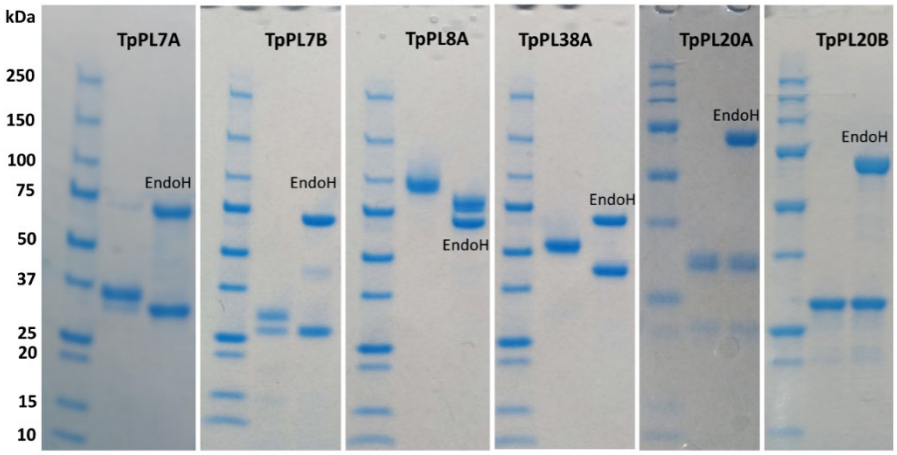
Supplementary information

Discovery of a novel glucuronan lyase system in *Trichoderma parareesei*

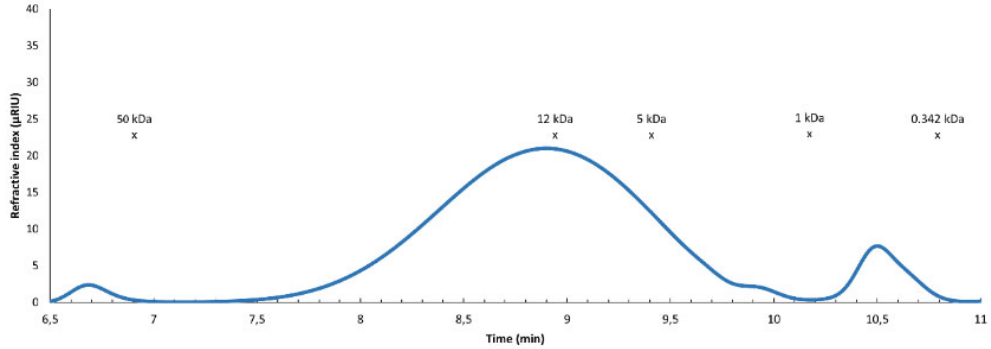
Bo Pilgaard, Marlene Vuillemin, Line Munk, Jesper Holck, Sebastian Meier, Casper Wilkens* & Anne S. Meyer*

Protein Chemistry and Enzyme Technology Section, DTU Bioengineering, Department of Biotechnology and Biomedicine, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.
DTU Chemistry, Department of Chemistry, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

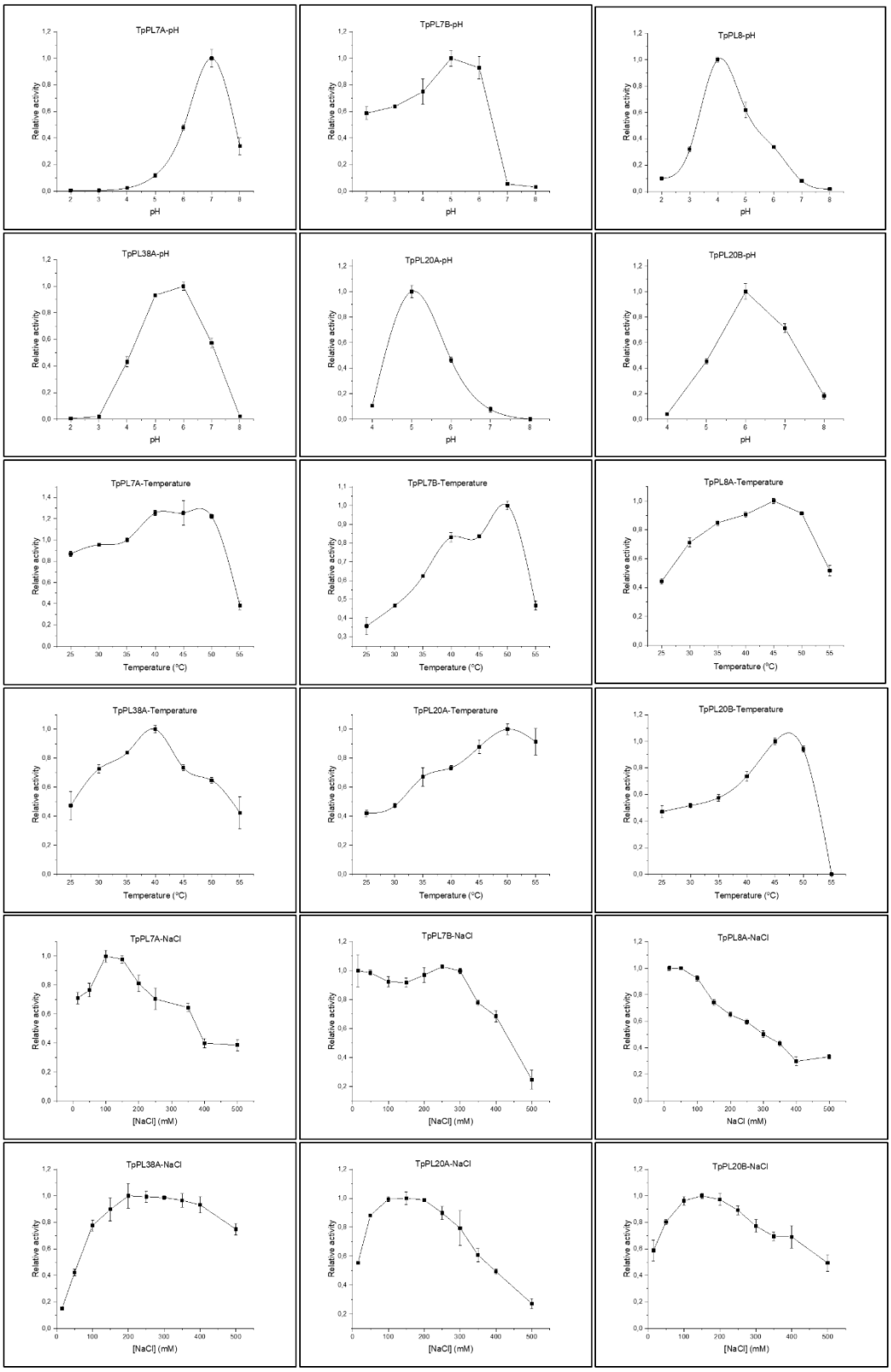
*Corresponding author. Address as above, e-mail: cwil@dtu.dk; asme@dtu.dk



