Induction of Genes Encoding Plant Cell Wall-Degrading Carbohydrate-Active Enzymes by Lignocellulose-Derived Monosaccharides and Cellobiose in the White-Rot Fungus Dichomitus squalens

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2 # lignocellulose-derived monosaccharides and cellobiose in the white-rot

3 # fungus *Dichomitus squalens*

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13 Running title: Induction of lignocellulolytic genes in *D. squalens*

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Abstract

Fungi can decompose plant biomass to small oligo- and monosaccharides to be used as carbon sources. Some of these small molecules may induce metabolic pathways and production of extracellular enzymes targeted for degradation of plant cell wall polymers. Despite extensive studies in ascomycete fungi, little is known about the nature of inducers for the lignocellulolytic systems of basidiomycetes. In this study, we analyzed six sugars, known to induce expression of lignocellulolytic genes in ascomycetes, for their role as inducer in the basidiomycete white-rot fungus *Dichomitus squalens* using a transcriptomic approach. This identified cellobiose and L-rhamnose to be the main inducers of cellulolytic and pectinolytic genes, respectively, of *D. squalens*. Our results also identified differences in gene expression patterns between a dikaryotic and monokaryotic strain of *D. squalens* cultivated on plant biomass derived monosaccharides and the disaccharide cellobiose. This suggests that despite conservation of the induction between these two genetic forms of *D. squalens*, the fine-tuning in gene regulation of lignocellulose conversion is differently organized in these strains.

Importance

Wood-decomposing basidiomycete fungi have a major role in the global carbon cycle and are promising candidates for lignocellulosic biorefinery applications. However, information on which components trigger enzyme production is currently lacking, which is crucial for efficient use of these fungi in biotechnology. In this study, transcriptomes of the white-rot fungus *Dichomitus squalens* from plant biomass derived monosaccharide and cellobiose cultures were studied to identify compounds that induce the expression of genes involved in plant biomass degradation.

Introduction

Plant biomass is an emerging source of fuels and chemicals since it is the most abundant renewable organic material on earth (1, 2). However, its depolymerization is usually required before it can be used in biotechnological applications. White-rot fungi are a group of basidiomycetes whose natural substrate is wood (3), and therefore they produce a set of enzymes that is ideally suited for depolymerization of components present in wood.

Wood cell walls consists mainly of four different polymers, cellulose, hemicellulose, pectin and lignin, with cellulose being the major structural component (4). All these polymers form a complex and compact lignocellulose network. White-rot fungi have the unique ability to efficiently degrade all the polymeric components of the plant cell walls, including the recalcitrant aromatic lignin. This makes them attractive candidates for various applications in the bio-based economy (5). The polysaccharide fraction of plant biomass, which comprises cellulose, hemicellulose and pectin, is composed of several different monomeric sugars. While cellulose consists solely of D-glucose, hemicelluloses (xylan, xyloglucan or mannan) and pectin consist of various sugar monomers, such as D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose, D-galacturonic acid, D-glucuronic acid and L-fucose (6).

Fungi produce a broad range of extracellular enzymes to facilitate depolymerization of plant biomass (7), most of which are classified in the Carbohydrate Active Enzyme database (CAZy, www.cazy.org) (8). The CAZy database divides the enzymes and associated modules in families and subfamilies and provides genomic, structural and biochemical information on them. Action of CAZy enzymes results in the formation of monomeric and small oligomeric components that are taken up into the fungal cells and catabolized for growth and reproduction.

The expression of the genes encoding plant biomass degrading enzymes is tightly controlled to ensure efficient utilization of the carbon and energy sources available in fungal habitats (9). Production of the

63   most suitable set of enzymes is achieved by inducing or repressing regulatory systems. Monosaccharides

64   or oligosaccharides from plant biomass can trigger signaling pathways resulting in the activation of a

65   transcriptional regulator. Several transcriptional activators and repressors related to lignocellulose

66   degradation have been characterized from ascomycete fungi (10, 11), but except for Ace3 and CRE1,

67   none of these have orthologs in basidiomycete fungi (12). Interestingly, the sets of genes that are

68   expressed in response to specific plant biomass substrates appear to be highly similar between

69   ascomycetes and basidiomycetes, despite the absence of orthologous regulators (13, 14). This similarity

70   in the expression profiles of the CAZyme encoding genes together with the absence of orthologous

71   regulators suggests parallel evolution of corresponding regulatory systems in these two fungal phyla

72   (14).

73   Uncovering regulatory systems driving wood degradation in the white-rot basidiomycetes is crucial to

74   understanding the abilities of this group of fungi and to fully exploit them in biotechnological

75   applications. A first step is the identification of the low molecular mass inducers of these regulators. In

76   this study, the white-rot fungus *Dichomitus squalens* was cultivated on six monosaccharides derived

77   from cellulose, hemicellulose and pectin, as well as on a disaccharide cellobiose. Transcriptomics was

78   used to identify which of these compounds induce the expression of genes encoding plant cell wall

79   polymer degrading enzymes. In nature, the predominant form of *D. squalens* is dikaryotic, but

80   colonization of wood initiates from a spore that forms monokaryotic mycelium until it encounters a

81   compatible mate. Previously, mono- and dikaryotic strains of *D. squalens* were suggested to have highly

82   diverse abilities to degrade plant biomass (15). To evaluate if the diversity between these forms is due

83   to regulatory level differences, comparative transcriptomics of a monokaryotic progeny of the dikaryotic

84   *D. squalens* strain was performed on the three carbon sources that were observed to induce plant

85   biomass degrading enzymes encoding genes in the dikaryon.

