PLANT POLYSACCHARIDE DEGRADATION BY FUNGI

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ABSTRACT

Plant biomass is the major carbon source for many fungal species. Due to its complex polymeric nature, degradation of this biomass to digestible monomers requires a large range of enzyme activities. With the availability of an increasing number of fungal genomes, new insights into the diversity of fungi with respect to plant biomass degradation have been obtained. Recent progress in this area will be reviewed in this paper.

Keywords: fungi, plant biomass, polysaccharide degradation, CAZy, growth profiling

PLANT BIOMASS COMPOSITION AND STRUCTURE

Plant biomass is the most abundant organic matter on earth and the major substrate for the majority of fungal species. It consists mainly of polysaccharides, but also contains proteins and the aromatic polymer lignin. Plant polysaccharides can be divided into plant cell wall polysaccharides (cellulose, hemicelluloses, pectin) and storage polysaccharides (e.g. starch, inulin, gums) [1]. They consist of many different monomeric components that are attached to each other by a variety of linkages.

Cellulose is the most abundant plant polysaccharide. It consists of a linear chain of β-1,4-linked D-glucose residues (Fig. 1). These chains are organised in bundles called microfibrils [2] that provide the main strength and structure for the plant cell wall.

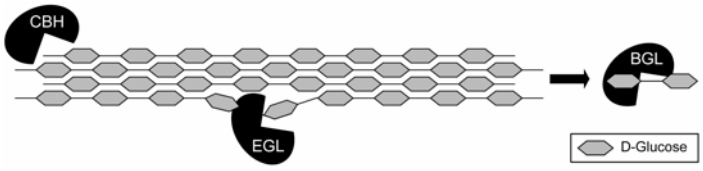

Figure 1: Schematic presentation of cellulose, its degradation and release of D-glucose. Reprinted from [3] with permission from the publisher (Copyright remains with the original publisher).

Hemicelluloses are more diverse in nature and three main types are present in plant cell walls: xylan, xyloglucan and mannan (Fig. 2). Xylan consists of a backbone of β-1,4-linked D-xylose residues [4, 5]. Other residues can be attached to this backbone, such as α-linked L-arabinose or (4-O-methyl-) D-glucuronic acid residues, α- or β-linked D-galactose residues and acetyl residues [5-8]. The L-arabinose residues can be further decorated with feruloyl residues [9-11]. Xyloglucan has a backbone that consists of β-1,4-linked D-glucose residues that are decorated with α-1,6-linked D-xylose residues [12]. Attached to the D-xylose residues can be L-arabinose, D-galactose and L-fucose residues. Mannan is often also referred to as galactomannan as this

hemicelluloses consists of a of β-1,4-linked D-mannose backbone with α-1,4-linked D-galactose residues and acetyl residues as decorations [13]. Depending on the plant species, the D-mannose backbone can also be interrupted with single D-glucose residues.