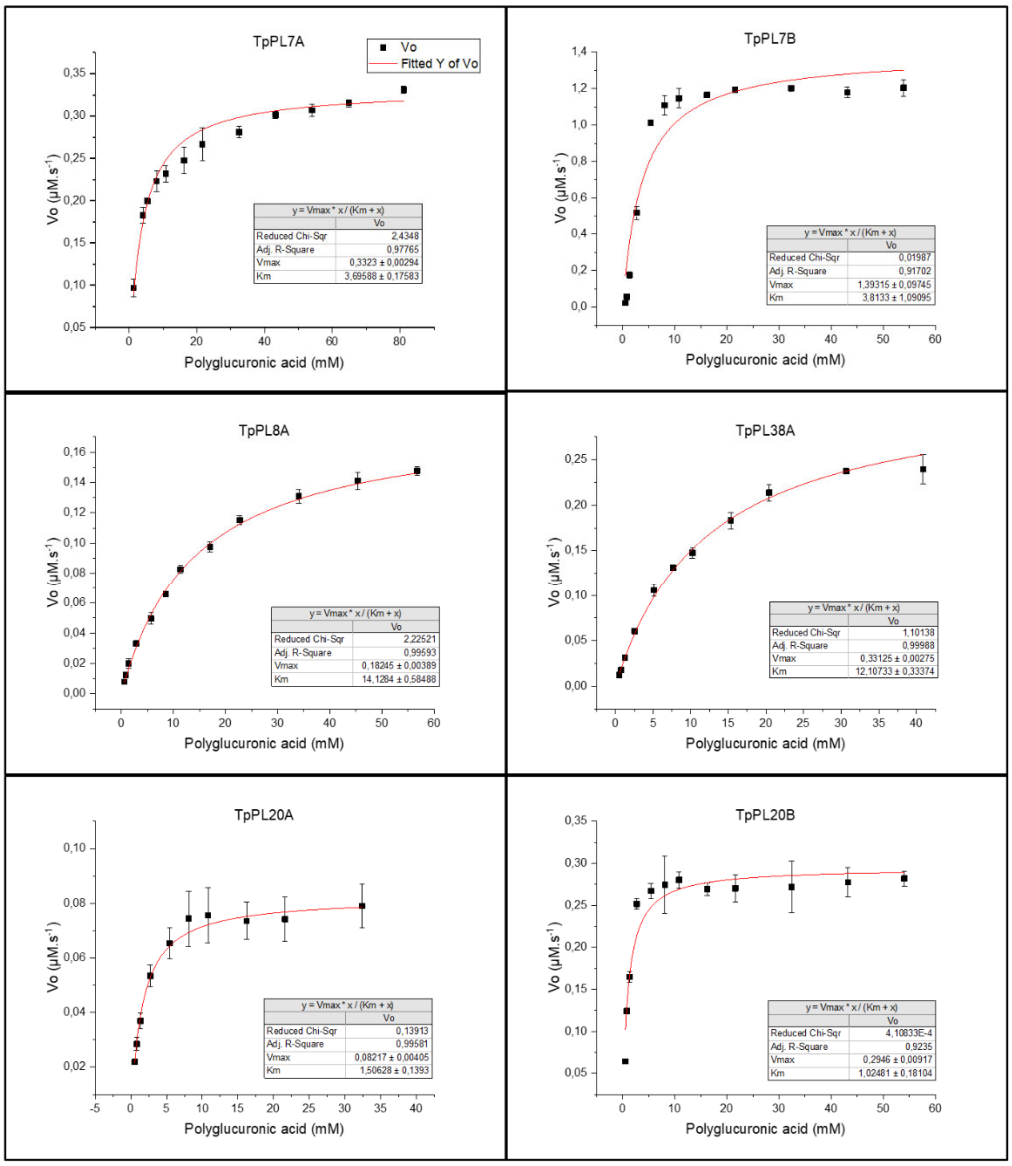
Supplementary Figure 1. 4-12% gradient SDS-page gels of the purified recombinant lyases before and after endoH treatment. EndoH is marked by text closest to the band representing it.



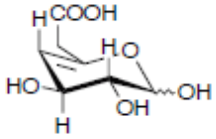
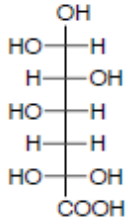
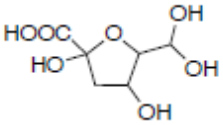
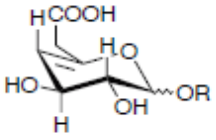
Supplementary Figure 2. HP-SEC of the TEMPO prepared polyglucuronic acid at a concentration of 10 g/L. The peaks of the pullulan size standards are marked by x and the size in kDa.



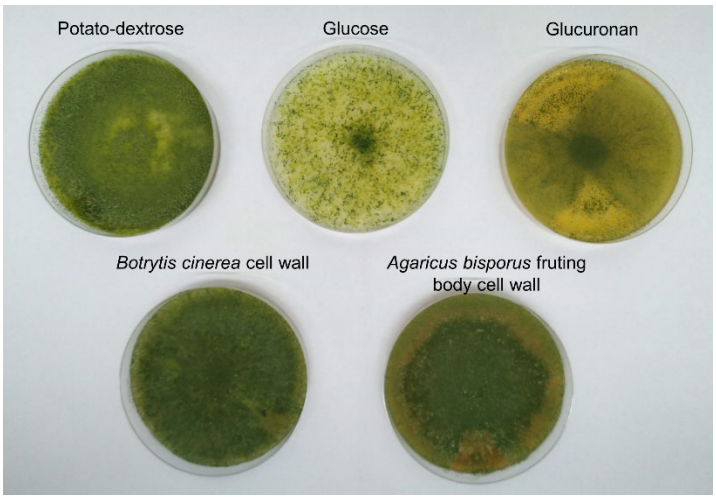
Supplementary Figure 3. Biochemical characterisations of the six recombinant glucuronan lyases from *T. parareesei*. Error bars represent standard deviations of triplicate experiments.



