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Comparative characterization of nine novel GH51, GH54 and GH62 α-L-arabinofuranosidases from *Penicillium subrubescens*

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α-L-Arabinofuranosidases (ABFs) are important enzymes in plant biomass degradation with a wide range of applications. The ascomycete fungus *Penicillium subrubescens* has more α-L-arabinofuranosidase-encoding genes in its genome compared to other Penicillia. We characterized nine ABFs from glycoside hydrolase (GH) families GH51, GH54 and GH62 from this fungus and demonstrated that they have highly diverse specificity and activity levels, indicating that the expansion was accompanied by diversification of the enzymes. Comparison of the substrate preference of the enzymes to the expression of the corresponding genes when the fungus was grown on either of two plant biomass substrates did not show a clear correlation, suggesting a more complex regulatory system governing L-arabinose release from plant biomass by *P. subrubescens*.

Keywords: arabinoxylan; pectin; *Penicillium subrubescens*; recombinant expression; α-L-arabinofuranosidases

L-Arabinose is a major constituent of plant biomass and is present in the side chains of pectin, xylan and xyloglucan [1]. α-L-Arabinofuranosidases (ABFs) are exo-acting enzymes, which release α-arabinofuranosyl residues from xylan, pectin and xyloglucan, and are therefore an important class of enzymes involved in degradation of plant biomass [1]. They are commonly produced by fungi during growth on plant biomass substrates. ABFs have many applications in plant biomass-based industrial processes, such as increasing digestibility of animal feeds [2], enhancing bread quality and texture [3], clarification of fruit juice [4], increasing aroma in wine [5], promoting pulp delignification [6], and improving saccharification for biofuel production [7] and prebiotics production [8].

Based on their amino acid sequence signatures, fungal ABFs have been assigned to four glycoside hydrolase (GH) families of the Carbohydrate-Active enZyme database (www.cazy.org) [9]: GH43, GH51, GH54 and GH62. GH43 is a more diverse family that contains various enzyme activities, including enzymes with ABF and/or β-xylosidase activity, while the other families in fungi only contain ABFs. Many studies have reported the differences between ABFs of the different GH families and revealed significant differences also within the specific GH families (see [10] and [11] for a review of these studies).

Genome sequencing has provided an unprecedented insight into the diversity of fungi with respect to their enzymatic potential and revealed significant differences in the number of genes for specific CAZy families [12]. While the evolutionary drivers for this diversity remain to be elucidated, it has been shown that expansion or reduction of enzymes related to a certain polysaccharide

Abbreviations
ABF, α-L-arabinofuranosidases; Axh, arabinoxylan arabinofurnaohydrolase; GH, glycoside hydrolase; *p*NPαAra, *para*-nitrophenyl-α-L-arabinofuranoside.

correlates with improved or reduced growth of the fungus on that polysaccharide [13–15].

Penicillium subrubescens stands apart from most other Penicillia, by an expanded set of genes encoding plant biomass degrading enzymes, in particular with respect to hemicellulase- and pectinase-encoding genes [16]. It was previously shown to be a promising industrial species [17] and has established genome editing methodology [18]. In a previous study, we compared six GH27 and GH36 α-galactosidases, revealing clear differences in substrate specificity and physical properties of the enzymes [19]. Among the expanded gene set of *P. subrubescens* are also genes encoding putative ABFs, five, four and four members of GH51, GH54 and GH62, respectively. While the number of GH51 genes is not unusual, most fungi analysed so far only have 0–2 GH54 and GH62 genes (unpublished data), suggesting a specific increase in the ability to release L-arabinose from plant biomass by *P. subrubescens*. To determine whether this expansion resulted in an increased set of functional enzymes and whether this led to redundancy or diversification, we compared nine ABFs from *P. subrubescens* in this study by heterologous production and biochemical characterization of the corresponding enzymes. The results demonstrate large variability between the enzymes and demonstrate that the expansion cannot be simply considered to be enzyme redundancy, but appears to have been accompanied with a functional diversification.