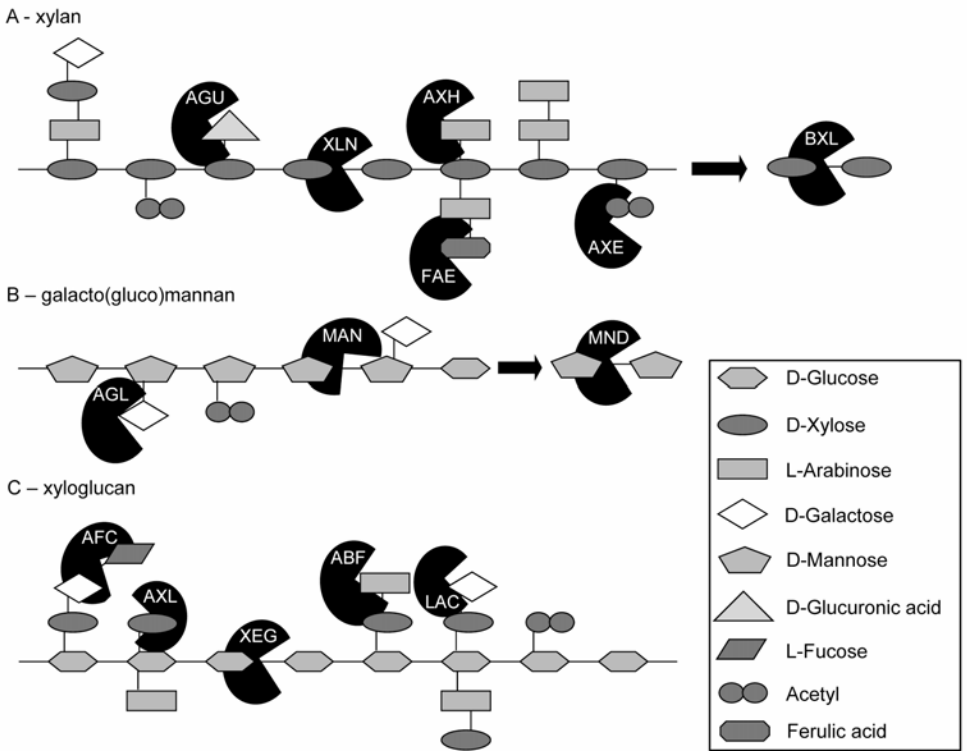

Figure 2: Schematic presentation of hemicellulose components (xylan, galacto(gluco)mannan, xyloglucan) and their degradation. Reprinted from [3] with permission from the publisher (Copyright remains with the original publisher).

Pectin is the third plant cell wall polysaccharide and it consists of several sub-structures (Fig. 3) [14, 15]. Homogalacturonan is a linear polymer of α-1,4-lined D-galacturonic acid residues that has various degrees of acetylation and/or methylation. Xylogalacturonan is a modified homogalacturonan with β-1,3-linked D-xylose residues attached to D-gaalcturonic acid. Rhamnogalacturonan 1 consists of a backbone of α-1,4-linked D-galacturonic acid and α-1,2-linked L-rhamnose residues. Side chains consisting of L-arabinose and/or D-galactose residues are attached to many of the L-rhamnose residues creating a structure often referred to as the 'hairy' region of pectin. Terminal feruloyl and *p*-coumaroyl residues can be attached to these side chains.

Starch is one of the main storage polysaccharides. It consists of an α-1,4-linked polymer of D-glucose residues that contain α-1,4-linked branching points [16]. Another major storage polysaccharide is inulin, that consist of a β-2,1-linked chain of D-fructose with a terminal d-glucose residue [17]. Another varied group of storage polysaccharides are the gums that contain many different structures [18]. Some gums (e.g. locust bean gum, guar gum) are highly similar in structure to cell wall galactomannan.