86

Materials and Methods

Fungal strains and cultivation

A dikaryotic *D. squalens* strain FBCC312 (the Fungal Biotechnology Culture Collection, FBCC, Department

of Microbiology, University of Helsinki) and a monokaryotic *D. squalens* strain CBS 464.89 (the CBS

collection, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands), which is a direct offspring

of the dikaryon FBCC312, were maintained on 2 % (w/v) malt extract 1.5 % (w/v) agar (MEA) plates. The

strains were grown on low-nitrogen asparagine-succinate (LN-AS) medium (16) 1.5% (w/v) agar plates

supplemented with main monosaccharides derived from plant biomass polysaccharides, i.e. 25 mM D-

glucose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, D-galactose, D-xylose, D-mannose or L-

arabinose, or 25 mM disaccharide cellobiose. For growth profiling, an agar plug (diam. 0.5 cm) from MEA

plate that was covered with fresh fungal mycelium was placed at the center of the LN-AS plate and

incubated at 28°C. As the mycelium of the dikaryon FBCC312 reached the edge of the D-glucose

supplemented plate after 5 d, this was chosen as incubation time for all the plates.

100

RNA isolation

Based on the growth profiling, six plant cell wall polysaccharides derived monosaccharides and

cellobiose were selected to be used as carbon sources to test the fungal response at gene expression

level. The dikaryon FBCC312 was cultivated as mentioned before on 25 mM D-glucose, L-rhamnose, D-

galactose, D-xylose, D-mannose, L-arabinose or cellobiose, but this time using a polycarbonate

membrane (Maine manufacturing, LCC) on the top of the agar plate to facilitate harvesting of the

mycelium. The monokaryon CBS 464.89 was cultivated similarly with L-rhamnose, D-xylose and

108   cellobiose as carbon sources. After 5 days of growth at 28°C, the most external mycelium from the edge

109   of the colony (1.5 cm) of each plate was harvested and transferred to 2 ml eppendorf tubes containing

110   two carbon steel balls (size 3/16") and frozen in liquid nitrogen. The tubes were placed in pre-cooled

111   adapters (Qiagen) and ground for 1 min at a frequency of 25 s^{-1} using a Tissuelyser II (Qiagen). Trizol

112   (Ambion) and Nucleo spin RNA extraction kit (Macherey-Nagel) were used for RNA isolation according to

113   the instructions of the manufacturers. RNA was eluted using RNAase-free H_2O and stored at –45°C.

114

115   **RNA sequencing analysis**

116   The quantity and quality of RNA was checked with a RNA6000 Nano Assay using the Agilent 2100

117   Bioanalyzer (Agilent Technologies). RNA samples were single-end sequenced using Illumina HiSeqTM

118   2000 platform (http://illumina.com). Purification of mRNA, synthesis of cDNA library and sequencing

119   were conducted in the BGI Tech Solutions Co., Ltd. (Hong Kong, China).

120   Raw reads were produced from the original image data by base calling. After data filtering, the adaptor

121   sequences, highly 'N' containing reads (>10% of unknown bases) and low quality reads (more than 50%

122   bases with quality value of <5%) were removed. After data filtering, on average 99.9% clean reads

123   remained in each sample. Clean reads were then mapped to the genome of *D. squalens* CBS 464.89

124   (http://genome.jgi.doe.gov/Dicsqu464_1) using Bowtie2 (17) and BWA software (18). In average, 64.9%

125   total mapped reads to the genome was achieved. The gene expression level was measured in

126   "fragments per kilobase of exon model per million mapped reads" (FPKM) (19) using RSEM tool (20).

127   Differential expression was identified by the DESeq2 (21) with a cut-off value of fold change ≥2.5, FPKM

128   ≥10 and adjusted p-value ≤0.01. The RNA-seq data have been submitted to Gene Expression Omnibus

129   (GEO) (22) with accession number GSE105076. For the comparison of the plant cell wall CAZy genes

130   considered highly expressed with previous parameters, a Venn diagram was constructed using the

131   online tool (23). For CAZy annotations, the JGI Mycocosm online website was used

132   (https://genome.jgi.doe.gov/mycocosm/proteins-browser/browse;qLeIA4?p=Dicsqu464_1).

133

Results

135   **Transcriptome analysis reveals cellobiose and L-rhamnose as main inducers of CAZy genes in a *D.***

136   ***squalens* dikaryon**

137   The dikaryotic and monokaryotic strains of *D. squalens* showed varied morphologies after five days of

138   growth on the main plant polysaccharide derived monomers and cellobiose (Fig. 1) (15). Both strains

139   grew well on D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose and L-rhamnose. While

140   mycelium of the dikaryon FBCC312 fully covered those plates, radial growth of the monokaryon CBS

141   464.89 was overall slower, except on L-rhamnose, and it formed a less dense mycelium. Compared to

142   the dikaryon, the colony diameter of the monokaryon was smaller also on cellobiose, but the mycelium

143   was denser, thus possibly indicating a better ability of the CBS 464.89 to convert this carbon source. In

144   contrast, compared to the monokaryon CBS 464.89, growth of the dikaryon FBCC312 was slow on D-

145   galacturonic acid and especially poor on D-glucuronic acid. Most possibly due to the poor growth,

146   extraction of high-quality RNA was not successful from these cultures, which were therefore omitted

147   from the RNAseq analysis.

148   When the putative plant biomass degrading CAZyme encoding genes induced by the different sugars in

149   *D. squalens* FBCC312 were compared to D-glucose (fold change ≥2.5 and p-value ≤0.01), 83 significantly

150   upregulated genes were detected among all the sugars (Fig. 2, Suppl. Table 1A-F). Of these, 26 were

151   unique to L-rhamnose and 27 to cellobiose. In contrast, 28 were shared at least in two different sugars.

152   On L-arabinose, D-xylose, L-rhamnose, D-galactose and D-mannose, a smaller number of upregulated

153   genes were present (Fig. 2). On one of the two pentoses studied, L-arabinose, 18 genes were

154   upregulated and all of them were also present in some of the other tested sugars. Similarly, none of the

155   13 genes upregulated in D-galactose were unique to this sugar. The growth of *D. squalens* on D-xylose,

156   the other pentose studied, resulted in 16 upregulated CAZy genes with only one gene unique to this

157   monosaccharide. On the hexose D-mannose only three genes were upregulated, and two of them were

158   also found on D-xylose or L-rhamnose. No upregulated CAZy genes common to all the sugars were

159   detected (Fig. 2). Down regulated genes were not analyzed.