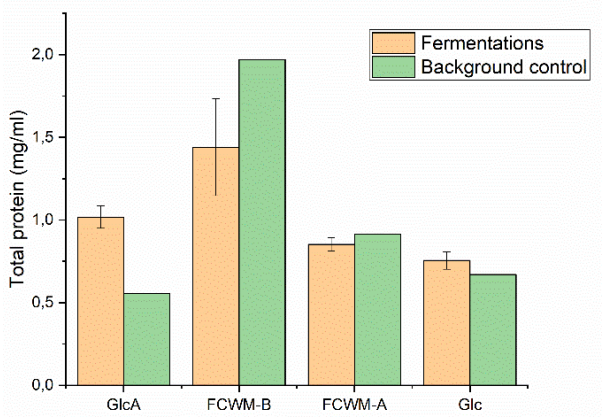
Supplementary Figure 4. Initial rates at optimum conditions under increasing substrate concentrations. Red lines are fitted Michaelis-Menten curves. Calculated V_{max} and K_m values are displayed in the grey boxes. Error bars represent standard deviations from triplicate experiments. The graphs and model-fittings were performed in Origin v. 2019b.

		^{1}H	^{13}C
β-glucuronan	C-1	4.49	102.3
	C-2	3.35	72.9
	C-3	3.61	74.4
	C-4	3.66	80.9
	C-5	3.85	75.3
	C-6		175.1
		5.21	92.7
		3.69	70.2
		4.20	65.9
		5.71	106.2
			145.5
			169.3
		4.89	93.1
		3.28	76.5
		3.91	68.1
		2.1/1.87	39.2
			95.9
			175.6
		5.17	89.5
		4.00	85.0
		4.42	72.0
		2.38/2.32	44.3
			103.8
			177.1
		5.08	100.1
		3.85	69.4
		4.12	n.d.
		5.86	107.2
			144.9
			169.1

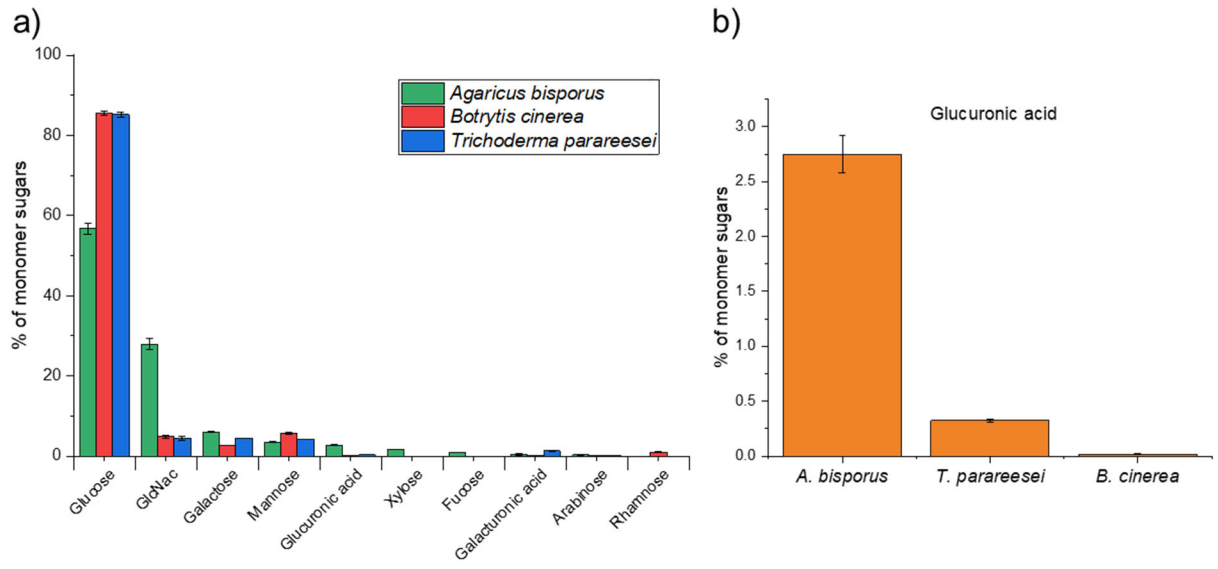
Supplementary Figure 5. NMR, ^{1}H and ^{13}C chemical shift assignments for the main isomers of the displayed molecules.



Supplementary Figure 6. Agar plates showing growth of *T. parareseei* after six days at 30°C on the various carbon sources used in this study.



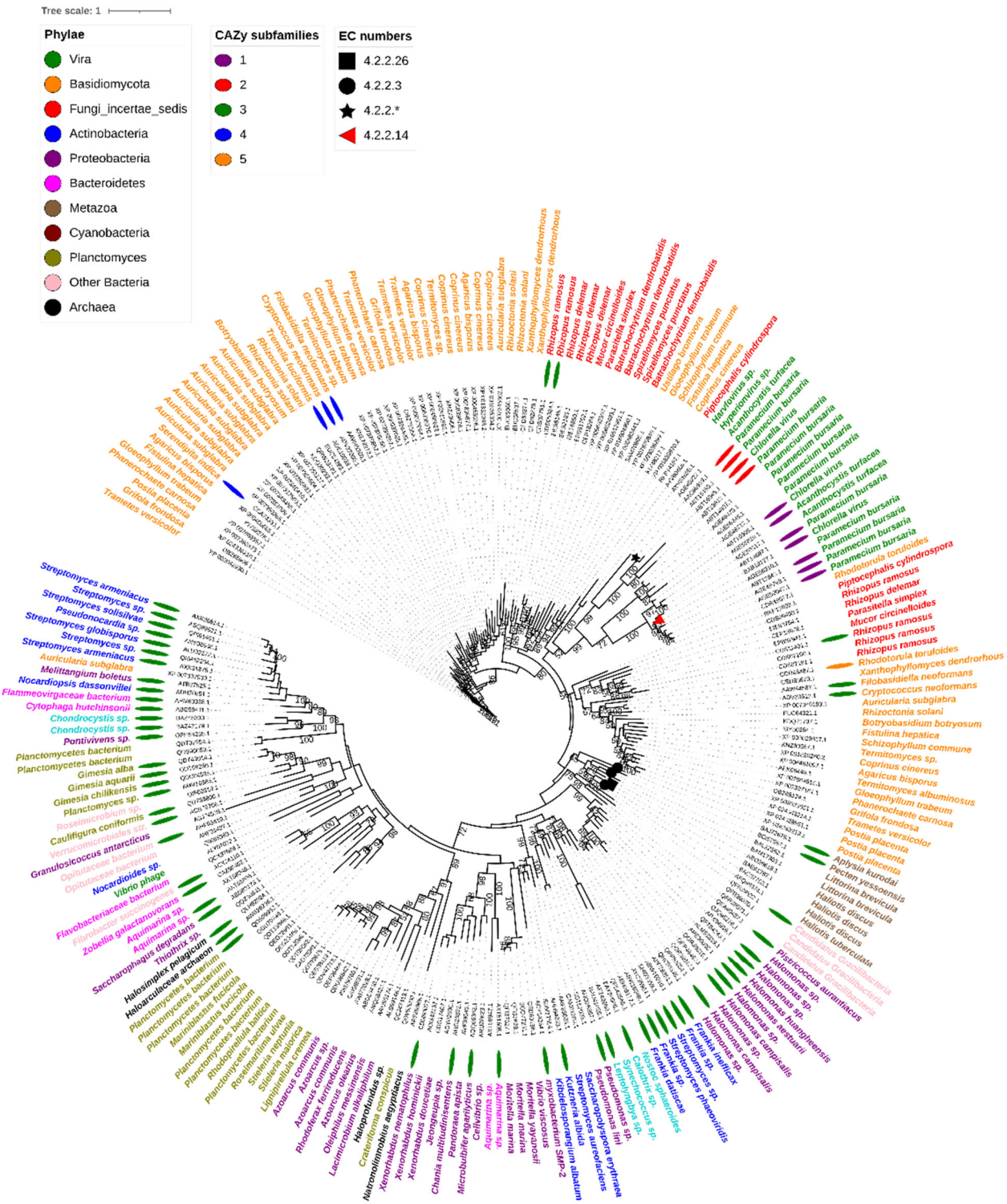
Supplementary Figure 7. Total protein content of fermentation supernatant. Estimated by BCA with BSA as standard. Error bars represent standard deviations of triplicate experiments.