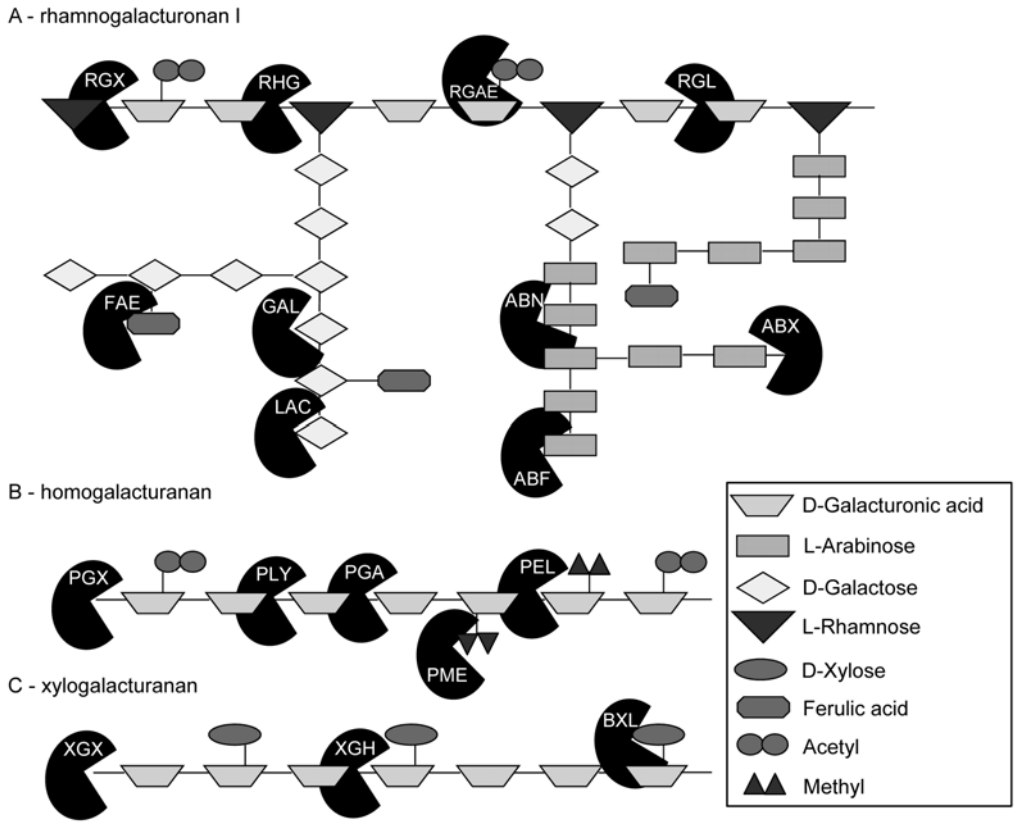

Figure 3: Schematic presentation of pectin components and their degradation. Reprinted from [3] with permission from the publisher (Copyright remains with the original publisher).

BIODEGRADATION OF PLANT POLYSACCHARIDES

Due to the diverse nature of plant polysaccharides, a large range of enzyme activities is required to degrade them to their monomeric components (Table 1) [1, 19]. These enzymes can be divided into families based on modules in their amino acid sequence resulting in a classification system called Carbohydrate Active enZymes (CAZy, www.cazy.org) [20]. Using the CAZy annotation pipeline for fungal genomes has been shown to be a highly powerful tool to obtain insight in the carbohydrate potential of a fungus and has been shown to be superior to manually annotated carbohydrate related genes of fungal genomes. The pipeline has been used for several genome annotations including several basidiomycetes [19, 21-35]. This demonstrated that significant differences exist in the plant polysaccharides degrading potential of *Schizophyllum commune*, *Coprinopsis cinerea*, *Laccaria bicolor*, *Postia placenta*, *Phanerochaete chrysosporium*, *Cryptococcus neoformans* and *Ustilago maydis* [25]. Overall, the genome of *C. neoformans* contained the lowest number of genes encoding putative plant cell wall (PCW) degrading enzymes, followed by the genomes of *U. maydis* and *L. bicolor*, which can be linked to their lifestyle as a animal/human pathogen, a biotrophic plant pathogen and a mycorrhizae, respectively. The saprobic basidiomycetes all contain significantly higher numbers of genes encoding putative PCW enzymes.

Table 1. Fungal enzymatic activities involved in plant polysaccharide degradation. The enzymes are sorted alphabetically on the enzyme code. Modified from [19].