160   The proportion of the induced genes encoding CAZymes putatively acting on different polymeric

161   components of plant biomass was determined from the transcriptome data (Fig. 2, Tables 1-3).

162   Cellobiose induced 34 CAZy genes of which most (68%) encode enzymes that putatively act on cellulose

163   (Table 1, Suppl. Fig. 1A, Suppl. Table 1A). CAZy family GH6 and GH7 cellobiohydrolases encoding genes

164   were highly expressed on cellulose, with *cel7c* (Protein Id: 944872) showing 27-fold higher expression

165   compared to D-glucose (Table 1, Suppl. Table 1A). In contrast, the only highly expressed cellulolytic

166   genes on other sugars were cellobiohydrolase genes *cel6a* (Protein Id: 803735) on L-arabinose (Fig. 2,

167   Suppl. Table 1A, C) and *cel7a* (Protein Id: 918783) on L-rhamnose, D-xylose (Tables 1-3) and L-arabinose

168   (Fig. 2, Suppl. Table 1). On L-rhamnose, 38% of the 49 induced genes encoded enzymes putatively

169   involved in pectin degradation (Table 2, Suppl. Fig. 1B, Suppl. Table 1B). The number of genes with

170   increased expression on D-xylose was lower (16) and thus its role as an inducer was less evident

171   compared to cellobiose and L-rhamnose. However, 25% of the CAZy genes induced on D-xylose were

172   related to xylan degradation (Table 3, Suppl. Fig. 1C, Suppl. Table 1D). Lignin degradation related

173   enzymes encoding CAZy genes were induced only on L-rhamnose (27%) and D-xylose (25%) cultures of

174   *D. squalens* FBCC312, but not on cellobiose (Fig. 2/Tables 1-3).

175

176 **L-Rhamnose resulted in significant differences in gene expression of mono- and dikaryotic strains of *D.***

177 ***squalens***

178 To compare possible regulatory differences in plant cell wall degradation between mono- and dikaryotic

179 strains of *D. squalens*, the *D. squalens* monokaryon CBS 464.89 was cultivated on cellobiose, L-rhamnose

180 and D-xylose. When the transcriptomes from the three sugars were analyzed, a considerably lower

181 amount of highly expressed (fold change ≥2.5, p-value ≤0.01 and FPKM ≥10) CAZy genes were detected

182 in D-xylose (7-11) (Suppl. Table 2) in comparison to cellobiose (21) and L-rhamnose (45) (Suppl. Tables

183 3B and 4B), and therefore the D-xylose transcriptome was excluded from further analyses. When the

184 putative plant biomass degrading CAZyme encoding genes induced by cellobiose (FPKM >10) were

185 compared to those induced by L-rhamnose (fold change ≥2.5 and p-value ≤0.01) in the two *D. squalens*

186 strains, 20 genes were highly expressed in the dikaryon, from which 11 (55%) are related to cellulose

187 degradation (Suppl. Table 3A). In the monokaryon, the number of genes (21) induced by cellobiose as

188 well as the proportion of the genes putatively targeted for cellulose degradation was similar (12, 57%),

189 but 1/3 of these were different genes in the two strains (Fig. 3, Suppl. Table 3B). Overall, when the CAZy

190 gene expression of the mono- and dikaryotic strains on cellobiose was compared to L-rhamnose, no big

191 differences were detected in the number of highly expressed genes targeting different putative plant

192 cell wall compounds (Fig. 3).

193 When the putative plant biomass degrading CAZyme encoding genes of *D. squalens* induced by L-

194 rhamnose (FPKM >10) were compared to those induced by cellobiose (fold change ≥2.5 and p-value

195 ≤0.01), the differences between the mono- and dikaryotic strains were evident. The number of the

196 expressed genes was higher in the dikaryon (55) than in the monokaryon (45) (Fig. 4, Suppl. Table 4).

197 Also, the proportion of the induced pectinolytic genes was higher in the monokaryon (44%) than in the

198 dikaryon (25%) (Fig. 4, Suppl. Table 4). Nearly all pectinolytic genes upregulated in the dikaryon were

199 also upregulated in the monokaryon, indicating that in the the monokaryon an additional set of genes

200   responds to L-rhamnose. Interestingly, the proportion of the lignin degradation related genes varied

201   notably between the strains, as 31% and 18% of the induced genes in the dikaryon and the monokaryon,

202   respectively, were putatively involved in lignin modification (Fig.4). Here the opposite pattern was

203   observed as for the pectinolytic genes, as all induced genes in the monokaryon were also induced in the

204   dikaryon, suggesting that in the dikaryon an additional set of ligninolytic genes responds to L-rhamnose.

205   The largest difference in gene expression between the mono- and dikaryon was observed on L-

206   rhamnose, since despite similar number of genes induced by L-rhamnose in comparison to cellobiose,

207   only 36 of those genes were common in the both strains. From the nine genes unique for the

208   monokaryon, seven (78%) were related to pectin degradation, whereas almost half (47%) from the 19

209   unique dikaryotic genes were related to lignin degradation (Fig. 4, Suppl. Table 4).

210

Discussion

211

212   Gene regulation has been extensively studied in fungi, especially in the representatives from phylum

213   Ascomycota (10, 11). In contrast, the understanding of gene regulation in basidiomycetes is less

214   extensive, largely due to the lack of characterized regulators and complications with gene

215   transformation systems in these fungi. With the recent development of a transformation system (24),

216   the white-rot fungus *D. squalens* has become an interesting species to study regulation of plant biomass

217   degradation in basidiomycetes. This species can degrade both hard- and softwood (3) and several

218   dikaryotic and related monokaryotic strains are available (15), as well as genome sequences for four

219   monokaryotic strains (25).

