDEs. The analysis of the PCWDEs could help to understand part of the fungal infection process and possibly find substances that can control its development.

Keywords

Carbohydrate-Degrading Enzymes, Phytopathogenic Fungi, Smut Fungi, Transcriptome, *Thecaphora frezii*

1. Introduction

At present, it is known that there are a large number of pathogenic microorganisms that affect plants, including fungi, some of which have an impact on crops of various plantations [1] [2]. One mechanism through which these microorganisms affect target plants is the secretion of enzymes that attack their cell walls. This wall is a great barrier that limits the action of microorganisms, and these enzymes are part of the so-called Plant Cell Wall-Degrading Enzymes (PCWDEs) [3], which are responsible for degrading several polysaccharides (cellulose, hemicellulose and pectins) and glycoproteins [4]. The complex nature of cellulose, hemicellulose, pectins, glycoproteins and their interactions within the cell wall make up a diverse structure and constitute an effective barrier against plant pathogens [5]. The composition of this cell wall may change based on the stage of development, growth and response of the plant to external conditions [6]. In nature, there are a large number of enzymes that make up a complex system that aims at degrading the carbohydrates that are components of the plant cell wall, which make this wall susceptible to attacks by numerous microorganisms. Among them, a family of proteins has been classified as demonstrating reasons that confer catalytic activity against carbohydrates to enzymes called Carbohydrate-Active enZyme (CAZyme) [7]. Within this wide enzymatic arsenal, there are the Glycoside Hydrolases (GH), Polysaccharide Lyases (PL), Carbohydrate Esterases (CE), enzymes with Auxiliary Activities (AA) and Non-catalytic Carbohydrate Binding Modules (CBM) [8] [9] [10]. The identification and comparison of these fungal CAZymes that have different nutritional modes or infection mechanisms can provide information for a better understanding of their lifestyle and infection patterns [11].

Peanuts (*Arachis hypogaea* L.) is one of the most important legume crops in the world, native to South America, distributed in Brazil, Paraguay, Bolivia, Argentina and Uruguay [12]. In Argentina, crops are mainly found in the province of Córdoba, with a contribution of 92% of the national primary production [13]. During the growing cycle, peanuts are frequently attacked by fungal diseases, especially, those that develop in the soil during the fruit formation stage, during harvest and in the drying and storage phases of the grain [14] [15] [16]. Some of the fungi responsible for diseases in peanuts include the genera *Aspergillus*, *Penicillum*, *Sclerotinia*, *Fusarium*, *Rhizopus* and *Thecaphora* [17] [18] [19]. One of

these diseases is called "peanut smut", which is caused by *Thecaphora frezii* (*T. frezii*), a biotrophic fungus (it infects its host without causing cell death to complete its life cycle) and single peanut host, which produces numerous yearly losses, with a higher incidence in the southern part of the province of Córdoba. The fungus was, first, detected by Carranza and Lindquist in wild diploid peanut germplasm from Aquidauana, Brazil [20], and, in Argentina, in the 1994/95 campaign, while its prevalence, incidence and severity have been increasing in the last 10 years, expanding, in addition, to other provinces of Argentina [21] [22]. There are three ontogenetic stages described for *T. frezii* life cycle: teliospores, which represent the resistance structures; basidiospores, formed after the germination of teliospores; and hyphae, formed by the fusion of compatible germinating basidiospores. These hyphae infect the stalks peg when it enters the ground by penetrating from the outside and by passing through the tissues until they reach the seeds [23]. Inside the plant, it closes its cycle by forming teliospores again, producing deformation and hypertrophy of the fruits, with the ability to affect only one seed or the entire fruit, transforming it into a carbonaceous mass of reddish-brown spores. Teliospores have poor germination *in vitro*, but Cazón and collaborators [24], achieved their germination in PDA with the addition of grain extract. However, by placing a large number of spores our team was able to obtain their germination in PDA [25]. Likewise, it was possible to obtain basidiospores *in vitro*, employing a poor-nutrient media [25]. To obtain basidiospores, their growth can be induced depending on the culture medium used, a nutritional fast, heat shock and the pH of the medium that are factors that induce the formation of basidiospores [26] [27].

The aim of this work was to identify the presence in *T. frezii*, and in its three stages; transcript for enzymes belonging to the PCWDE group with the potential to degrade the polysaccharides presents in the cell wall of the peanut plant. In turn, the relative mRNA quantification was done (the quantification that is carried out is indicative since it is done from an "*in vitro*" culture for basidiospores and hyphae, and for teliospores from infected pods). Despite this, the results could contribute to understanding whether there is a correlation between the level of expression and the infective process, since an increase in expression in the basidiospore stage, but more particularly in the hyphae stage, would be strongly suggestive of this action [28]. This phenomenon is also noted in other fungi, *Ustilago maydis* (*U. maydis*) and *Magnaporthe oryzae*, which infect maize and rice plants, respectively. In both cases, an increased expression of genes coding for cellulases, hemicellulases and pectate lyases was observed [29] [30]. Meanwhile, some organisms can adapt to their environment and synthesize specific enzymes according to the carbon source that is available. The enzyme function that could be regulated by the glucose levels is the non-fermentative sucrose protein kinase serine threonine (Snf1) [31] [32]. Snf1 also has roles in several cellular mechanisms [33]. We try to identify at least partially the Snf1 transcript in *T. frezii* and its expression levels which could condition the expression levels of some PCWDEs.

2. Materials and Methods

2.1. Collection, Isolation and Cultivation of *Thecaphora frezii*

Teliospores of *T. frezii* were obtained from peanut pods showing symptoms of disease (hypertrophy, because we have not been able to grow teliospores in culture without changing their ontogenetic stage). The pods were superficially disinfected with 0.5% NaOCl (v/v), plated on potato-dextrose agar (Britannia PDA) and incubated at 26˚C in the dark until germination. Once the hyphae had developed, they were multiplied in potato broth (Neogen) for 24 hours while stirring at 26˚C. To obtain the basidiospores, an inoculum of hyphae from the liquid culture was transferred to agar/water (1.5% w/v) and incubated for 10 days at 26˚C in the dark until the formation of the basidiospores, which was corroborated by optical microscopy (Figure 1).