Enzyme class	Enzyme code	Substrate	CAZy families
α-L-arabinofuranosidase	ABF	Xyloglucan, xylan, pectin	GH51,54
endoarabinanase	ABN	Pectin	GH43
exoarabinanase	ABX	Pectin	GH93
α-L-fucosidase	AFC	Xyloglucan	GH29,95
α-1,4-D-glucosidase	AGD	Starch	GH31
α-1,4-D-galactosidase	AGL	Xyloglucan, xylan, galactomannan	GH27,36
α-glucuronidase	AGU	Xylan	GH67,115
α-amylase	AMY	Starch	GH13
acetyl xylan esterase	AXE	Xylan	CE1
arabinoxylan arabinofuranohydrolase	AXH	Xylan	GH62
α-D-xylosidase	AXL	Xyloglucan	GH31
β-1,4-D-glucosidase	BGL	Cellulose	GH1,3
β-1,4-D-xylosidase	BXL	Xylan, pectin	GH3,39,43
cellobiohydrolase	CBH	Cellulose	GH6,7
β-1,4-D-endoglucanase	EGL	Cellulose	GH5,7,12,61
feruloyl esterase	FAE	Xylan, pectin	CE1
β-1,4-endogalactanase	GAL	Pectin	GH53
Glucuronyl esterase	GE	Xylan	CE15
Glucoamylase	GLA	Starch	GH15
β-1,6-endogalactanase	GLN	Pectin	
galactomannan acetyl esterase	GMAE	Galactomannan	
Endo-inulinase	INU	Inulin	GH32
Exo-inulinase	INX	Inulin	GH32
β-1,4-D-galactosidase	LAC	Xyloglucan, xylan, pectin, galactomannan	GH2,35
β-1,4-D-endomannanase	MAN	Galactomannan	GH5,26
β-1,4-D-mannosidase	MND	Galactomannan	GH2
pectin acetyl esterase	PAE	Pectin	
pectin lyase	PEL	Pectin	PL1
rhamnogalacturonan lyase	RGL	Pectin	PL4,11
endopolygalacturonase	PGA	Pectin	GH28
exopolygalacturonase	PGX	Pectin	GH28
pectate lyase	PLY	Pectin	PL1,3,9
pectin methyl esterase	PME	Pectin	CE8
rhamnogalacturonan acetyl esterase	RGAE	Pectin	CE12
rhamnogalacturonan galaturonohydrolase / exorhamnogalacturonase	RGX	Pectin	GH28
α-rhamnosidase / rhamnogalacturonan rhamnohydrolase	RHA	Pectin	GH78
rhamnogalacturonan hydrolase / endorhamnogalacturonase	RHG	Pectin	GH28
Invertase / fructofuranosidase	SUC	Inulin	GH32
d-4,5 unsaturated -glucuronyl hydrolase	UGH	Pectin	GH88
unsaturated rhamnogalacturonan hydrolase	URH	Pectin	GH105
β-1,4-exogalactanase	XFG	Pectin	
xyloglucan-active β-1,4-D-endoglucanase	XEG	Xyloglucan	GH12,74
xyloglucan acetylesterase	XGAE	Xyloglucan	
β-1,4-D-endoxylanase	XLN	Xylan	GH10,11
β-1,6-exogalactanase	XSG	Pectin	
β-1,3-exogalactanase	XTG	Pectin	

EXPANSION OF BASIDIOMYCETE GENOMES

Basidiomycete species form a relative small section among the fungi for which genome annotations have been published as could be seen from the comparison mentioned above. This prevents in depth comparisons as have been published for ascomycete species as the coverage of the basidiomycete tree of line is insufficient to draw conclusions on evolutionary changes in these species related to plant polysaccharide degradation. However, a much larger set of basidiomycete genomes is currently in progress which will soon enable such detailed studies (Table 2). With the rapidly reducing costs of genome sequencing, this number of genomes is likely to grown exponentially over the next years.

Table 2. Basidiomycete genomes that are finished or in progress.

Species	Order	Sequencing Institute
Agaricus bisporus var bisporus	Agaricomycotina	JGI
Agaricus bisporus var. burnettii	Agaricomycotina	JGI
Auricularia delicata	Agaricomycotina	JGI
Ceriporiopsis subvermispora	Agaricomycotina	JGI
Coniophora puteana	Agaricomycotina	JGI
Coprinopsis cinerea	Agaricomycotina	Broad
Cryptococcus neoformans	Agaricomycotina	Broad
Dacryopinax sp.	Agaricomycotina	JGI
Dichomitus squalens	Agaricomycotina	JGI
Fomitiporia mediterranea	Agaricomycotina	JGI
Fomitopsis pinicola	Agaricomycotina	JGI
Ganoderma sp.	Agaricomycotina	JGI
Gloeophyllum trabeum	Agaricomycotina	JGI
Hemileia vastatrix	Pucciniomycotina	Genoscope
Heterobasidion annosum	Agaricomycotina	JGI
Laccaria bicolor	Agaricomycotina	JGI
Malassezia globosa	Ustilaginomycotina	JGI
Melampsora laricis-populina	Pucciniomycotina	JGI
Microbotryum violaceum	Pucciniomycotina	Genoscope
Phanerochaete carnosa	Agaricomycotina	JGI
Phanerochaete chrysosporium	Agaricomycotina	JGI
Phlebia brevispora	Agaricomycotina	JGI
Pleurotus ostreatus	Agaricomycotina	JGI
Postia placenta	Agaricomycotina	JGI
Puccinia graminis	Pucciniomycotina	Broad
Punctularia strigosozonata	Agaricomycotina	JGI
Rhodotorula graminis	Pucciniomycotina	JGI
Schizophyllum commune	Agaricomycotina	JGI
Serpula lacrymans	Agaricomycotina	JGI
Sporobolomyces roseus	Pucciniomycotina	JGI
Stereum hirsutum	Agaricomycotina	JGI
Trametes versicolor	Agaricomycotina	JGI
Tremella mesenterica Fries	Agaricomycotina	JGI
Ustilago maydis	Ustilaginomycotina	Broad
Wolfiporia cocos	Agaricomycotina	JGI