220   In response to different conditions, fungi have the ability to up- or downregulate the expression of

221   specific genes in order to adapt to their environment. Since the two major fungal phyla, Ascomycota and

222   Basidiomycota, separated 500 million years ago (26), we may expect different responses in their gene

223   expression. In addition, that dichotomy is possibly a reason for the scarcity of orthologous

224   transcriptional regulators between ascomycetes and basidiomycetes (12, 14). The present study reveals

225   detailed insights into the induction system of plant cell wall polymer degradation in *D. squalens*.

226   Similar to what has been observed in ascomycetes (27), genes encoding enzymes active on a variety of

227   plant polymers were upregulated in *D. squalens* when cultivated on cellobiose, L-rhamnose and D-

228   xylose. However, the distribution of these genes revealed a clear pattern for two of these compounds.

229   On cellobiose, more than half of the upregulated genes encode enzymes active on cellulose, while on L-

230   rhamnose more than 1/3 of the upregulated genes encoded enzymes active on pectin. In contrast, on D-

231   xylose, a more randomly distributed set of genes was upregulated, including a very small number of

232   cellulolytic genes. At this point, it is not clear whether a specific inducer for (subsets of) hemicellulolytic

233   genes exists in *D. squalens*.

234   Therefore, our results demonstrate that the dimer cellobiose is the primary inducer for cellulose

235   degradation in *D. squalens*, while L-rhamnose is an inducer for pectin degradation. This role for

236   cellobiose was previously suggested for the basidiomycete brown-rot fungus *Postia placenta* where

237   cellulases were induced by soluble sugars, especially cellobiose, after repression of oxidoreductases

238   (28).

239   In the well-studied white-rot fungus *Phanerochaete chrysosporium*, cellotriose and cellotetraose, but

240   not cellobiose, were suggested to result in the strongest induction of cellulases (29). In light of the

241   results of our study, we had a closer look at the data from Suzuki et al. (29) and do not think that those

242   results exclude a role for cellobiose as inducer. In that study, cellobiose is accumulating after 1 h

243   cultivation of *P. chrysosporium* on cellotriose and cellotetraose. This is also the time point at which high

244   induction of the cellobiohydrolase encoding genes *cel7C*, *cel7D*, *cel7F/G* and *cel6A* is observed on

245 cellotriose and cellotetraose cultures (29), suggesting that in fact the accumulated cellobiose may have

246 caused induction of these genes. Microscrystalline cellulose (Avicel) has been found to upregulate the

247 expression of cellobiohydrolase encoding genes in *D. squalens* FBCC312, with the strongest effect on the

248 gene *cel7c* (Protein Id: 944872) (30, 31). This observation is in agreement with our results of this

249 particular gene in the cellobiose cultures of *D. squalens* FBCC312 , indicating that the results from the

250 Avicel cultures may in fact also reflect the induction by cellobiose released from this substrate.

251 Cellobiose has also been proposed as an inducer for several, but not all, ascomycete species (12),

252 suggesting that there possibly are some similarities in the signaling mechanism of ascomycete and

253 basidiomycete fungi, even if no orthologs for ascomycete cellobiose-responsive regulators were

254 identified in basidiomycete genomes.

255 Considering the broad range of pectinolytic genes that were upregulated in the presence of L-rhamnose,

256 this sugar appears to be a main inducer for pectinolytic gene expression in *D. squalens*. An L-rhamnose

257 responsive pectinolytic regulator (RhaR) has been identified in the ascomycete fungus *Aspergillus niger*

258 (32), but this regulator seems to have a more narrow role in activating pectinolytic genes than our data

259 suggests for *D. squalens* (33). Furthermore, *D. squalens* does not have an ortholog of RhaR, nor of the

260 other two *A. niger* pectinolytic regulators GaaR and AraR (12), indicating significant differences in

261 pectinolytic regulation in *D. squalens*. In *A. niger*, genes encoding pectinolytic enzymes have recently

262 been shown to be induced mainly by D-galacturonic acid (33), while L-rhamnose and L-arabinose affect

263 smaller sets of pectinolytic genes (34). Although we were not able to assess the role of D-galacturonic

264 acid as a pectinolytic inducer in *D. squalens* due to the low quality of RNA extracted from those cultures,

265 our results demonstrated L-rhamnose induction for a wide set of pectinolytic genes, especially from the

266 Glycoside Hydrolase (GH) families. Considering the low percentage of pectin in wood (35), the

267 development of an efficient regulatory system for pectin degradation in *D. squalens* may seem less

268 important than that for cellulose degradation. However, it has been previously reported that pectin in

269   wood is particularly present at high levels in bordered pits, which connect adjacent xylem parenchyma

270   cells, and are often the point of entry for basidiomycete hyphae to the wood cell walls (36). Therefore,

271   this polysaccharide may still be a significant target for white-rot fungi during the onset of wood

272   colonization.

273   Differences of gene expression between the *D. squalens* mono- and dikaryon were observed especially

274   on L-rhamnose. This effect was not limited to pectinolytic genes, but observed across all CAZy genes.

275   When both strains were exposed to L-rhamnose, the dikaryon showed higher upregulation for genes

276   encoding lignin-modifying enzymes than the monokaryon. The monokaryon instead, showed higher

277   upregulation for pectinolytic genes than the dikaryon. The proportion of induced ligninolytic genes

278   during growth on L-rhamnose correlated with a previously reported comparison of enzymatic activities

279   in mono- and dikaryotic strains of *D. squalens* (15). In addition, the dikaryon had a broader set of

280   upregulated genes encoding CAZymes, like those active on starch and xyloglucan, which were not

281   expressed in the monokaryon, as well as a larger number of genes involved in mannan degradation.

282   On cellobiose, the strains showed a similar pattern of CAZy gene expression, both having a large

283   proportion of cellulolytic genes upregulated, but showing differences between individual genes.

284   However, genes encoding mannanases and expansin-like proteins were only induced in the dikaryon.

285   The broader induction of CAZyme encoding genes in the dikaryon indicates that this form of the fungus

286   has better ability to grow or utilize plant biomass in nature compared to the monokaryon. This was not

287   only observed during growth on plant biomass substrates for *D. squalens* (15), but also for another

288   white-rot species (*Pleurotus ostreatus*), a litter-degrading species (*Agaricus bisporus*) and a brown-rot

289   species (*Serpula lacrymans*) (www.fung-growth.org).