2.2. RNA Extraction

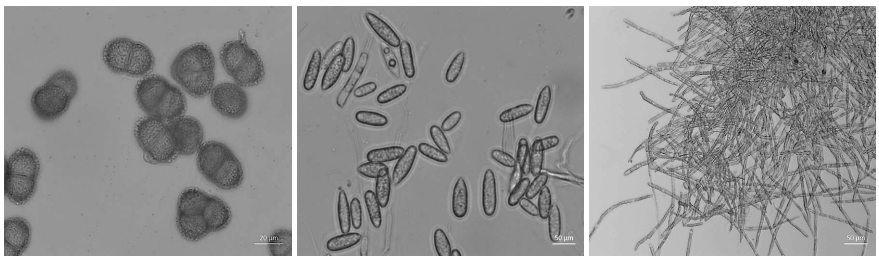
Total RNA from the three stages of *T. frezii* (three independent cultivars were pooled for each stage) was extracted with TRIzol (Invitrogen, California) according to the manufacturer's recommendations. gDNA was removed by on-column digestion with DNase (Qiagen, Germany) at twice the manufacturer's recommended concentration. Possible RNA degradation and impurities were monitored on a 1.5% (w/v) agarose gel and RNA purity was confirmed using NanoPhotometer spectrophotometer (Implen, California). RNA concentration was measured using the Qubit RNA assay kit and the Qubit Fluorometer 2.0 (Life Technologies, California).

2.3. Library Preparation and RNA-Seq

The cDNA library was prepared using the RNA of the three stages of *T. frezii*. They were performed using the NEB Next Ultra RNA kit for Illumina (Nebraska) according to the manufacturer's instructions.

2.4. RNA-Seq Data Analysis

After cluster generation, the cDNA libraries were sequenced on Illumina HiSeq 1500 to obtain 2 × 150 bp pair-end reads at the service facilities of INDEAR



(A) (B) (C)

Figure 1. Optical microscopy images from the different *T. frezii* ontogenetic stages: (A) Teliospores, (B) Basidiospores and (C) Hyphae (600×).

(Rosario, Argentina). Briefly, a de novo transcriptome was assembled with all reads, then gene expression in each condition was calculated using counts per million (CPM) of reads. RNA-Seq read quality was checked using FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads of the three ontogenetic stages of the fungus were performed and compared (basidiospores, hyphae and teliospores).

Fold change (FC) and statistical significance for all comparisons was determined by General Linear Model statistics using the EdgeR package 3.4.2 from Bioconductor [34] in the R environment (3.0.2, [35]).

2.5. Identification of *T. frezii* PCWDEs

From the analysis of the RNA-Seq data (mRNA), the sequences of the *T. frezii* proteins were deduced, which were screened to detect the presence of active carbohydrate modules using the Carbohydrate-active enzyme ANnotation (dbCAN, [36] [37]). The annotation of CAZyme modules by this program uses E-value, alignment length and coverage, with an E-value of <1e−5 for alignments of >80 amino acids and an E-value of <1e−3 for alignments of <80 amino acids [38].

To eliminate proteins that were identified by dbCAN but were not truly CAZyme, all proteins with CAZyme motifs were examined individually using MyHits [39], Prosite [40] and BLASTp of non-redundant protein sequences in National Center for Biotechnology Information (NCBI) (National Center for Biotechnology Information. Available at: https://www.ncbi.nlm.nih.gov/. Accessed: 15 March 2021 [41]). Proteins, for which the alignment coverage was less than 0.5, were also examined individually. Putative PCWDEs identified were those containing GH, PL, CE, CBM and AA modules known to be associated with the degradation of carbohydrates in the cell wall. To identify PCWDEs that may have been missed by dbCAN, additional searches, including keyword and PFAM [42] domain searches and tBLASTn analyzes [43] were performed with characterized proteins listed on the CAZy site [38] [44]. When identification of a PCWDE was not clear, CAZymes Analysis Toolkit (CAT) was used [45].

2.6. Identities Comparison of the *Thecaphora frezii* PCWDE's with their Orthologues from *U. maydis*, *Thecaphora thlaspeos* (*T. thlaspeos*), *Anthracocystis flocculosa* (*A. flocculosa*), *Sporisorium reilianum* (*S. reilianum*), *Kalmanozyma brasiliensis* (*K. brasiliensis*), *Ustilago hordei* (*U. hordei*) and *Moesziomyces antarcticus* (*M. antarcticus*)

PCWDEs identities were compared and analyzed by multiple proteins sequence and pairwise alignments, omitting the SP (signal peptide) if it is present [46]. Proteins were considered to be homologues if they shared 25% or more identity with an alignment length greater >80 amino acids [47].

2.7. qPCR Analysis of Selected Genes

The transcripts of genes presents in the PCWDE families were quantified by

real-time PCR. The oligonucleotides were designed using the program Primer-Blast (NCBI, NIH) (Table 1 shows the sequences of the primers of the ten genes with the highest FC, the rest, 30 genes, were in Table S1). Quantification of gene expression was performed using the StepOne Plus Real-Time PCR Detection System® (Thermo Fisher Scientific™, Massachusetts).

The cDNA was prepared from the same RNA samples used for RNA-Seq analysis using the enzyme SuperScript® III Reverse Transcriptase (Invitrogen; California) according to the manufacturer's recommendations. qPCR was performed with three technical replicates using the Sybr® Green Master Mix Kit (Applied Biosystems; Thermo Fisher Scientific™, California) according to the manufacturer's recommendations.

Relative gene expression was performed using the actin transcript as the reference gene for expression normalization (this gene is commonly used for normalization of fungal gene expression [48] [49] [50] [51] [52]). The program used for all targets was: 95 C for 3 minutes, 40 cycles of 95 C for 10 seconds and 60 C for 30 seconds. After this step, the fluorescence was read. At the end of the program, the temperature was reduced from 95 C to 65 C with a rate ramp of 0.1 C/s, which allowed the evaluation of the melting curves for each reaction. The specificity of the amplicon was verified by analysis of the melting curves and by sequencing of the fragments obtained. The change in expression of the target gene relative to actin expression was calculated using the $2^{-\Delta\Delta CT}$ method [53]. The mean and SE (±) were, then, determined for each of the different samples.

2.8. Statistical Analysis

Statistical analysis was performed using InfoStat software [54]. All data were calculated as mean ± standard deviation. Data were analyzed for statistical significance using the t-test.

Table 1. Real time PCR oligonucleotides.