GROWTH PROFILING OF FUNGI VALIDATES DIFFERENCES IN FUNGAL GENOME SEQUENCES RELATED TO PLANT POLYSACCHARIDE DEGRADATION

Recently a novel fungal database has been initiated (www.fung-growth.org) to host growth profiles on a set of 35 carbon sources related to plant biomass. These growth profiles have already been used to provide biological support for differences observed in ascomycete genomes with respect to plant polysaccharide degradation [19, 22-24, 27]. The database currently contains growth profiles for more than 100 fungal species with genome sequences that are finished or in progress and has already shown significant differences in the ability of basidiomycete fungi to use various plant polysaccharides. These comparisons are part of several basidiomycete genome papers that are currently submitted or in progress and will provide a better understanding of the evolutionary changes in basidiomycete fungi.

CONCLUDING REMARKS

Considering the importance of plant biomass as a carbon source for many fungi, a better understanding of the diversity of fungi with respect to carbon utilisation will provide a significant increase in our understanding of fungal evolution. The expansion of basidiomycete genome sequences and other datasets (e.g. transcriptomics, proteomics) will enable studies into these fungi to much higher level and is likely to result in new strategies for improving mushroom cultivation.

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GENOME SEQUENCE, FUNCTIONAL GENOMICS OF SHIITAKE MUSHROOM *LENTINULA EDODES*

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ABSTRACT

Lentinula edodes (Shiitake/Xianggu) is a popular cultivated mushroom species. Understanding the genomics and functional genomics of *L. edodes* is essential to improve its cultivation and quality. Genome sequencing of *L. edodes* provides numerous molecular genetic markers for breeding and genetic manipulation. We sequenced the genome of *L. edodes* monokaryon L54A using Roche 454 and ABI SOLiD. Sequencing reads of about 1011 Mb were *de novo* assembled into a 39.8 Mb genome. We compiled the genome sequences into a searchable database with which we have been annotating the genes and analyzing the metabolic pathways. Over 13,000 gene models were predicted from the genome sequence. The gene models were annotated by BLASTX and categorized according to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). For functional genomics, we have been using many molecular techniques including RNA arbitrarily primed-PCR, SAGE, LongSAGE, EST sequencing and cDNA microarray to analyze genes differentially expressed during development. Protein families of *L. edodes* genome sequence compared across genomes of several fungi identified protein families conserved to mushroom-forming fungi. We are learning more about the molecular biology and genetics of this economically important mushroom.

Keywords: Shiitake Mushroom, genome sequence, transcriptome, fruiting body development

INTRODUCTION

Lentinula edodes (Berk.) Pegler, or the shiitake mushroom, is the second most cultivated mushroom worldwide, especially in China and Japan. Understanding the genomics and functional genomics of *L. edodes* is essential to improve its cultivation and quality. *Lentinula edodes* follows a typical basidiomycete life cycle. Two monokaryotic mycelia with compatible mating types fuse to form a dikaryon. Under appropriate environmental conditions, dikaryotic mycelia aggregate to form a primordium and then mature into a fruiting body. The molecular biology of Agaricales fruiting body initiation and development remains to be elucidated.

Since 2008, reports of genome sequences from mushroom-forming basidiomycete fungi (Agaricales) has been emerging, including *Laccaria bicolor* [1], *Coprinopsis cinerea* [2], *Schizophyllum commune* [3]. Comparison of the mushroom genome sequences will help distinguish genes shared by different mushroom species from those that are specific to individual species. Further characterization of the conserved genes may provide insights into the complex developmental process.