290   The results of this study are an important step towards dissecting the regulatory network driving the

291   expression of genes encoding plant biomass degrading enzymes in the white-rot fungus *D. squalens* and

292 possibly other basidiomycetes. Together with the recent development of a transformation system for *D.*

293 *squalens* (24) this species is a good candidate as a model system for the molecular biological analysis of

294 wood decay by white-rot fungi.

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401

402   **Tables**

403   **Table 1. Expression of genes encoding putative plant biomass polysaccharide degrading enzymes**

404   **during the growth of *D. squalens* dikaryon FBCC312 on cellobiose compared to D-glucose.** Only genes

405   of which the expression was at least 2.5 fold higher compared to D-glucose with p-value ≤0.01 and had a

406   FPKM value >10 are included.

| Protein Id	Enzyme code	Gene name	Fold change over D-glucose	*p*-value over D-glucose	FPKM cellobiose	Putative substrate	CAZy family
829728	BGL		18.7	1.32E-75	229.2	cellulose	GH1
1001790	BGL		7.2	5.90E-30	38.5	cellulose	CBM1-GH3
922530	BGL		3.5	1.79E-15	44.3	cellulose	GH3
931309	BGL		3.0	4.16E-20	96.5	cellulose	GH3
944872	CBH	*cel7c*	27.3	3.19E-88	969.9	cellulose	GH7
918783	CBH	*cel7a*	14.6	1.83E-19	203.0	cellulose	GH7
803735	CBH	*cel6a*	9.6	7.73E-46	451.4	cellulose	CBM1-GH6
946952	CBH	*cel7b*	8.6	3.96E-11	252.4	cellulose	GH7
1015695	CDH		12.4	6.64E-47	107.7	cellulose	AA8-AA3_1
931204	EGL		19.7	7.98E-44	182.3	cellulose	GH131-CBM1
830363	EGL		18.1	6.75E-69	199.8	cellulose	CBM1-GH5_5
955303	EGL		9.6	1.09E-63	426.1	cellulose	CBM1-GH5_5
946842	EGL		8.2	5.82E-23	52.8	cellulose	CBM1-GH5_5
991840	EGL		5.0	3.33E-12	140.6	cellulose	GH45
399056	LPMO	*lpmo2*	20.3	1.86E-56	417.2	cellulose	AA9
815832	LPMO		14.3	5.92E-34	99.1	cellulose	AA9-CBM1
948592	LPMO		14.2	1.73E-45	269.7	cellulose	AA9-CBM1
465156	LPMO		9.1	1.09E-33	340.1	cellulose	AA9
816464			23.8	3.88E-40	160.6	cellulose	CBM1
1017331	LPMO		20.7	8.23E-52	280.7	cellulose/hemicellulose	AA9
512221	LPMO		18.3	3.22E-54	417.8	cellulose/hemicellulose	AA9
928670	LPMO		12.0	6.85E-26	195.7	cellulose/xylan	AA9
972934	LPMO		2.8	0.003	17.6	cellulose/xyloglucan	AA9
38831	EXPN		2.8	0.0001	24.5	expansin-like	#N/A
1040576	AXE/GMAE		16.2	1.08E-33	79.1	hemicellulose	CBM1-CE16
938389	AGL		3.4	5.43E-13	109.6	mannan	GH27
825072	MAN		3.5	8.65E-18	82.4	mannan	CBM1-GH5_7
811661	MAN		2.8	1.99E-11	134.1	mannan	GH5_7
938021	BXL		7.0	4.32E-22	101.1	xylan	GH5_22
945667	XLN		9.7	9.97E-34	82.2	xylan	CBM1-GH10
810461	XLN		6.4	7.48E-07	11.5	xylan	CBM1-GH10
725679	XG-EGL		36.0	7.12E-53	200.8	xyloglucan	GH12
805675	XG-EGL		13.8	5.31E-48	243.5	xyloglucan	GH74-CBM1
417119	XG-EGL		3.8	8.38E-07	67.7	xyloglucan	GH12

407   **Table 2. Expression of genes encoding putative plant biomass polysaccharide degrading enzymes**

408   **during the growth of _D. squalens_ dikaryon FBCC312 on L-rhamnose compared to D-glucose.** Only genes

409   of which the expression was at least 2.5 fold higher compared to D-glucose with p-value ≤0.01 and had a