Supplementary Figure 8. HPAEC-PAD analysis of sugar monomer composition of fungal cell walls after acid hydrolysis. Values are normalized to total amount of detected sugars. Error bars represent standard deviation of triplicate analysis. **a)** All sugars. **b)** Subset of a) showing only glucuronic acid.

						Glucuronan					GAG	Pectin						
Phylum	Class	Order	Family	Organism	Major ecology	PL7	PL8	PL20	PL38	PL14	PL35	PL1	PL3	PL4	PL9	PL11	PL26	PL27
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma arundinaceum*	Mycotrophic / generalistic	2	1	2	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma asperellum*	Mycotrophic / generalistic	3	1	2	2	0	0	1	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma atroviride*	Mycotrophic / generalistic	3	1	2	2	0	0	2	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma gamsii*	Mycotrophic / generalistic	3	1	2	2	0	0	2	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma harzianum*	Mycotrophic / generalistic	3	1	2	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma longibrachiatum*	Mycotrophic / generalistic	2	1	2	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma parareesei*	Mycotrophic / generalistic	2	1	2	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma reesei QMA*	Mycotrophic / generalistic	2	1	2	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma virens*	Mycotrophic / generalistic	3	1	2	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Cladobotryum protrusum*	Mycotrophic	4	1	2	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces perniciosus*	Mycotrophic	2	1	1	1	0	0	0	0	0	0	0	0	1
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces rosellus*	Mycotrophic	4	1	2	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Escovopsis weberi*	Mycotrophic	1	1	0	2	0	0	2	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium acridum*	Entomopathogenic	1	1	1	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium album*	Entomopathogenic	1	1	1	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium brunneum*	Entomopathogenic	1	1	1	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium robertsii*	Entomopathogenic	1	1	1	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Pochonia chlamydosporia*	Nematophagous	5	1	1	2	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps fumosorosea*	Entomopathogenic	1	1	1	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps militaris*	Entomopathogenic	1	1	1	0	0	1	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Purpureocillium lilacinum*	Entomopathogenic	1	1	1	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Ophiocordyceps sinensis*	Entomopathogenic	2	0	0	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Clonostachys rosea*	Mycotrophic / generalistic	0	0	1	0	0	0	18	11	4	1	2	1	3
Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Geosmithia morbida*	Phytopathogenic	0	0	1	0	0	0	2	1	1	0	0	2	0
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Ustilaginoidea virens*	Phytopathogenic	0	0	0	0	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium fujikuroi*	Phytopathogenic	0	0	0	0	0	0	11	5	2	2	0	1	0
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium graminearum*	Phytopathogenic	0	0	1	0	0	0	9	7	3	1	0	1	0
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium oxysporum*	Saprobic	0	0	0	0	0	0	10	7	4	2	1	1	0
Ascomycota	Sordariomycetes	Diaporthales	Cryphonectriaceae	*Cryphonectria parasitica*	Phytopathogenic	1	0	0	0	0	0	5	1	2	0	0	0	0
Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe citri*	Phytopathogenic	0	0	0	0	0	0	18	8	7	1	0	1	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum aenigma*	Phytopathogenic	0	0	1	0	0	0	16	14	7	3	1	1	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum fructicola*	Phytopathogenic	0	0	1	0	0	0	18	14	7	3	2	2	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum graminicola*	Phytopathogenic	0	0	0	0	0	0	7	4	3	1	0	1	1
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum higginsianum*	Phytopathogenic	0	0	0	0	0	0	18	16	7	4	1	1	1
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum karsti*	Phytopathogenic	0	0	0	0	0	0	18	15	8	3	1	1	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum orchidophilum*	Phytopathogenic	0	0	0	0	0	0	14	9	6	3	0	2	0
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum scovillei*	Phytopathogenic	0	0	1	0	0	0	15	13	6	3	1	2	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum siamense*	Phytopathogenic	0	0	1	0	0	0	17	14	7	3	1	2	2
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum truncatum*	Phytopathogenic	0	0	0	0	0	1	22	15	9	5	1	2	2
Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	*Verticillium alfalfae*	Phytopathogenic	0	0	0	0	0	0	16	11	4	2	1	1	0
Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	*Verticillium dahliae*	Phytopathogenic	0	0	0	0	0	0	16	11	5	2	1	1	0
Ascomycota	Sordariomycetes	Magnaporthales	Magnaporthaceae	*Gaeumannomyces tritici*	Phytopathogenic	0	0	1	0	0	0	3	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Magnaporthales	Pyriculariaceae	*Pyricularia grisea*	Phytopathogenic	0	0	1	0	0	0	2	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Magnaporthales	Pyriculariaceae	*Pyricularia oryzae*	Phytopathogenic	0	0	1	0	0	0	2	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Magnaporthales	Pyriculariaceae	*Pyricularia pennisetigena*	Phytopathogenic	0	0	1	0	0	0	2	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Microascales	Microascaceae	*Scedosporium apiospermum*	Saprobic	0	0	0	0	0	0	1	0	1	0	0	0	0
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Grosmannia clavigera*	Wood-rot	0	0	0	0	0	0	2	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Sporothrix brasiliensis*	Saprobic	0	0	0	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Sporothrix schenckii*	Saprobic	0	0	0	1	0	0	0	0	0	0	0	0	0
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium globosum*	Saprobic	0	0	1	0	0	0	7	4	2	1	0	1	0
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium thermophilum*	Saprobic	0	0	0	0	0	0	1	3	1	0	0	0	0
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Podospora anserina*	Saprobic	0	0	0	0	0	1	4	2	1	0	0	0	0
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thermothelomyces thermophilus*	Saprobic	0	0	1	0	0	0	5	1	1	0	0	1	0
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thermothielavioides terrestris*	Saprobic	1	0	0	0	0	0	0	0	2	0	0	1	0
Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora crassa*	Saprobic	0	0	1	0	0	0	1	1	1	0	0	0	0
Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora tetrasperma*	Saprobic	0	0	1	0	0	0	1	1	1	0	0	0	0
Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Sordaria macrospora*	Saprobic	0	0	1	0	0	0	1	2	1	0	0	0	0
Ascomycota	Sordariomycetes	Togniniales	Togniniaceae	*Phaeoacremonium minimum*	Phytopathogenic	0	0	1	0	0	0	4	3	3	1	0	0	0
Ascomycota	Sordariomycetes	Xylariales	Hypoxylaceae	*Daldinia childiae*	Wood-rot	0	0	2	0	0	0	2	0	5	0	0	1	0
Ascomycota	Sordariomycetes	Xylariales	Pseudomassariaceae	*Pseudomassariella vexata*	Saprobic	0	0	0	0	0	0	10	8	7	1	0	1	0
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Pestalotiopsis fici*	Phytopathogenic	0	0	0	0	0	0	19	7	7	1	1	3	1
Ascomycota	Sordariomycetes	Xylariales	Cephalothecaceae	*Phialemoniopsis curvata*	Saprobic	0	0	1	0	0	0	1	1	2	0	0	2	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus aculeatus*	Saprobic	1	0	0	0	0	0	6	0	3	0	0	1	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus flavus*	Saprobic	1	0	2	0	0	0	12	3	3	1	0	2	1
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus fumigatus*	Saprobic	0	0	1	1	0	0	6	3	3	1	0	1	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus nidulans*	Saprobic	0	0	2	0	0	0	8	5	4	1	1	1	1
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus niger*	Saprobic	0	0	0	0	0	0	6	0	2	0	0	0	1
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium chrysogenum*	Saprobic	0	0	1	0	0	0	5	1	2	0	0	1	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium digitatum*	Phytopathogenic	0	0	0	0	0	0	3	1	2	0	0	0	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium expansum*	Phytopathogenic	0	0	2	0	0	0	4	1	2	0	0	0	0
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium roqueforti*	Saprobic	0	0	0	0	0	0	2	0	2	0	0	0	0
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces marneffei*	Saprobic	1	0	1	0	0	1	2	0	0	0	0	0	0
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces verruculosus*	Saprobic	1	0	1	0	0	0	3	0	0	0	0	1	0
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Paradendryphiella salina*	Saprobic (marine)	3	1	0	0	0	0	6	5	5	0	0	1	0
Ascomycota	Leotiomycetes	Leotiomycetes i.s.	Pseudeurotiaceae	*Pseudogymnoascus verrucosus*	Saprobic	3	0	0	3	0	0	9	4	4	1	1	2	0
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Botrytis cinerea*	Phytopathogenic	1	0	0	0	0	0	7	2	0	0	0	0	0
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Sclerotinia sclerotiorum*	Phytopathogenic	1	0	0	0	0	0	4	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus bisporus*	Saprobic	0	1	0	1	4	1	2	1	1	0	0	1	0
Basidiomycota	Agaricomycetes	Agaricales	Fistulinaceae	*Fistulina hepatica*	Wood-rot	0	1	0	0	4	1	1	0	1	0	0	0	0
Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	*Termitomyces sp*	Saprobic	0	3	0	1	7	1	2	1	1	0	0	0	0
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Lentinula edodes*	Wood-rot	2	0	0	2	3	1	3	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinopsis cinerea*	Saprobic	0	1	0	3	7	3	1	2	2	0	0	0	0
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum commune*	Wood-rot	0	1	0	1	2	1	5	4	3	1	0	1	0
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia subglabra*	Wood-rot	1	2	0	6	13	2	2	1	1	0	0	1	0
Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	*Botryobasidium botryosum*	Wood-rot	0	1	0	0	3	1	4	3	1	0	0	0	0
Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia solani*	Phytopathogenic	0	3	0	5	5	1	28	18	10	2	0	1	0
Basidiomycota	Agaricomycetes	Gloeophyllales	Gloeophyllaceae	*Gloeophyllum trabeum*	Wood-rot	0	1	0	1	6	1	0	0	2	0	0	0	0
Basidiomycota	Agaricomycetes	Polyporales	Dacryobolaceae	*Postia placenta*	Wood-rot	0	0	0	1	5	1	0	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	Polyporales	Grifolaceae	*Grifola frondosa*	Wood-rot	0	2	0	2	4	2	0	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phanerochaete carnosa*	Wood-rot	0	1	0	2	4	1	0	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes versicolor*	Wood-rot	0	2	0	2	6	2	0	0	1	0	0	0	0
Basidiomycota	Tremellomycetes	Tremellales	Cryptococcaceae	*Cryptococcus gattii*	Saprobic	0	0	0	2	2	1	0	0	1	0	0	0	0
Basidiomycota	Pucciniomycetes	Pucciniales	Pucciniaceae	*Puccinia striiformis*	Phytopathogenic	0	0	0	0	0	1	3	0	0	0	0	0	0
Basidiomycota	Pucciniomycetes	Pucciniales	Pucciniaceae	*Puccinia triticina*	Phytopathogenic	0	0	0	0	0	1	4	0	0	0	0	0	0
Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Anthracocystis flocculosa*	Phytopathogenic	0	0	0	0	0	1	1	1	1	0	0	0	0
Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Moesziomyces antarcticus*	Saprobic	0	0	0	0	1	1	1	0	0	0	0	0	0
Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Ustilago maydis*	Phytopathogenic	0	0	0	0	0	1	1	0	0	0	0	0	0
Mucoromycota	Mucoromycetes	Mucorales	Lichtheimiaceae	*Lichtheimia ramosa*	Saprobic	0	0	0	4	6	0	0	0	0	0	0	0	0
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor circinelloides*	Saprobic	0	2	0	2	2	0	0	0	0	0	0	0	0
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Parasitella parasitica*	Mycoparasitic	0	2	0	2	2	0	0	0	0	0	0	0	0
Mucoromycota	Mucoromycetes	Mucorales	Rhizopodaceae	*Rhizopus delemar*	Saprobic	0	1	0	3	5	0	0	0	0	0	0	0	0
Chytridiomycota	Chytridiomycetes	Rhizophlyctidales	Rhizophlyctidaceae	*Rhizophlyctis rosea*	Saprobic	0	0	0	2	1	0	4	4	2	0	0	0	0
Chytridiomycota	Chytridiomycetes	Rhizophydiales		*Batrachochytrium dendrobatidis*	Dermatophyte	0	0	0	1	4	0	0	0	0	0	0	0	0
Chytridiomycota	Chytridiomycetes	Spizellomycetales	Spizellomycetaceae	*Spizellomyces punctatus*	Parasitic	0	0	0	2	2	0	0	0	0	0	0	0	0
Chytridiomycota	Monoblepharidomycetes	Monoblepharidales	Gonapodyaceae	*Gonapodya prolifera*	Saprobic	0	0	0	0	0	0	6	3	0	0	0	0	0
Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	*Piptocephalis cylindrospora*	Mycoparasitic	0	0	0	3	3	0	0	0	0	0	0	0	0
Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	*Syncephalis pseudoplumigaleata*	Mycoparasitic	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Figure 9. Genome annotation of PL genes in a broad selection of fungi covering major ecological niches and taxonomic groups. The putative substrate categories, glucuronan, GlycosAmino Glycans (GAG) and pectin are inferred by substrate specificities of experimentally characterized members of the CAZy families and phylogenetic analysis of the protein sequences.