Materials and methods

Bioinformatic analysis

Amino acid sequences of all characterized fungal ABFs from GH51, GH54 and GH62 were obtained from the CAZy database (http://www.cazy.org/) [9] and combined with the *P. subrubescens* amino acid sequences of candidate secreted ABFs in a multiple sequence alignment using MAFFT v7.0 (https://www.ebi.ac.uk/Tools/msa/mafft/). Phylogenetic analysis was computed using the maximum likelihood (ML) method with the Poisson correction distance of substitution rates of the Molecular Evolutionary Genetics Analysis (MEGA v7.0) program [20]. Neighbour-joining (NJ) and minimum evolution (ME) trees were conducted both using the Poisson model with uniform rates and complete deletion. Bootstrap values were generated based on the 500 resampled data sets, using a 50% value as cut-off. All positions containing gaps and missing data were eliminated. The optimal tree from ML method was used as support for the other displayed NJ and ME trees, indicating their bootstrap values in the branches of the ML trees.

Theoretical isoelectric point (p*I*) and molecular weights (Mw) were calculated by ExPASy–ProtParam tool (https://web.expasy.org/compute_pi/).

cDNA cloning of *P. subrubescens* ABF encoding genes and production in *Pichia pastoris*

Specific total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fischer Scientific, Carlsbad, CA, USA) and purified by NucleoSpin RNA (Macherey-Nagel, Düren, Germany). Full-length cDNA was obtained using ThermoScript Reverse Transcriptase (Invitrogen). Mature ABF encoding cDNAs, without the native signal peptide, were amplified by PCR from RNA obtained from *P. subrubescens* grown on sugar beet pulp as described previously [19]. All PCR products were assembled in pPICZαA cloning vector using NEBuilder HiFi DNA Assembly Mix (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's protocol. The resulting plasmids were transformed, propagated into *Escherichia coli* DH5α competent cells (Invitrogen, Thermo Scientific) and confirmed by sequence analysis (Macrogen, Amsterdam, the Netherlands). After linearization of the plasmids with *Pme*I or *Sac*I (Promega, Madison, WI, USA), the DNA was transformed into *Pichia pastoris* X-33 cells using electroporation.

Pichia pastoris transformants were selected and cultured, and the proteins subsequently purified as described previously [19]. Fractions containing enzyme were pooled, concentrated, and buffer-exchanged to 20 mM HEPES, pH 7.0, using 10 kDa cut-off ultrafiltration units Amicon (Merck, Darmstadt, Germany). All purification steps were performed at 4 °C.

Physical properties of ABFs

The molecular mass of the purified enzymes was estimated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (12% w/v, SDS/PAGE) using Mini-PROTEAN Tetra Cell (Bio-Rad, Hercules, CA, USA) and the standard marker, PageRuler™ Plus Prestained Protein ladder (Thermo Fisher Scientific) with Coomassie Brilliant Blue staining (Bio-Rad). Deglycosylation was performed by treating the native enzymes with endoglycosidase H (New England Biolabs, Rowley, MA, USA) according to the manufacturer instructions. The protein concentration was determined by a Bradford assay with bovine serum albumin (Pierce, Thermo Scientific, Waltham, MA, USA) as standard.

Enzyme activity assays and enzyme stability

For assessment of α-arabinofuranosidase activity, *p*-nitrophenyl α-L-arabinofuranoside (*p*NPαAra) (Sigma-Aldrich, Zwijndrecht, The Netherlands) was used as a substrate. The

activities were assayed in a total volume of 100 µL reaction mixtures containing 10 µL of 2 mM *p*NPαAra in 50 mM sodium acetate buffer, pH 5.0, and 0.2–0.3 nM purified enzymes at 30 °C. The release of *p*-nitrophenol was spectrophotometrically quantified by following the absorbance at 405 nm in a FLUOstar OPTIMA microtiter plate reader (BMG LabTech, Ortenberg, Germany) up to 30 min with a 2 min interval. The linear range was used for calculation of enzyme activity. One unit of enzymatic activity was defined as the amount of protein required to release 1 µmol of the corresponding product per minute, under the assay condition used.

The effect of pH on the recombinant α-arabinofuranosidases was determined over different pH range of 2.0–12.0 using 40 mM Britton-Robinson buffer (adjusted to the required pH) at 30 °C, under the conditions described above, except that the reaction was stopped after 30 min with 100 µL 0.25 M Na_2CO_3. The pH stability was analysed by incubating the enzymes in the same buffer system in the range from pH 2.0 to pH 12.0 for 1 h and then determining their residual activities by the standard assay in 50 mM sodium acetate, pH 5.0, at 30 °C. The effect of temperature on the recombinant α- arabinofuranosidases was determined over the temperature range of 20–90 °C at their optimum pH values, essentially as above. Thermostability was investigated by measuring the enzyme activity remaining after incubation for 1 h at 20–90 °C.