410   FPKM value >10 are included.

Protein Id	Enzyme code	Gene name	Fold change over D-glucose	_p_-value over D-glucose	FPKM L-rhamnose	Putative substrate	CAZy family
972434	BGL		2.7	5.64E-09	36.4	cellulose	GH3
1001790	BGL		3.7	5.41E-13	18.1	cellulose	CBM1-GH3
918783	CBH	_cel7a_	3.3	1.66E-04	33.5	cellulose	GH7
882755	EGL		7.5	3.31E-68	714.4	cellulose	GH45
993254	EGL		3.4	7.38E-05	21.6	cellulose	GH45
214438	EXPN		2.7	5.02E-13	543.1	expansin-like	
904543	EXPN		4.0	1.56E-06	130.5	expansin-like	
38831	EXPN		2.8	1.44E-04	22.4	expansin-like	
920797	AXE/GMAE		3.5	1.67E-11	93.9	hemicellulose	CE16
932464	AXE/GMAE		2.6	1.64E-07	69.8	hemicellulose	CE16
863600	AXE/GMAE		3.6	1.05E-04	15.5	hemicellulose	CE16
818957	CRO		22.6	2.94E-65	567.6	lignin	AA5_1
455342	CRO		11.0	1.85E-49	432.9	lignin	AA5_1
819102	CRO		5.9	1.18E-19	186.2	lignin	AA5_1
551897	CRO		40.8	5.43E-63	153.0	lignin	AA5_1
930013	GMC		3.3	3.20E-06	20.1	lignin	AA3_2
823531	LCC	_lcc8_	2.6	2.79E-05	19.9	lignin	AA1_1
979936	MnP	_mnp7_short_	76.4	2.19E-153	3594.2	lignin	AA2
808604	MnP	_mnp4_extralong_	7.2	4.16E-14	2539.5	lignin	AA2
825018	MnP	_mnp5_short_	6.6	1.04E-10	2001.9	lignin	AA2
578774	MnP	_mnp2_extralong_	2.8	6.53E-03	560.0	lignin	AA2
577408	MnP	_mnp3_extralong_	7.2	2.31E-26	153.4	lignin	AA2
934487	MnP	_mnp1_extralong_	2.7	1.19E-03	13.9	lignin	AA2
928470	AOX		6.2	1.09E-65	4376.5	lignin	AA3_3
939846	AGL		7.2	7.47E-40	448.6	mannan	GH27
918405	MND		3.3	4.75E-08	14.2	mannan	GH2
191860	ABN		6.0	6.28E-34	744.9	pectin	GH43
972003	BXL		7.6	2.06E-41	142.4	pectin	GH43-CBM35
928226	ABN		2.5	1.53E-03	16.5	pectin	GH43
1040936	LAC		11.1	4.39E-104	1101.3	pectin	GH35
841467	PGA		4.0	2.30E-07	49.1	pectin	GH28
960291	PGX		3.7	1.68E-09	59.3	pectin	GH28
929479	PME		2.8	7.92E-08	50.6	pectin	CE8

978188	RGAE	2.7	7.88E-04	26.2	pectin	CE12
929528	RGAE	3.5	1.66E-04	21.7	pectin	CE12
961557	RGX	3.7	5.27E-13	89.4	pectin	GH28
973573	RGX	4.0	6.67E-10	27.6	pectin	GH28
937177	RHA	2.9	5.64E-08	23.4	pectin	GH78
816118	RHA	3.1	2.31E-08	21.5	pectin	GH78
50651	PGA	2.9	2.11E-06	39.6	pectin	GH28
849572	ABF	2.6	1.78E-10	75.9	pectin/xylan	GH51
501043	FAE	3.1	1.59E-16	211.5	pectin/xylan	
723513	FAE	3.2	1.82E-18	208.0	pectin/xylan	
829508	FAE	4.5	2.20E-34	152.1	pectin/xylan	
912876	AMY	3.0	1.16E-23	296.9	starch	GH13_1
933193	GE	20.4	8.94E-50	94.5	xylan	CBM1-CE15
513778	XLN	31.9	2.07E-66	161.1	xylan	CBM1-GH10
933288	XLN	14.4	2.35E-17	33.3	xylan	GH10
815375	AFC	2.5	2.04E-17	275.5	xyloglucan	GH95

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Table 3. Expression of genes encoding putative plant biomass polysaccharide degrading enzymes

414   **during the growth of *D. squalens* dikaryon FBCC312 on D-xylose compared to D-glucose.** Only genes of

415   which the expression was at least 2.5 fold higher compared to D-glucose with p-value ≤0.01 and had a

416   FPKM value >10 are included.

Protein Id	Enzyme code	Gene name	Fold change over D-glucose	*p*-value over D-glucose	FPKM L-rhamnose	Putative substrate	CAZy family
918783	CBH	*cel7a*	4.0	3.1E-05	44.555	cellulose	GH7
949690	EXPN		3.5	9.3E-04	21.47	expansin-like	EXPN
920797	AXE/GMAE	2.6		2.2E-06	72.855	hemicellulose	CE16
932464	AXE/GMAE	2.5		2.5E-06	70.8	hemicellulose	CE16
930013	GMC		4.2	5.6E-08	27.8	lignin	AA3_2
979936	MnP	*mnp7*_short	4.0	5.5E-15	177.31	lignin	AA2
952617	AOX		2.8	2.1E-08	346.085	lignin	AA3_3
928470	AOX		2.8	2.5E-20	2079.17	lignin	AA3_3
939846	AGL		3.4	7.3E-15	221.745	mannan	GH27
1040936	LAC		3.2	5.7E-24	335.375	pectin	GH35
973573	RGX		2.5	2.6E-04	17.97	pectin	GH28
191860	ABN		3.1	1.8E-13	409.23	pectin	GH43
829508	FAE		3.0	2.0E-17	107.605	pectin/xylan	
933193	GE		4.2	2.4E-10	19.25	xylan	CBM1-CE15
513778	XLN		5.5	7.2E-15	27.48	xylan	CBM1-GH10
945667	XLN		2.7	4.1E-06	22.06	xylan	CBM1-GH10

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Figures and legends
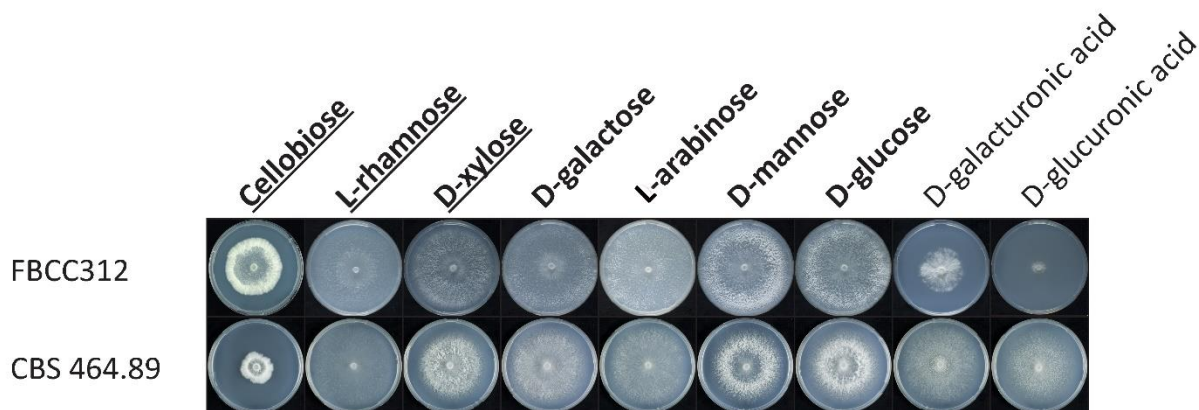
Figure 1. Growth profiles of *D. squalens* dikaryon FBCC312 and monokaryon CBS 464.89 on different sugars that were considered as potential inducers of plant cell wall degrading CAZymes, based on studies in ascomycetes (12). The plate cultures that were subsequently used for transcriptomics in FBCC312 are in bold, and in CBS 464.89 in bold and underlined.