GenBank Accession Numbers	Potential Protein Name	Primer Forward (5'→3')	Primer Reverse (5'→3')	Amplification Size (bp)
MW602834	putative GMC oxidoreductase	CTCAAGAAGACGCTCAAGGC	GATGATGCTCTCAGGGTGGT	183
MW602838	putative GMC oxidoreductase	CAGGGCTACAACCTCACGTA	GCAGACCTCCAAAGCTGATG	100
MW602839	putative GMC oxidoreductase	ACCCGAGAACAAGCCCATAA	GGAACCATGGAAAGGATGCC	135
MW602840	putative GMC oxidoreductase	GCAACCCTTACCTGCTGAAG	GATGGACGAGTCGACAAACG	182
MW602841	putative GMC oxidoreductase	ACCGACGTTGGCAAATATGG	GATGAGGGTGGCGAGGTTAT	192
MW602847	putative exo-beta-glucanase	CACCACAATCCAGGACCTCT	GAGTGAGGGATTCTGCCAGT	121
MW602848	putative glucan-beta-glucosidase	CGTGGAGCGACTTTGTCATT	AACCACTGAGTACTCGCCAA	182
MW602849	putative exo-beta-glucanase	CAGCACCTCAACACGTTCAT	TTCAGCAGGTCGTACTGGTT	149
MW602850	putative exo-1,3-beta-glucanase	AACTGGCTGCTGTTTGAAGG	ACTCGTCAAAGTGCTTCTGC	166
MW602851	putative glycoside hydrolase family	TCAACGAGCCCAACAACATC	GGTAGAGGCGGTAGACCTTG	147
MW602863	Actin	CTACGTTGCCCTCGACTTTG	CGTTTCCGACAGTGATGACC	107

3. Results

3.1. Identification of *T. frezii* PCWDEs

The analysis of the transcriptomes of the three ontogenetic stages of *T. frezii* (teliospores, basidiospores and hyphae), allowed us to search for enzymes that can degrade the plant cell wall (PCWDEs). Teliospores of *T. frezii* fungus was isolated and cultured *in vitro* to obtain its others ontogenetic stages (basidiospores and hyphae, **Figure 1**). The total RNA of the three stages was extracted and from there, the data generated by RNA-Seq was performed and analyzed. Based on these results, the respective protein sequences were deduced and transcripts were quantified (it must be taken into account that the quantifications carried out are made from the samples obtained according to the experimental conditions used, that is, culture for basidiospores and hyphae; and for teliospores, from peanut pods showing symptoms of disease).

Using the dbCAN2 metaserver (a web server), the presence of carbohydrate-active enzyme ANnotation modules were identified and these were analyzed complementarily with the MyHits, Prosite and BLASTp sites. From there, we focused on identifying the various enzymes that comprised the GH, PL, CE, CBM and AA modules. More than 135 translated mRNA were identified that had these features, but only 40 of them were compatible with the functions of the PCWDEs. 27 GH, 2 PL, 3 CE and 8 AA were detected (all sequences data were deposited into GenBank at https://www.ncbi.nlm.nih.gov/genbank/ site). Among them, 18 enzymes would degrade cellulose; 1 cutin; 1 galactomannan; 9 pectins; 7 xylans and 4 xyloglucans (**Table 2** and **Figure 2**). In addition, this table compares the expressions of the different transcripts using the FC between basidiospore versus teliospore, and hyphae versus teliospore stages and shows the presumed EC number of enzymes and the CAZyme family to which they belong.

Cellulose: *T. frezii* expressed genes whose encoded proteins would potentially act on cellulose, eight were from AA and ten were from GH (**Table 2** and **Figure 2**).

CAZy auxiliary activity family 3 (AA3) includes enzymes from the glucose-methanol-choline (GMC) family of oxidoreductases that support the activity of other AA family enzymes via their reaction products or support the action of glycoside hydrolases in lignocellulose degradation [55].

The transcript levels for five expressed AA3 genes were highest in the basidiospores and hyphae stages compared to teliospores, and only three are decreased in both stages. Under our conditions, a *T. frezii* AA3 enzyme (putative GMC oxidoreductase, MW602841) was the most highly expressed PCWDE gene.

The GH5 family has a range of activities and target substrates and has recently been classified into 51 subfamilies [56].

The β-glucosidases are predominantly found in the GH1 and GH3 families. However, these families also contain other glycosidases. A feature typical of most, but not all, cellulases, and also found in some other PCWDEs, is the presence of

Table 2. *T. frezii* PCWDE genes expressed at teliospores, basidiospore or hyphal stages.

GenBank Accession Numbers	Overexpression (FC[a] B vs. T)	Overexpression (FC[a] H vs. T)	Substrate	EC Number	CAZyme family[b]	mRNA expression (Basidiospore)[c]	mRNA expression (Hyphae)[c]	mRNA expression (Teliospore)[c]
MW602835	0.91	0.17	Cellulose	1.1.99.18	AA3	0.0703	0.0135	0.0773
MW602836	0.47	0.32	Cellulose	1.1.99.18	AA3	0.0042	0.0029	0.0091
MW602837	0.64	0.58	Cellulose	1.1.99.18	AA3	0.0792	0.0712	0.1233
MW602842	0.30	0.25	Cellulose	3.2.1.-	GH5	0.0089	0.0075	0.0297
MW602843	1.07	0.62	Cellulose	3.2.1.-	GH5	0.1832	0.1052	0.1708
MW602844	1.01	0.46	Cellulose	3.2.1.-	GH5	0.0665	0.0302	0.0658
MW602845	0.25	0.18	Cellulose	3.2.1.78	GH5	0.0164	0.0117	0.0646
MW602846	0.21	0.16	Cellulose	3.2.1.58	GH5	0.0453	0.0343	0.2179
MW602852	0.34	0.39	Cutin	3.1.1.74	CE5	0.0506	0.0592	0.1508
MW602853	1.50	1.15	Galactomannan	3.2.1.22	GH27	0.0009	0.0007	0.0006
MW602854	0.33	0.37	Pectin	3.2.1.172	GH105	0.0061	0.0069	0.0187
MW602855	0.59	0.60	Pectin	3.2.1.31	GH2	0.1867	0.1894	0.3174
MW602856	0.09	0.18	Pectin	3.2.1.15	GH28	0.0016	0.0030	0.0173
MW602857	3.26	0.30	Pectin	3.2.1.15	GH28	0.2733	0.0250	0.0839
MW602858	0.94	0.90	Pectin	3.2.1.15	GH28	0.0350	0.0336	0.0374
MW602859	0.36	0.75	Pectin	3.2.1.99	GH43	0.0584	0.1232	0.1640
MW602860	0.11	0.14	Pectin	3.2.1.89	GH53	0.0418	0.0547	0.3930
MW602861	0.38	0.05	Pectin	4.2.2.2	PL1	0.0076	0.0010	0.0200
MW602862	0.52	0.26	Pectin	4.2.2.23	PL4	0.0277	0.0140	0.0537
MW602864	6.58	2.08	Xylan	3.2.1.8	GH10	0.0276	0.0087	0.0042
MW602865	7.30	0.58	Xylan	3.2.1.99	GH43	0.0382	0.0031	0.0052
MW602866	2.23	1.02	Xylan	3.2.1.55	GH43	0.1381	0.0628	0.0618
MW602867	0.68	0.60	Xylan	3.2.1.8	GH43	0.1000	0.0876	0.1461
MW602868	0.08	0.07	Xylan	3.2.1.55	GH51	0.0106	0.0095	0.1315
MW602869	0.74	0.52	Xylan	3.1.1.72	CE1	0.0313	0.0218	0.0423
MW602870	2.45	0.93	Xylan	3.1.1.73	CE7	0.0292	0.0111	0.0119
MW602871	1.92	2.29	Xyloglucan	3.2.1.177	GH31	0.0409	0.0487	0.0213
MW602872	0.42	0.17	Xyloglucan	3.2.1.177	GH31	0.0358	0.0147	0.0853
MW602873	0.07	0.45	Xyloglucan	3.2.1.23	GH42	0.0003	0.0018	0.0040
MW602874	0.26	0.37	Xyloglutan	3.2.1.23	GH35	0.0338	0.0473	0.1283