Supplementary Figure 10. Maximum likelihood phylogenetic tree of protein sequences from the PL14 family. All sequences were subjected to redundancy, fragmentation and alignment checks. Characterized members are highlighted by symbols at the node ending. Subfamily annotations are highlighted by the colored ellipsis next to the species names. Branch numbers indicate bootstrap values above 50.

Supplementary Table 1. Experimental determination of the extinction coefficient (E) for the enzymatic 4-5 double bond formation in glucuronan. Glucuronic acid was used as quantitative standard in the reducing end assay. TpPL7B was used for producing stable double bonds in the substrate under standard assay conditions.

Glucuronan (mg/mL)	A_{235}	s.d.	Reduced ends (mM)	s.d.	E
0.8	0.221	0.0025	0.0641	0.0419	6621
1.0	0.515	0.0064	0.1650	0.0074	6013
1.5	0.322	0.0006	0.0957	0.0298	6470
Mean					**6368 ± 317**

Supplementary Table 2. Subset of the most abundant CAZymes found in the proteomics analysis. The standard deviations are based on triplicate experiments.

					Normalized intensities							
Accession	**CAZy family**	**EC number**	**Enzyme description**	**Substrate**	**Glucuronan**	**Std. d**	**FCWM-B**	**Std. d**	**FCWM-A**	**Std. d**	**Glucose**	**Std. d**
OTA07244.1	GH15	3.2.1.3	glucan 1,4-α-glucosidase	Starch	0.221	0.011	0.326	0.139	0.058	0.031	0.097	0.020
OTA07404.1	GH13_1	3.2.1.1	α-amylase	Starch	0.000	0.000	0.212	0.067	0.000	0.001	0.012	0.010
OTA02846.1	GH31	-	-	Starch	0.265	0.020	0.102	0.042	0.121	0.085	0.294	0.089
OTA01276.1	GH79	-	-	Proteoglycan	0.342	0.085	1.231	0.158	0.004	0.002	0.004	0.002
OTA07787.1	GH79	3.2.1.31	β-glucuronidase	Proteoglycan	0.572	0.088	0.892	0.185	0.062	0.046	0.091	0.097
OTA03228.1	GH89	3.2.1.50	α-N-acetylglucosaminidase	Proteoglycan	0.032	0.001	0.067	0.078	0.034	0.018	0.061	0.020
OTA08831.1	GH20	3.2.1.52	β-N-acetylhexosaminidase	Proteoglycan	0.270	0.070	0.014	0.017	0.024	0.009	0.099	0.054
OTA01106.1	GH20	3.2.1.52	β-N-acetylhexosaminidase	Proteoglycan	0.053	0.004	0.000	0.000	0.081	0.028	0.071	0.016
OTA02666.1	AA2	-	-	Oxidase	0.064	0.022	0.220	0.044	0.101	0.016	0.144	0.062
OTA07476.1	AA7	-	-	Oxidase	1.083	0.183	0.209	0.069	4.863	1.046	2.833	0.093
OTA07907.1	AA5	-	-	Oxidase	0.380	0.041	0.073	0.036	0.923	0.174	0.382	0.068
OTA02290.1	AA14	-	-	Oxidase	0.039	0.006	0.059	0.016	0.131	0.026	0.049	0.012
OTA02426.1	AA7	-	-	Oxidase	0.267	0.014	0.049	0.017	0.231	0.098	0.080	0.066
OTA03014.1	AA2	-	-	Oxidase	0.175	0.011	0.025	0.026	0.260	0.059	0.107	0.056
OTA03614.1	AA2	1.11.1.5	cytochrome-c peroxidase	Oxidase	0.098	0.004	0.006	0.002	0.038	0.006	0.070	0.017
OTA03261.1	GH38	3.2.1.24	α-mannosidase	α-1,6-mannan	0.078	0.018	0.000	0.000	0.077	0.016	0.119	0.044
OTA05230.1	GH92	3.2.1.24	α-mannosidase	α-1,6-mannan	0.023	0.002	0.000	0.000	0.052	0.012	0.057	0.016
OTA02561.1	GH71	3.2.1.59	glucan endo-1,3-α-glucosidase	α-1,3-glucan	0.004	0.001	0.006	0.003	0.007	0.005	0.015	0.007
OTA00561.1	GH71	3.2.1.59	glucan endo-1,3-α-glucosidase	α-1,3-glucan	0.040	0.012	0.000	0.000	0.016	0.006	0.036	0.012
OSZ99865.1	CBM24	3.2.1.59	glucan endo-1,3-α-glucosidase	α-1,3-glucan	0.038	0.006	0.000	0.000	0.005	0.004	0.129	0.163
OTA05307.1	GH54	-	-	Xylan	0.163	0.019	0.467	0.109	0.272	0.214	0.399	0.125
OTA05875.1	GH115	3.2.1.131	xylan α-1,2-glucuronosidase	Xylan	0.066	0.013	0.000	0.000	0.023	0.002	0.067	0.031
OTA01922.1	GH18	3.2.1.96	syl-glycoprotein endo-β-N-acetylglucosami	Glycoprotein	0.165	0.016	0.251	0.126	0.062	0.042	0.188	0.068