Unique to L-rhamnose

Protein Id	Enzyme code	Putative substrate
972434	BGL	cellulose
882755	EGL	cellulose
904543	EXPN	expansin-like
863600	AXE/GMAE	hemicellulose
455342	CRO	lignin
551897	CRO	lignin
818957	CRO	lignin
819102	CRO	lignin
823531	LCC	lignin
577408	MnP	lignin
808604	MnP	lignin
825018	MnP	lignin
934487	MnP	lignin
918405	MND	mannan
928226	ABN	pectin
841467	PGA	pectin
960291	PGX	pectin
929479	PME	pectin
978188	RGAE	pectin
816118	RHA	pectin
937177	RHA	pectin
501043	FAE	pectin/xylan
723513	FAE	pectin/xylan
912876	AMY	starch
933288	XLN	xylan
815375	AFC	xyloglucan

Unique to D-mannose

857208	GMC	lignin

Unique to Cellobiose

Protein Id	Enzyme code	Putative substrate
829728	BGL	cellulose
922530	BGL	cellulose
931309	BGL	cellulose
944872	CBH	cellulose
946952	CBH	cellulose
1015695	CDH	cellulose
830363	EGL	cellulose
931204	EGL	cellulose
946842	EGL	cellulose
955303	EGL	cellulose
991840	EGL	cellulose
399056	LPMO	cellulose
815832	LPMO	cellulose
948592	LPMO	cellulose
816464		cellulose
512221	LPMO	cellulose/hemicellulose
1017331	LPMO	cellulose/hemicellulose
928670	LPMO	cellulose/xylan
972934	LPMO	cellulose/xyloglucan
1040576	AXE/GMAE	hemicellulose
938389	AGL	mannan
811661	MAN	mannan
938021	BXL	xylan
810461	XLN	xylan
417119	XG-EGL	xyloglucan
725679	XG-EGL	xyloglucan
805675	XG-EGL	xyloglucan

Unique to D-xylose

949690	EXPN	expansin-like

Venn diagram (5-set) of overlapping induced genes among Cellobiose, D-xylose, L-rhamnose, D-mannose, D-galactose, L-arabinose.

Bar graph (total number of induced CAZy genes by each sugar):

L-rhamnose	Cellobiose	L-arabinose	D-xylose	D-galactose	D-mannose
49	34	18	16	13	3
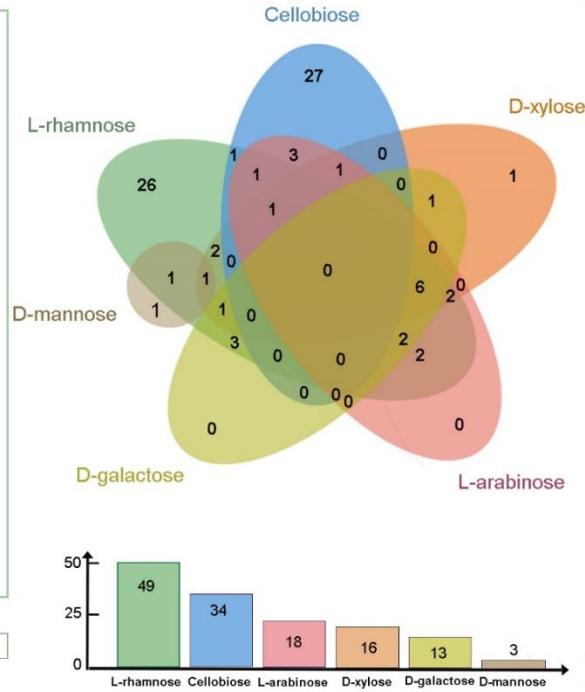
Protein Id	Enzyme code	Putative substrate	Common sugars inducing gene expression
1001790	BGL	cellulose	Cellobiose L-rhamnose
918783	CBH	cellulose	Cellobiose L-rhamnose L-arabinose D-xylose
803735	CBH	cellulose	Cellobiose L-arabinose
993254	EGL	cellulose	L-rhamnose L-arabinose
465156	LPMO	cellulose	Cellobiose L-arabinose
38831	EXPN	expansin-like	Cellobiose L-rhamnose L-arabinose
214438	EXPN	expansin-like	L-rhamnose L-arabinose D-galactose
920797	AXE/GMAE	hemicellulose	L-rhamnose L-arabinose D-xylose D-galactose
932464	AXE/GMAE	hemicellulose	L-rhamnose D-xylose
928470	AOX	lignin	L-rhamnose L-arabinose D-xylose D-galactose
952617	AOX	lignin	D-xylose D-galactose
930013	GMC	lignin	L-rhamnose D-xylose D-mannose
979936	MnP	lignin	L-rhamnose L-arabinose D-xylose
578774	MnP	lignin	L-rhamnose D-mannose
939846	AGL	mannan	L-rhamnose L-arabinose D-xylose D-galactose
825072	MAN	mannan	Cellobiose L-arabinose
191860	ABN	pectin	L-rhamnose D-xylose D-galactose
972003	BXL	pectin	L-rhamnose L-arabinose D-galactose
1040936	LAC	pectin	L-rhamnose L-arabinose D-xylose D-galactose
50651	PGA	pectin	L-rhamnose D-galactose
929528	RGAE	pectin	L-rhamnose D-galactose
961557	RGX	pectin	L-rhamnose D-galactose
973573	RGX	pectin	L-rhamnose D-xylose
849572	ABF	pectin/xylan	L-rhamnose L-arabinose
829508	FAE	pectin/xylan	L-rhamnose L-arabinose D-xylose
933193	GE	xylan	L-rhamnose L-arabinose D-xylose D-galactose
945667	XLN	xylan	Cellobiose L-arabinose D-xylose
513778	XLN	xylan	L-rhamnose L-arabinose D-xylose D-galactose