[a]FC, fold change difference between basidiospore (B) or hyphae (H) compared to teliospore (T) stage. [b]GH, glycoside hydrolases; AA, Auxiliary Activities; CE, Carbohydrate Esterases; PL, Polyssacharides Lyases. Numbers associated with CAZy types refer to the family number. [c]Mean of three technical replicates normalized by actin expression.

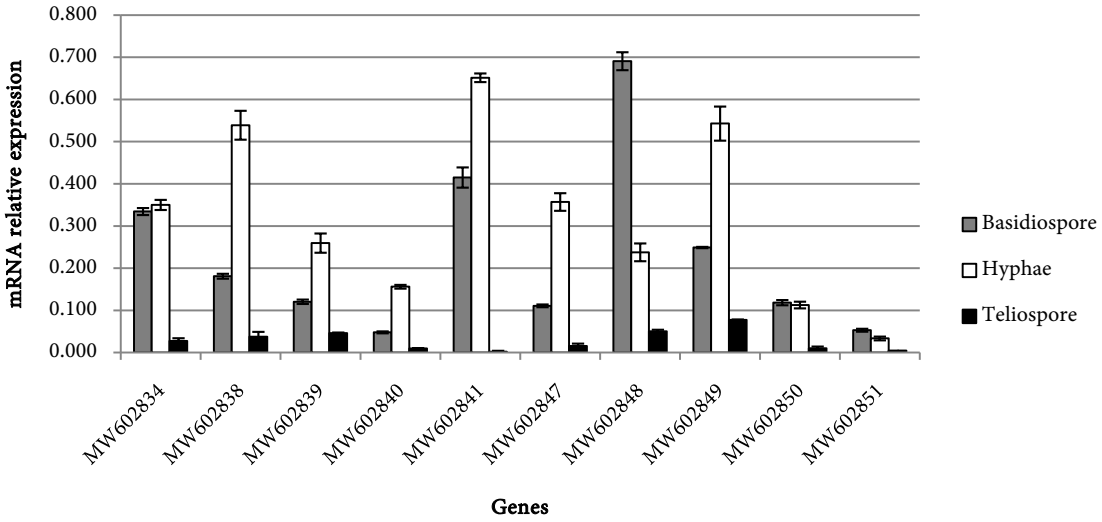


Figure 2. PCWDE mRNA expression levels from *T. frezii* according to their ontogenetic stages. The mean and SE (±) were determined for each of the different samples (technical triplicates normalized with actin transcript). List of genes from which the corresponding proteins are deduced, all having cellulose as a substrate: 1) Putative GMC oxidoreductase (MW602834); 2) Putative GMC oxidoreductase (MW602838); 3) Putative GMC oxidoreductase (MW602839); 4) Putative GMC oxidoreductase (MW602840); 5) Putative GMC oxidoreductase (MW602841); 6) Putative exo-beta-glucanase (MW602847); 7) Putative glucan-beta-glucosidase (MW602848); 8) Putative exo-beta-glucanase (MW602849); 9) Putative exo-1,3-beta-glucanase (MW602850); and 10) Putative glycoside hydrolase family (MW602851). The first 5 genes belong to the AA3 family, from genes 6 to 9 to the GH5 family and the gene 10 to the GH1 family.

a polysaccharide that binds to a domain linked by a hinge region that aids in the binding of cellulases to their insoluble substrates [8]. We found high expression of a GH1 (putative glycoside hydrolase family 1, MW602851) in both basidiospores and hyphae stages (**Table 2** and **Figure 2**).

Galactomannan: Alpha-galactosidases catalyze the hydrolysis of terminal alpha-1,6-galactosyl units from galacto-oligosaccharides and polymeric galactomannans and are assigned to glycosyl hydrolase family 27 (GH27), for which several members are structurally known (CAZy) [57]. We found only one transcript (putative alpha-galactosidase, MW602853) with these properties and its expression was 1.15- and 1.50-fold higher in hyphae and basidiospores, respectively, than in teliospores (**Table 2**).

Xylan: Many proteins encoded by genes of the GH and CE families act on hemicellulose as well as a number of other polysaccharides. Although some genes targeting hemicellulose were expressed during all ontogenetic stages, the highest transcript levels were observed mainly during the basidiospores stage (**Table 2** and **Figure 2**).

GH10 proteins are endo-β-1,4-xylanases that act on xylans, glucuronoxylans and glucuronoarabinoxylans [3], and we found one whose gene expression peaked during the basidiospore stage (**Table 2** and **Figure 2**).

Three different GH43s were found, arabinase; alpha-l-arabinofuranosidase and endo-beta-1,4-xylanase (MW602865; MW602866 and MW602867, respectively). The MW602865 transcript was found to be seven FC more expressed in

basidiospores compared to teliospores (Table 2 and Figure 2).

Another enzyme of the glycoside hydrolase family expressed in different stages of *T. frezii* was alpha-L-arabinofuranosidase (GH51) (MW602868), whose level was 13- to 12-fold higher in teliospores compared with hyphae and basidiospores, respectively (Table 2 and Figure 2). Other gene family whose expression was slowly increased in basidiospores and is thought to encode hemicellulose-directed enzymes was CE7 esterase, which remove the acetyl moiety of xylans (Table 2 and Figure 2).

Xyloglucan: As shown in Table 2 and Figure 2, two alpha-xylosidases releasing alpha-xylose from xyloglucan oligosaccharides belonging to the GH31 family were identified.

Two beta-galactosidases were found (MW602873 and MW602874), belonging to the GH42 and GH35 families, respectively. Their expressions are decreasing from teliospores, passing through hyphae and their lowest expression was found in basidiospores (Table 2 and Figure 2).

Pectin: The transcriptome of *T. frezii* contains nearly nine PCWDEs that are likely to act specifically on pectins. These nine PCWDEs are derived from seven CAZyme families. Only one transcript is overexpressed in the basidiospore stage compared to what is expressed in teliospores (MW602857) and all transcript were down-regulated in the hyphae stage, also compared to the expression that occurs in teliospores (MW602854, MW602855, MW602856, MW602857, MW602858, MW602859, MW602860, MW602861, MW602862).