OTA05399.1	GH47	3.2.1.113	iannosyl-oligosaccharide 1,2-α-mannosidas	Glycoprotein	0.225	0.019	0.220	0.162	0.267	0.142	0.427	0.237
OTA07247.1	PL20_B	4.2.2.14	glucuronan lyase	Glucuronan	0.412	0.016	0.863	0.453	0.003	0.002	0.007	0.003
OTA00986.1	PL7_4_A	-	-	Glucuronan	0.822	0.102	0.236	0.209	0.019	0.003	0.124	0.182
OTA01262.1	PL7_4_B	-	-	Glucuronan	0.049	0.023	0.083	0.099	0.001	0.000	0.001	0.000
OTA06285.1	PL38	4.2.2.14	glucuronan lyase	Glucuronan	0.348	0.151	0.067	0.048	0.010	0.007	0.009	0.003
OTA05836.1	PL8_4	-	-	Glucuronan	0.959	0.181	0.049	0.026	0.018	0.000	0.024	0.014
OSZ99978.1	-	3.2.1.22	α-galactosidase	Galactan	0.141	0.038	0.000	0.000	0.014	0.003	0.024	0.012
OTA00159.1	GH2	3.2.1.146	β-galactofuranosidase	idophosphogalactomar	0.252	0.074	0.000	0.000	0.034	0.025	0.043	0.024
OTA07992.1	CBM66	3.2.1.146	β-galactofuranosidase	idophosphogalactomar	0.077	0.002	0.000	0.000	0.174	0.048	0.200	0.095
OTA00212.1	GH75	3.2.1.132	chitosanase	Chitosan	0.423	0.087	1.053	0.229	0.015	0.004	0.034	0.006
OTA04600.1	GH18	3.2.1.14	chitinase	Chitin	0.325	0.027	0.146	0.045	0.425	0.095	0.654	0.402
OTA00069.1	GH2	3.2.1.165	exo-1,4-β-D-glucosaminidase	Chitosan	0.631	0.114	0.043	0.027	0.142	0.094	0.045	0.021
OTA08247.1	GH18	3.2.1.14	chitinase	Chitin	0.220	0.007	0.000	0.000	0.247	0.040	0.392	0.104
OTA06834.1	GH18	3.2.1.14	chitinase	Chitin	0.107	0.015	0.000	0.000	0.194	0.058	0.229	0.128
OTA01114.1	CE4	-	-	Chitin	0.029	0.005	0.000	0.000	0.102	0.032	0.033	0.020
OTA00434.1	GH18	3.2.1.14	chitinase	Chitin	0.017	0.005	0.000	0.000	0.009	0.002	0.020	0.006
OTA04876.1	GH5_5	3.2.1.4	cellulase	β-1,4-glucan	0.006	0.008	0.037	0.022	0.037	0.035	0.153	0.119
OTA02682.1	GH3	-	-	β-1,4-glucan	0.003	0.000	0.005	0.006	0.002	0.001	0.000	0.000
OSZ99894.1	GH3	-	-	β-1,4-glucan	0.016	0.002	0.000	0.000	0.015	0.005	0.017	0.004
OTA01208.1	GH30_3	3.2.1.75	glucan endo-1,6-β-glucosidase	β-1,6-glucan	0.147	0.007	1.567	0.488	0.045	0.004	0.064	0.010
OTA00769.1	GH55	3.2.1.58	glucan 1,3-β-glucosidase	β-1,3-glucan	0.875	0.203	0.335	0.165	0.228	0.097	0.488	0.225
OTA05146.1	GH55	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.443	0.042	0.238	0.124	0.051	0.014	0.016	0.009
OTA02562.1	GH64	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.661	0.060	0.229	0.067	0.828	0.566	0.869	0.328
OTA08417.1	GH128	-	-	β-1,3-glucan	0.000	0.000	0.147	0.070	0.000	0.000	0.000	0.000
OTA08148.1	GH55	-	-	β-1,3-glucan	2.400	0.284	0.108	0.078	0.032	0.020	0.014	0.011
OTA01266.1	GH55	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.023	0.001	0.065	0.013	0.014	0.007	0.011	0.008
OTA01439.1	GH128	-	-	β-1,3-glucan	0.005	0.001	0.038	0.008	0.008	0.002	0.011	0.006
OTA04977.1	GH55	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.024	0.004	0.020	0.022	0.016	0.003	0.016	0.001
OTA04295.1	GH81	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.000	0.000	0.019	0.019	0.001	0.000	0.000	0.000
OTA01419.1	GH132	3.2.1.x	-	β-1,3-glucan	0.354	0.045	0.000	0.000	0.337	0.022	0.388	0.091
OTA06148.1	GH16_3	3.2.1.6	endo-1,3(4)-β-glucanase	β-1,3-glucan	0.238	0.031	0.000	0.000	0.536	0.065	0.265	0.071
OTA06837.1	GH128	-	-	β-1,3-glucan	0.121	0.071	0.000	0.000	0.121	0.024	0.130	0.093
OTA05871.1	GH81	3.2.1.39	glucan endo-1,3-β-D-glucosidase	β-1,3-glucan	0.011	0.002	0.000	0.000	0.006	0.002	0.010	0.003
OTA01208.1	GH30_3	3.2.1.75	glucan endo-1,6-β-glucosidase	β-1,6-glucan	0.147	0.007	1.567	0.488	0.045	0.004	0.064	0.010
OTA07339.1	GH5_15	3.2.1.75	glucan endo-1,6-β-glucosidase	β-1,6-glucan	0.004	0.000	0.000	0.000	0.015	0.005	0.017	0.004

Supplementary Table 3. Genomes downloaded from NCBI and used in this study.