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Figure 2. Comparison of the number of the plant cell wall degrading CAZy genes induced by the tested sugars in *D. squalens* FBCC312. The total number of induced CAZy genes by each sugar is listed in the bar graph below the Venn diagram. CAZy genes considered to be induced had a fold change ≥2.5 compared to D-glucose with p-value ≤0.01 and had a FPKM value >10. See Suppl. Material 3 for explanation of the

431   enzyme codes.
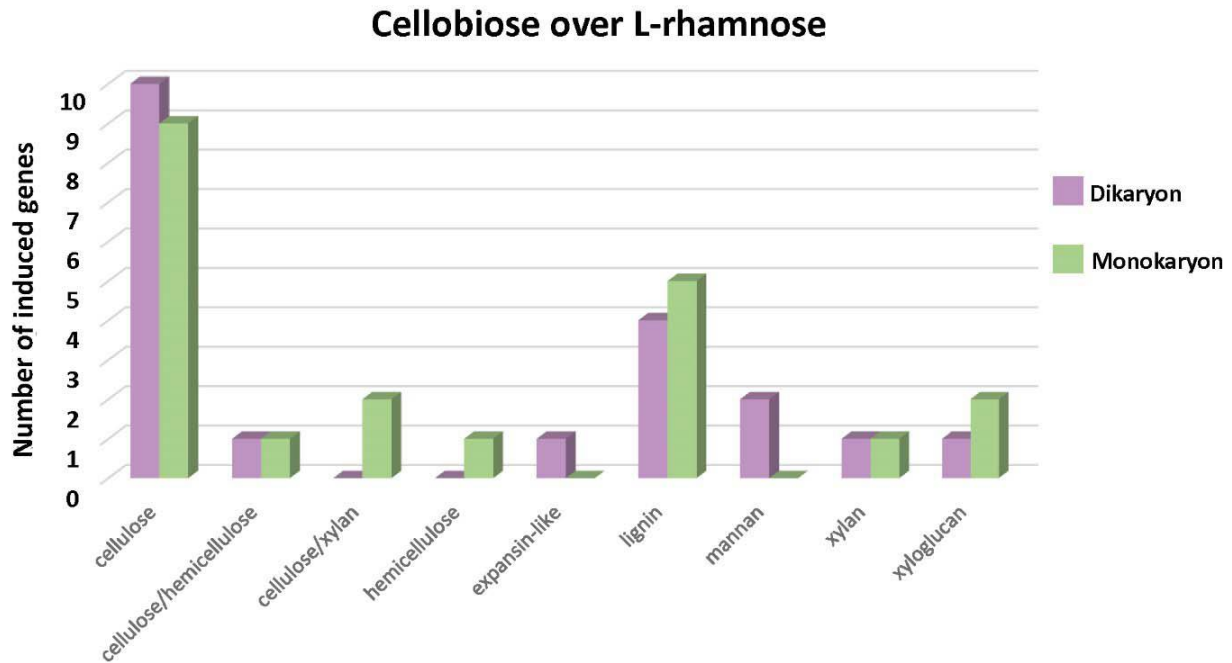
Figure 3. Plant biomass degradation related CAZy genes induced by cellobiose in comparison to

L-rhamnose in the *D. squalens* dikaryon FBCC312 and monokaryon CBS 464.89. CAZy genes

considered to be induced by cellobiose were expressed at 2.5-fold higher level (p-value ≤0.01)

compared to cellobiose and FPKM >10.

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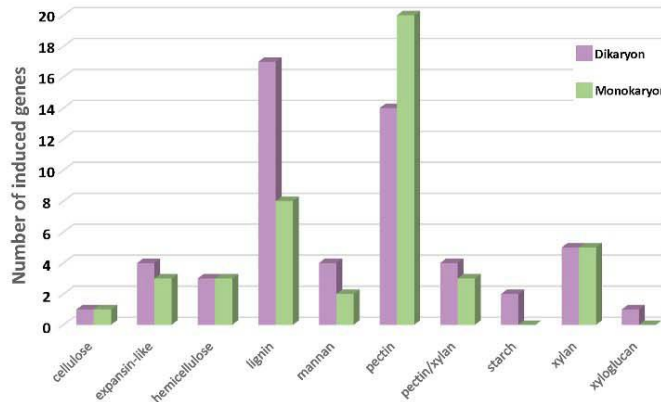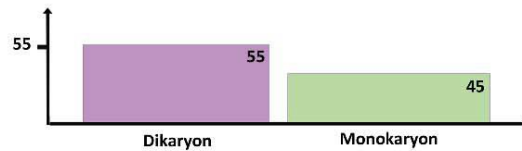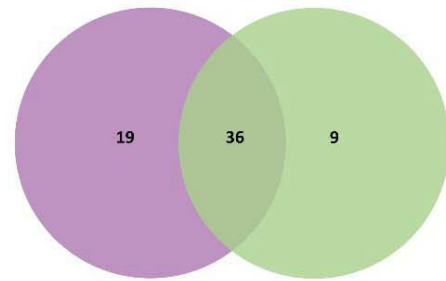
Figure 4. Common and strain-specific plant cell wall degradation related CAZy genes of *D. squalens* dikaryon FBCC312 (purple) and monokaryon CBS 464.89 (green) induced by L-rhamnose in comparison to cellobiose. The genes considered to be induced by L-rhamnose were expressed at 2.5 fold higher level (p ≤0.01) compared to cellobiose and FPKM value >10. See Suppl. Material 3 for explanation of the enzyme codes.