Within the families of glycoside hydrolases, we found one GH105 with unsaturated rhamnogalacturonyl hydrolase (MW602854); one GH2 with beta-glucuronidase (MW602855); three GH28s with endo-polygalacturonase (MW602856, MW602857, and MW602858); one GH43 with arabinan endo-1,5-alpha-L-arabinanase (MW602866); and one GH53 with arabinogalactan endo-beta-1,4-galactanase (MW602860) activities. We also found two pectin lyases, PL1 and PL4, with pectate lyase (MW602861) and rhamnogalacturonan endolyase (MW602862) similarities, respectively; both express mainly in teliospores (Table 2 and Figure 2).

Cutin: Cutin is composed of hydroxy and hydroxyepoxy fatty acids. Cutinases (family CE5) catalyze the cleavage of the ester bonds of cutin to release cutin monomers. There are several fungi that express these enzymes [11]. In our samples, we found similar levels of expression in basidiospores and hyphae and approximately 3 fold higher in teliospores (Table 2).

Although in the *T. frezii* transcriptome the number of genes with CAZy domains was higher than those we showed (more than 135), we only list transcripts that we could detect possible PCWDE activity using websites tools. An additional complication we encountered was that the genome of *T. frezii* is unknown. If it were available, it would allow us to make more and better comparisons, particularly with similar species.

In addition, we compared the identity of each of the derived enzymes of *T.*

frezii with their orthologues from *U. maydis*, *T. thlaspeos*, *A. flocculosa*, *S. reilianum*, *K. brasiliensis*, *U. hordei* and *M. antarcticus* (because they belong to the same taxonomic class (Ustilaginomycetes), and some of them are phytopathogenics, generating smut diseases (Table 3 and Table S2).

Table 3. Analysis of *T. frezii* PCWDE (length and identities comparison with *U. maydis*, *T. thlaspeos*, *A. flocculosa* and *U. hordei*).

PCWDE GenBank Accession Numbers	Deduced Protein Length (number of amino acids)	Percentage (%) of Protein Identity/Accession Number of Reference Sequence			
Thecaphora frezii		*Ustilago maydis*	*Thecaphora thlaspeos*	*Anthracocystis flocculosa*	*Ustilago hordei*
MW602834	600	83/XP_011386298.1	80/UWYS01000020.1	87/XP_007882019.1	83/XP_041409722.1
MW602835	710	75/XP_011387932.1	82/UWYS01000014.1	84/XP_007879028.1	74/XP_041411102.1
MW602836	564	55/XP_011389669.1	76/UWYS01000011.1	75/XP_007881316.1	54/XP_041415878.1
MW602837	610	75/XP_011387685.1	82/UWYS01000003.1	86/XP_007879328.1	76/XP_041411430.1
MW602838	692	57/XP_011391368.1	62/UWYS01000029.1	68/XP_007878618.1	54/XP_041413656.1
MW602839	628	60/XP_011387810.1	72/UWYS01000022.1	78/XP_007882371.1	29/XP_041415878.1
MW602840	697	68/XP_011390038.1	76/UWYS01000009.1	79/XP_007878320.1	43/GAC71446.1
MW602841	623	55/XP_011389987.1	70/UWYS01000009.1	73/XP_007878214.1	54/XP_041411913.1
MW602842	818	63/XP_011386174.1	69/UWYS01000001.1	69/XP_007876186.1	62/XP_041409506.1
MW602843	882	57/XP_011390266.1	68/UWYS01000006.1	77/XP_007879865.1	59/XP_041413492.1
MW602844	476	55/XP_011391775.1	67/UWYS01000014.1	74/XP_007880014.1	54/XP_041412014.1
MW602845	511	67/XP_011387602.1	57/UWYS01000003.1	69/XP_007879449.1	ND/ND
MW602846	463	64/XP_011386164.1	59/UWYS01000001.1	73/XP_007876246.1	62/XP_041409492.1
MW602847	535	31/XP_011386896.1	73/UWYS01000015.1	75/XP_007881477.1	35/XP_041409492.1
MW602848	632	52/XP_011392454.1	64/UWYS01000007.1	65/XP_007877815.1	52/XP_041412344.1
MW602849	517	59/XP_011391862.1	72/UWYS01000023.1	73/XP_007881381.1	57/XP_041414386.1
MW602850	628	65/XP_011386896.1	73/UWYS01000029.1	76/XP_007878549.1	66/XP_041415648.1
MW602851	409	ND/ND	72/UWYS01000006.1	76/XP_007881827.1	ND/ND
MW602852	259	61/XP_011386312.1	65/UWYS01000015.1	59/XP_007880759.1	62/CCF54195.1
MW602853	365	57/XP_011390836.1	ND/ND	60/XP_007878151.1	39/CCF50716.1
MW602854	396	66/XP_011387870.1	69 7 UWYS01000003.1	75/XP_007882544.1	67/XP_041411152.1
MW602855	664	ND /ND	ND/ND	60/XP_007878519.1	53/XP_041415678.1
MW602856	376	55/XP_011388929.1	43/UWYS01000002.1	56/XP_007879026.1	57/XP_041414954.1
MW602857	462	26/XP_011388929.1	57/UWYS01000017.1	60/XP_007880267.1	ND/ND
MW602858	358	64/XP_011388929.1	65/UWYS01000014.1	65/XP_007879026.1	64/XP_041414954.1
MW602859	315	ND/ND	59/UWYS01000014.1	67/XP_007880158.1	ND/ND
MW602861	388	47/XP_011391598.1	66/UWYS01000006.1	66/XP_007876158.1	ND/ND
MW602862	685	ND/ND	58/UWYS01000001.1	61/XP_007879517.1	45/XP_041412196.1

Continued

MW602860	355	ND/ND	53/UWYS01000016.1	68/XP_007879956.1	ND/ND
MW602864	340	50/XP_011389883.1	53/UWYS01000019.1	51/XP_007880950.1	47/XP_041411767.1
MW602865	300	50/XP_011389888.1	81/UWYS01000010.1	79/XP_007878036.1	30/XP_041414991.1
MW602866	406	62/XP_011387715.1	69/UWYS01000010.1	81/XP_007878081.1	ND/ND
MW602867	388	69/XP_011391222.1	65/UWYS01000001.1	76/XP_007876316.1	65/XP_041413719.1
MW602868	317	48/XP_011386874.1	64/UWYS01000013.1	66/XP_007878998.1	50/XP_041415698.1
MW602869	311	64/XP_011391122.1	56/UWYS01000015.1	71/XP_007881474.1	63/XP_041414473.1
MW602870	594	ND/ND	53/UWYS01000019.1	53/XP_007881882.1	27/XP_041412944.1
MW602871	1069	73/XP_011391102.1	81/UWYS01000023.1	80/SPO40945.1	72/XP_041414441.1
MW602872	880	61/XP_011387863.1	91/UWYS01000003.1	74/XP_007882546.1	61/XP_041411161.1
MW602873	787	60/XP_011388548.1	77/UWYS01000001.1	79/XP_007875969.1	62/XP_041413828.1
MW602874	547	65/XP_011388663.1	73/UWYS01000008.1	71/XP_007877405.1	60/XP_041412864.1

ND: no data.