Strain name	NCBI assembly accession nb.	*Strain name*	NCBI assembly accession nb.
Achlya hypogyna	GCA_002081595.1	*Pyricularia oryzae*	GCF_000002495.2
Agaricus bisporus	GCA_000300575.1 Agabi_varbisH97_2	*Grosmannia clavigera*	GCF_000143105.1
Aspergillus aculeatus	GCF_001890905.1_Aspac1	*Chaetomium globosum*	GCF_000143365.1
Aspergillus flavus	GCA_000006275.3	*Gaeumannomyces tritici*	GCF_000145635.1
Aspergillus fumigatus	GCF_000002655.1_ASM265v1	*Colletotrichum graminicola*	GCF_000149035.1
Aspergillus nidulans	GCF_000149205.2_ASM14920v2	*Verticillium dahliae*	GCF_000150675.1
Aspergillus niger	GCF_000002855.3_ASM285v2	*Verticillium alfalfae*	GCF_000150825.1
Auricularia subglabra	GCF_000265015.1_Auricularia_subglabra_SS-5_V1.0	*Sordaria macrospora*	GCF_000182805.2
Batrachochytrium dendrobatidis	GCF_000203795.1_v1.0	*Neurospora crassa*	GCF_000182925.2
Botryobasidium botryosum	GCA_000697705.1_Botbo1	*Metarhizium acridum*	GCF_000187405.1
Botrytis cinerea	GCF_000143535.2_ASM14353v4	*Metarhizium robertsii*	GCF_000187425.2
Catenaria anguillulae	GCA_002102555.1_Catan2	*Neurospora tetrasperma*	GCF_000213175.1
Cladobotryum protrusum	GCA_004303015.1_ASM430301v1	*Chaetomium thermophilum*	GCF_000221225.1
Clonostachys rosea	GCA_000963775.2_ASM96377v2	*Cordyceps militaris*	GCF_000225605.1
Coprinopsis cinerea	GCF_000182895.1_CC3	*Thermothelomyces thermophilus*	GCF_000226095.1
Cordyceps fumosorosea	GCF_001636725.1_ISF_1.0	*Thermothielavioides terrestris*	GCF_000226115.1
Cordyceps militaris	GCF_000225605.1	*Podospora anserina*	GCF_000226545.1
Cryptococcus gattii	GCF_000185945.1_ASM18594v1	*Beauveria bassiana*	GCF_000280675.1
Escovopsis weberi	GCA_001278495.1_ASM127849v1	*Phaeoacremonium minimum*	GCF_000392275.1
Fistulina hepatica	GCA_000934395.1_Fishe1	*Pestalotiopsis fici*	GCF_000516985.1
Fusarium fujikuroi	GCF_900079805.1_Fusarium_fujikuroi_IMI58289_V2	*Ustilaginoidea virens*	GCF_000687475.1
Fusarium graminearum	GCF_000240135.3_ASM24013v3	*Scedosporium apiospermum*	GCF_000732125.1
Fusarium oxysporum	GCF_000149955.1_ASM14995v2	*Metarhizium album*	GCF_000804445.1
Gloeophyllum trabeum	GCF_000344685.1_Glotr1_1	*Metarhizium brunneum*	GCF_000814965.1
Gonapodya prolifera	GCA_001574975.1_Ganpr1	*Sporothrix brasiliensis*	GCF_000820605.1
Grifola frondosa	GCA_001683735.1_ASM168373v1	*Sporothrix schenckii*	GCF_000961545.1
Hypomyces perniciosus	GCA_008477525.1_ASM847752v1	*Drechmeria coniospora*	GCF_001625195.1
Hypomyces rosellus	GCA_011799845.1_ASM1179984v1	*Cordyceps fumosorosea*	GCF_001636725.1
Lentinus edodes	GCA_002003045.1	*Pochonia chlamydosporia*	GCF_001653235.2
Lichtheimia ramosa	GCA_000945115.1_Lramosa_hybrid_454_Illumina	*Purpureocillium lilacinum*	GCF_001653265.1
Lichtheimia ramosa	GCA_000945115.1	*Colletotrichum higginsianum*	GCF_001672515.1
Moesziomyces antarcticus	GCF_000747765.1_ASM74776v1	*Colletotrichum orchidophilum*	GCF_001831195.1
Mucor circinelloides	GCA_000401635.1_Muco_sp_1006Ph_V1	*Pseudomassariella vexata*	GCF_002105095.1
Ophiocordyceps sinensis	GCA_012934285.1_ASM1293428v1	*Sodiomyces alkalinus*	GCF_003711515.1
Paradendryphiella salina	GCA_900634815.1	*Verticillium nonalfalfae*	GCF_003724135.2
Parasitella parasitica	GCA_000938895.1_PP.v1	*Pyricularia pennisetigena*	GCF_004337985.1
Penicillium chrysogenum	GCA_000710275.1_ASM71027v1	*Phialemoniopsis curvata*	GCF_004353045.1
Penicillium digitatum	GCF_000315645.1_PdigPd1_v1	*Pyricularia grisea*	GCF_004355905.1
Penicillium expansum	GCF_000769745.1_ASM76974v1	*Daldinia childiae*	GCF_008694065.1
Penicillium roqueforti	GCA_000513255.1_PROQFM164_20130607	*Colletotrichum fructicola*	GCF_009771025.1
Phanerochaete carnosa	GCF_000300595.1_Phanerochaete_carnosa_HHB-10118-Sp_v1.0	*Colletotrichum scovillei*	GCF_011075155.1
Piptocephalis cylindrospora	GCA_003614145.1_Pipcy3_1	*Cryphonectria parasitica*	GCF_011745365.1
Pochonia chlamydosporia	GCF_001653235.2_ASM165323v2	*Colletotrichum karsti*	GCF_011947395.1
Podospora anserina	GCF_000226545.1	*Geosmithia morbida*	GCF_012550715.1
Postia placenta	GCF_002117355.1_PosplRSB12_1	*Colletotrichum aenigma*	GCF_013390185.1
Pseudozyma flocculosa	GCF_000417875.1_Pflocc_1.0	*Colletotrichum siamense*	GCF_013390195.1
Puccinia striiformis	GCA_001191645.1	*Colletotrichum truncatum*	GCF_014235925.1
Puccinia triticina	GCA_000151525.2_P_triticina_1_1_V2	*Diaporthe citri*	GCF_014595645.1
Rhizoctonia solani	GCA_000524645.1_Rhizoctonia_solani_AG-3	*Pseudogymnoascus verrucosus*	GCA_001662655.1
Rhizophlyctis rosea	GCA_002214945.1		
Rhizopus delemar	GCA_000149305.1_RO3		
Schizophyllum commune	GCF_000143185.1_v1.0		
Sclerotinia sclerotiorum	GCF_000146945.2_ASM14694v2		
Spizellomyces punctatus	GCF_000182565.1_S_punctatus_V1		
Syncephalis pseudoplumigaleata	GCA_003614755.1_Synps1		
Talaromyces marneffei	GCF_000001985.1		
Talaromyces verruculosus	GCA_001305275.1		
Termitomyces	GCA_001263195.1_TerJ1321.0		
Trametes versicolor	GCF_000271585.1_Trametes_versicolor_v1.0		
Trichoderma asperellum	GCF_003025105.1_Trias_v._1.0		
Trichoderma atroviride	GCF_000171015.1_TRIAT_v2.0		
Trichoderma gamsii	GCF_001481775.2_TGAM01v2		
Trichoderma harzianum	GCF_003025095.1_Triha_v1.0		
Trichoderma longibrachiatum	GCA_003025155.1_Trilo3		
Trichoderma parareesei	GCA_001050175.1_Tpara_v1.0		
Trichoderma reesei QM6	GCF_000167675.1_v2.0		
Trichoderma virens	GCF_000170995.1_TRIVI_v2.0		
Ustilago maydis	GCF_000328475.2_Umaydis521_2.0		
Verticillium alfalfae	GCF_000150825.1_ASM15082v1		
Verticillium dahliae	GCF_000150675.1_ASM15067v2		