3.2. Snf1 Expression

In *T. frezii* transcriptome, we found a transcript that, when translated into a protein, has a sequence compatible with the Snf1 protein (MW691285) because it has 763 amino acids in length, the conserved threonine at position 202 (which is critical for activation by upstream kinases), and has 68% identity to the *U. maydis* orthologue (**Figure S1**). Furthermore, the identity at the protein level found with other Ustilaginomycetes was: *T. thlaspeos* (71%), *A. flocculosa* (76%), *S. reilianum* (69%), *K. brasiliensis* (71%), *U. hordei* (69%) and *M. antarcticus* (69%). It is also interesting to note that the relative expression levels in basidiospores (0.016) and hyphae (0.022) were significantly lower compared to teliospores (0.325).

4. Discussion

The plant surface is the first line of defense to prevent pathogens from penetrating and causing infection. The composition and structure of cell walls make it difficult for the pathogen to advance. Cell walls are made up of celluloses, hemicelluloses, pectins, structural proteins, and middle lamellae, which consist mainly of pectins. To overcome this line of defense, fungi generally secrete a mixture of hydrolytic enzymes including cutinases, cellulases, pectinases, and proteases.

Some of the fungi responsible for diseases in peanuts include the genera *Aspergillus*, *Penicillium*, *Sclerotinia*, *Fusarium*, *Rhizopus* and *Thecaphora* [17] [18] [19] [58]. For example, *Fusarium oxysporum* produces several enzymes that act upon the pectic and cellulose components of cell walls of host plant [59].

The phytopathogenicity of some fungi is related to the expression of certain enzymes, which are classified as Plant Cell Wall-Degrading Enzymes. Many fungi expressing these enzymes have different diets; they can be saprophytic, hemibiotrophic and necrotrophic [3].

Through bioinformatics analysis, we identified transcripts in *T. frezii* that have the potential to translate and produce enzymes that form the PCWDE group. In parallel, our comparisons were mainly made with the enzymes expressed by the fungus *U. maydis*, *T. thlaspeos*, *A. flocculosa*, *S. reilianum*, *K. brasiliensis*, *U. hordei* and *M. antarcticus* (fungi of the same class). The highest identities of the deduced PCWDEs were found with their orthologues of *A. flocculosa*, *T. thlaspeos* and *U. maydis*, respectively. This could mean a greater phylogenetic closeness between these fungi. At the time of our analysis and based on the data deposited in the NCBI NIH database, there were only 4 proteins and 276 nucleotide sequences deposited for the taxonomy corresponding to *Thecaphora*. Therefore, we believe that the contribution of these sequences from the PCWDE of *T. frezii* may contribute to constructing some phylogenetic distances with fungi of the same class.

Forty candidate genes with PCWDE potential contributing to plant interaction and pathogenicity were expressed in *T. frezii*. They were classified into 27 glycoside hydrolases, two polysaccharide lyases, three carbohydrate esterases and eight enzymes with auxiliary activities.

For the degradation of cellulose, galactomannans, xylans, xyloglucans and pectin, fungi belonging to the phylum Basidiomycetes expressed the enzymes AA3, GH1 and GH5; GH27; GH10, GH43; GH31, GH35; GH105, GH2, GH28, GH43, GH53, PL1 and PL4, respectively, which is consistent with their occurrence in *T. frezii* [60].

On the other hand, it is well known that the Snf1 protein kinase is conserved in eukaryotic organisms. According to Ludin [61], in *Saccharomyces cerevisiae*, the SNF1 gene is essential for the transcription of genes repressed by glucose. The direct interaction between Snf1 and its activating subunit, Snf4, within the kinase complex is regulated by the glucose signal. In addition, they demonstrated that the catalytic domain of Snf1 presents a critical conserved amino acid threonine, at position 220. Our results in *T. frezii* show the presence of threonine at the critical site.

At the same time, according to the evidence by Ahuatzi [62], the Snf1 kinase is activated under low glucose conditions. Interestingly, in our work, we found very high levels of Snf1 in teliospores that, curiously, are not exposed to high levels of glucose, since they are found directly in the peanut pods. Meanwhile, low levels of expression of this gene are found in hyphae and basidiospores stages that are grown in medium with dextrose.

In this work, we sought to identify the presence of multiple PCWDEs (in the three ontogenetic stages of *T. frezii*) and we compare their identities with other fungi of the same class. The expression analyzed in this work is not necessarily the expression that occurs in the peanut plant, because the cultivation conditions of the three ontogenetic stages are not the same or similar to what happens in the plant, and this can also differentially regulate the expression of certain PCWDEs. Their expressions could be affected by transcriptional regulation dependent on genes such as SNF1 (whose expression varies depending on the car-

bon source that the fungus uses). In our experimental conditions, basidiospores and hyphae are cultured with glucose, while teliospores are obtained directly from peanut fruit (teliospores cannot be cultured because they change their ontogenetic stage in the process). In this sense, it is logical to find a high level of Snf1 transcript in teliospores, compared to that of basidiospores and hyphae.

This is the first work that describes the expression of some enzymes with potential cell wall degradation activity in the peanut plant by the fungus *T. frezii*. These findings could contribute to understanding part of the mechanism by which *T. frezii* begins its infection process in peanut plants.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary Material

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T.frezii        ----MSSSARSPGHAADANARA-SAKAASASTLGLGSGGKAAPKQPVRIGQYTLQQTLGT        55
U.maydis521     MSGRDASSRRQPSDHPDGAASSSRSSSRHPSRSGSVHRTKESTKQPVRIGQYILQQTLGA        60
                     :** *.*..  *. * :  :.:   *  *     * : ********* ******:
T.frezii        GSFGKVKLATHSLTGHRVAMKIINRRKISSLDMGGRVKREIQYLKLLRHPHIIKLYEVIT        115
U.maydis521     GSFGKVKLATHALTGHRVAMKIINRRKISSLDMGGRVKREIQYLKLLRHPHIIKLYEVIT        120
                ***********:**************************************************
T.frezii        TPSDIIMVIEYAGGELFQYIVDRGRMSENEARRFFQQIICAIEYCHRHKIVHRDLKPENL        175
U.maydis521     TPNDIIMVIEYAGGELFQYIVDRGRMPEHEARRFFQQVICAMEYCHRHKIVHRDLKPENL        180
                **.**********************.*:********:***:******************
T.frezii        LLDEYLNVKIGDFGLSNIMTDGDFLKTSCGSPNYAAPEVISGRLYAGPEIDIWSCGVILY        235
U.maydis521     LLDEYLNVKIGDFGLSNIMTDGDFLKTSCGSPNYAAPEVISGRLYAGPEIDIWSCGVILY        240
                ****************************************************************
T.frezii        VMLCGRLPFDDEYIPTLFKKINGGIYTLPSFLSQEARHLLSQMLVVDPVKRITISEIRGH        295
U.maydis521     VMLCGRLPFDDEYIPTLFKKINNGIYTLPSYLSQEARHLLSQMLVVDPVKRITIQEIRQH        300
                **********************.*******:**********************.*** *
T.frezii        PWFNVDLPAYLRPLPPTPAVENAGFNFGISAMSECASAEDSSTSPS---------AAVAS        346
U.maydis521     PWFNVDLPAYLRPLPPTPATENHGFHFGMTASPADTGSPADVTSPTNSSSAANSGASSQT        360
                ********************.** **:**::*    :.:  . ***:         *:  :
T.frezii        GSFSSRPGSQGQPTPDLGMIDPDIVDELVGKMVGFDREDVLHHLREKGDNQVKVAYQLVR        406
U.maydis521     SAPASRPGSQPIVTGDLGTIENDIVDELVGKMMGFNRDELMHHLTEKGDNQVKVAYQLVR        420
                .: :******   * *** *: **********:**:*::::*** ***************
T.frezii        DHHRMLSIHHMEDQHGMENFLAQSPPPWNEGLDGVMARSTSLKRKPKLLERIATREGGNA        466
U.maydis521     DHRRMLQIHHMEDAHGMETFLAQSPPAWNEGLEGFMGRSTSIRRKNRDKEAAAVTSNKDQ        480
                **:***.****** ****.******* *****:*.*.****::** :  *  *. .. :
T.frezii        V----------------------------AEEEAETSIMEPDAESDDGNETFASEDDDGL        498
U.maydis521     VPPLPTSAQAHVHAQAQAQAHAAVSGAGEDEGDSFTADVAEPEPSDDGNDTLASDDDDVL        540
                *                              * :: *: :     *****:*:**:*** *
T.frezii        SDDDAHLIEEDDTEGARHTGIAVLETSLPGYLRAREAERLVTPSVERQPAWPKAGA---G        555
U.maydis521     TDDDGQLTDVEDGSAERIVRIAVLETSLPGFLRAREAERLATPSAEK-TSWPACTPIASA        599
                :***.:* : :* .. * . **********:*********.***.*:  :** .     .
T.frezii        ITPVPLPPTTAQIHKKPRSRWHFGIRSRSPPMEIMLELYRTLQVLGMEWRAKPSQAAAKA        615
U.maydis521     ATAALAQASTAQMHKKPRSRWHFGIRSRSPPMEIMLELYRTLQSLGMEWRAKPAAKQAKG        659
                 * .    :***:******************************** *********:   **.
T.frezii        RDE-----------DRKETHPEDASKGEELFFLETRWKVGDVLVRMDLQLYHVDSANFLV        664
U.maydis521     SGGDGAAGADDAKEKGKNASGSAVSKGEELFFLETRWKVGHVLVRMDLQLYHVDAANYLV        719
                 .            . *::  . .****************.**************:**:**
T.frezii        DFRNVGYTRLQRDASEA----------------------DDAGSSPPPTVDANKLEAAFD        702
U.maydis521     DFRNVGYTTQETSFGSDDEHDGHDDQRDSAGNKSAAMEDGAGGAGGTAIMDVSKLEAAFD        779
                      ********  : . ..                              . .*:.    :*..*******
T.frezii        EVMTASQQALSDD-GLRKHRFDGSGRKPSLAPAVPAGRKEVNSPFLFLECATRLIVELAG        761
U.maydis521     KAMTEAQQAMQDGEHAGQHRFDGTRIKPSLAPAVPAGRKEVNSPFLFLECATRLIVELAG        839
                :.** :***:.*.    :*****:  **********************************
T.frezii        GS                                                            763
U.maydis521     GA                                                            841
                *:
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Figure S1. Sequence alignment of *T. frezii* and *U. maydis* Snf1 proteins. In the rectangle, the threonine conserved amino acid, at position 202. * conserved amino acid, : strong amino acid conservation and, . weak amino acid conservation.

Table S1. Real Time PCR oligonucleotides.

GenBank Accession Numbers	Potential Gene Name	Primer Forward (5'→3')	Primer Reverse (5'→3')	Amplification Size (bp)
MW602835	putative GMC oxidoreductase	GCCGCCTTCATGACTAACTG	GATCGAACCAGCCGAAACAA	141
MW602836	putative GMC oxidoreductase	GGCCCAACATCAAGGTCTTC	GAGGCCCGACTTATCCTTGA	110
MW602837	putative GMC oxidoreductase	GAGGATCGAGGCATCATTGC	GCTGCAGCTTGATACCTTCC	160
MW602842	putative Cellulase	GAGCTTCCGGCTAGGAGTAG	GCTCCTTTGACAGCCAGATG	128
MW602843	putative Cellulase	TACGTCAAATCCTGGCCGAT	TTTCATCCCACTCCCAGACC	175
MW602844	putative Cellulase	ATCCTGATGCACCTCGACAA	GCGAGATGGTCTGGATGTTG	169
MW602845	putative Cellulase	CATTTCCTGGGCCAACAACA	GTCGACGTAGTCGCTAAACG	139
MW602846	putative Cellulase	ATTGCGCAAGGTACCTCAAC	AACATTTGGCGCAGGTAGTC	151
MW602852	putative Cutinase	CTTCATCCTGCAGGGCTACT	GTTACCGATGAGCACGACAC	109
MW602853	putative Alpha 1,4 galactosidase	TGGAACACCTACACGGTCAA	GAGAAGCGCGAGACAAAGTC	104
MW602854	putative Glycosyl hydrolase family 88	GGTCGCATCATCGACACAAA	CATGCGGTCTTCGAACCATT	141
MW602855	putative Glycosyl hydrolases family 2	CTCGTCACCATCCACGACTA	GGCGACAAAGATCGGATAGC	108
MW602856	Putative Glycosyl hydrolases family 28	AATGGGATCAGCCTCACCAA	AATCCCTGCAACTTCCCTCA	106
MW602857	Putative Glycosyl hydrolases family 28	AGCTGGTGATCTCCGACTTC	AAGCGTGGTTGATGGTGATG	177
MW602858	Putative Glycosyl hydrolases family 28	GCTTCGACTGTTGCTGTTCT	TGGATGTTGCTCAGGGTGAT	131
MW602859	Putative Glycosyl hydrolases family 43	GTTTGGAACGGGCATCTACC	CAGCACCTGTCAAACGAGAG	171
MW602860	Putative Glycosyl hydrolases family 53	ATCCAGGTGGTGGAAACCAA	TGCTCTTCCAGAAGGGTTCC	184
MW602861	Putative Pectate lyase	TGTCACCGGCTATGACAACT	TGTTGGCAAACGTGATCTGG	146
MW602862	Putative Polysaccharide lyase family 4	CATCACGTCGGTCAACGAAA	TGTAGTAGCGCGCAAAGTTC	158
MW602864	Putative Glycosyl hydrolase family 10	CCAGGCTTGCAAGAGTATCG	GCGGGCTTCTTGTTGTAGTT	123
MW602865	Putative Glycosyl hydrolase family 32	CCATCTATCCCACCAGCGAT	GTTCTCGTCCTTGGTGAAGC	191
MW602866	Putative Glycosyl hydrolase family 43	AAATGGAAGCCAGCTGTACG	TTAGCCTCGTTGCGGTAGAT	102
MW602867	Putative Glycosyl hydrolase family 43	CGTACCTCACCTACCACCAG	CGTGAAATGGCGAAGGATGT	100
MW602868	Putative Alpha-L-arabinofuranosidase	AGGTGATCAACACCAAGGCT	ATACCCGTCAGCTGCCATAG	106
MW602869	Putative Acetylxylan esterase	TCGAGAAGCTCGAACAAGGT	GATCCTCGGGCCTAAGTTGA	96
MW602870	Putative Feruloyl esterase	GGAAAGACATTCACGTGCGA	AGGTTGGTGTCATAGCCGAA	143
MW602871	Putative Glycosyl hydrolases family 31	TACTACATCGGCGAGATCGG	ATACGGCTTATCCTCGGCAA	93
MW602872	Putative Glycosyl hydrolases family 31	CAGAACCTACCCAGAAGCCA	ATCGAGATCAAGGCGAGTGT	108
MW602873	Putative Beta-galactosidase	TCGGACATCGTCATCGAGTT	TAGTGGTCGAAGTCGGTAGC	110
MW602874	Putative Glycosyl hydrolases family 35	GTATCACGAAGCCACCAACC	TGATTGGCCACCTTGGAGAA	138

Table S2. Analysis of *T. frezii* PCWDE (length and identities comparison with *Sporisorium reilianum*, *Kalmanozyma brasiliensis* and *Moesziomyces antarcticus*).

PCWDE GenBank Accession Numbers	Deduced Protein Length (number of amino acids)	Percentage (%) of Protein Identity/Accession Number of Reference Sequence		
Thecaphora frezii		*Sporisorium reilianum*	*Kalmanozyma brasiliensis*	*Moesziomyces antarcticus*
MW602834	600	83/SJX60469.1	83/XP_016295029.1	83/XP_014659543.1
MW602835	710	75/SJX62136.1	74/XP_016292951.1	75/XP_014658543.1
MW602836	564	55/CBQ73647.1	28/XP_016292807.1	55/XP_014657062.1
MW602837	610	76/SJX61802.1	75/XP_016292663.1	72/GAC76671.1
MW602838	692	59/CBQ69373.1	57/XP_016294204.1	58/XP_014653303.1
MW602839	628	57/CBQ72101.1	57/XP_016292807.1	60/XP_014658379.1
MW602840	697	66/CBQ73979.1	67/XP_016294579.1	66/GAC73024.1
MW602841	623	52/SJX63798.1	56/XP_016294560.1	55/XP_014656193.1
MW602842	818	62/SJX60288.1	64/XP_016294861.1	62/GAC73485.1
MW602843	882	58/CBQ68442.1	58/XP_016293046.1	58/XP_014655816.1
MW602844	476	55/CBQ69840.1	54/XP_016293731.1	54/XP_014655209.1
MW602845	511	66/SJX61686.1	68/XP_016292559.1	67/GAC76557.1
MW602846	463	64/CBQ67637.1	64/XP_016294848.1	61/XP_014659737.1
MW602847	535	35/CBQ67637.1	37/XP_016291715.1	35/XP_014659737.1
MW602848	632	54/SJX66403.1	51/XP_016292381.1	54/XP_014654407.1
MW602849	517	55/SJX64821.1	55/XP_016294313.1	59/XP_014655490.1
MW602850	628	65/SJX60939.1	65/XP_016295177.1	69/GAC72911.1
MW602851	409	69/CBQ73807.1	ND/ND	67/XP_014656356.1
MW602852	259	60/CBQ67856.1	66/XP_016295047.1	62/XP_014659518.1
MW602853	365	56/SJX64569.1	56/XP_016293526.1	57/XP_014655410.1
MW602854	396	67/SJX62056.1	68/XP_016292880.1	68/GAC76931.1
MW602855	664	ND/ND	55/XP_016295198.1	52/GAC72934.1
MW602856	376	57/CBQ72875.1	ND/ND	58/XP_014657530.1
MW602857	462	24/SJX62708.1	ND/ND	26/XP_014657530.1
MW602858	358	69/CBQ72875.1	ND/ND	70/XP_014657530.1
MW602859	315	ND/ND	ND/ND	26/XP_014654444.1
MW602861	388	ND/ND	ND/ND	ND/ND
MW602862	685	47/SJX65314.1	ND/ND	50/XP_014655028.1
MW602860	355	ND/ND	ND/ND	ND/ND
MW602864	340	48/CBQ73812.1	26/XP_016292247.1	49/XP_014656336.1
MW602865	300	32/SJX60889.1	ND/ND	48/GAC73181.1
MW602866	406	62/SJX61842.1	ND/ND	62/SPO44190.1
MW602867	388	67/CBQ69308.1	68/XP_016294261.1	68/GAC71400.1

Continued

MW602868	317	49/CBQ70225.1	48/XP_016295216.1	52/XP_014658559.1
MW602869	311	55/SJX64888.1	64/XP_016294378.1	64/XP_014655560.1
MW602870	594	ND/ND	37/XP_016291482.1	29/XP_014657903.1
MW602871	1069	73/CBQ69224.1	73/XP_016294356.1	73/XP_014655537.1
MW602872	880	60/CBQ72182.1	60/XP_016292886.1	61/GAC76924.1
MW602873	787	62/CBQ72762.1	63/XP_016289797.1	64/GAC72061.1
MW602874	547	60/CBQ71096.1	65/XP_016290857.1	60/XP_014657670.1

ND: no data.