Enzyme kinetics

Kinetic parameters of the Michaelis–Menten constant (K_m), maximum enzyme velocity (V_{max}), turnover number (k_{cat}), and the catalytic efficiency (k_{cat}/K_m) were measured by determining the enzyme initial activities over a defined concentration range (0.25–7.0 mM) of *p*NPαAra. The *p*NPαAra enzyme initial activities were determined during 30 min using the same experimental and assay conditions described above for each enzyme.

Activity towards plant biomass substrates

Hydrolysis of arabinan was measured using 3 $\mu g \cdot mL^{-1}$ of recombinant enzyme and 1% of wheat arabinoxylan, (sugar beet) arabinan or debranched arabinan (Megazyme), or sugar beet pectin (Pectin Betapec RU301 Herbstreith & Fox KG, Neuenbürg, Germany) in 50 mM sodium acetate buffer (pH 4.0). The samples were incubated for 24 h at 30 °C and 100 r.p.m. Saccharification reactions were stopped by incubation at 95 °C for 15 min after which the samples were centrifuged (10 min, 4 °C, 13 500 *g*), and the supernatant was diluted 10-fold in Milli-Q water prior the analysis. The released arabinose was quantified using HPAEC-PAD (Dionex ISC-5000+ system; Thermo Fisher Scientific, Sunnyvale, CA, USA), equipped with a CarboPac PA1 (250 mm × 4 mm i.d.) column (Thermo Fisher Scientific). The column was pre-equilibrated with 18 mM NaOH followed by a multi-step gradient: 0–20 min: 18 mM NaOH, 20–30 min: 0–40 mM NaOH and 0–400 mM sodium acetate, 30–35 min: 40–100 mM NaOH and 400 mM to 1 M sodium acetate, 35–40 min: 100 mM NaOH and 1 M to 0 M sodium acetate followed by re-equilibration of 18 mM NaOH for 10 min (20 °C; flow rate: 0.30 $mL \cdot min^{-1}$). 5–250 µM L-arabinose (Sigma-Aldrich) was used as standards for quantification. The data obtained are the results of two independent biological replicates, and for each replicate, three technical replicates were assayed. The arabinose released was calculated as a percentage of the highest hydrolysis reached for each treatment, which was set to 100%.

Results and Discussion

Phylogenetic diversity of fungal ABFs

Annotation of the *P. subrubescens* genome predicted five, four and four members of GH51, GH54 and GH62, respectively [16]. However, two of the GH51 members (8514 and 8515) are in fact two parts of a single gene surrounding a gap in the genome sequence, and 4850 (GH54) appears to be an incomplete gene model. These genes were therefore excluded from the study. Naming of the other genes was done in such a way that the genes that were most similar to the well-characterized AbfA, AbfB and AxhA from *Aspergillus niger* [21–23] were given the corresponding name, while the other genes were named consecutively.

To evaluate the diversity of these ABFs from *P. subrubescens* in more detail, phylogenetic trees were constructed for each of the families (Fig. 1) in which all characterized fungal members of the families from the CAZy database (www.cazy.org) were included as well as all the members of a selection of fungal genomes from Mycocosm (https://mycocosm.jgi.doe.gov/).

The four GH51 members from *P. subrubescens* separated clearly in the phylogenetic tree (Fig. 1), with 1–2 members being present in each major branch of ascomycete enzymes. AbfC and AbfH were both present in the same branch, but while AbfH clustered with other Eurotiomycete sequences. AbfC was most similar to an enzyme from the Sordariomycete fungus *Thermothelomyces thermophilus*, suggesting that *P. subrubescens* may have obtained this gene through horizontal gene transfer. AbfA and AbfD are present in separate branches of the tree, but each within a group of Eurotiomycetes enzymes, indicating that they are evolutionary conserved. The significant distance from each other in the tree indicates a high likelihood for functional differences among the GH51 enzymes of *P. subrubescens*. AbfD appears to be an intracellular enzyme

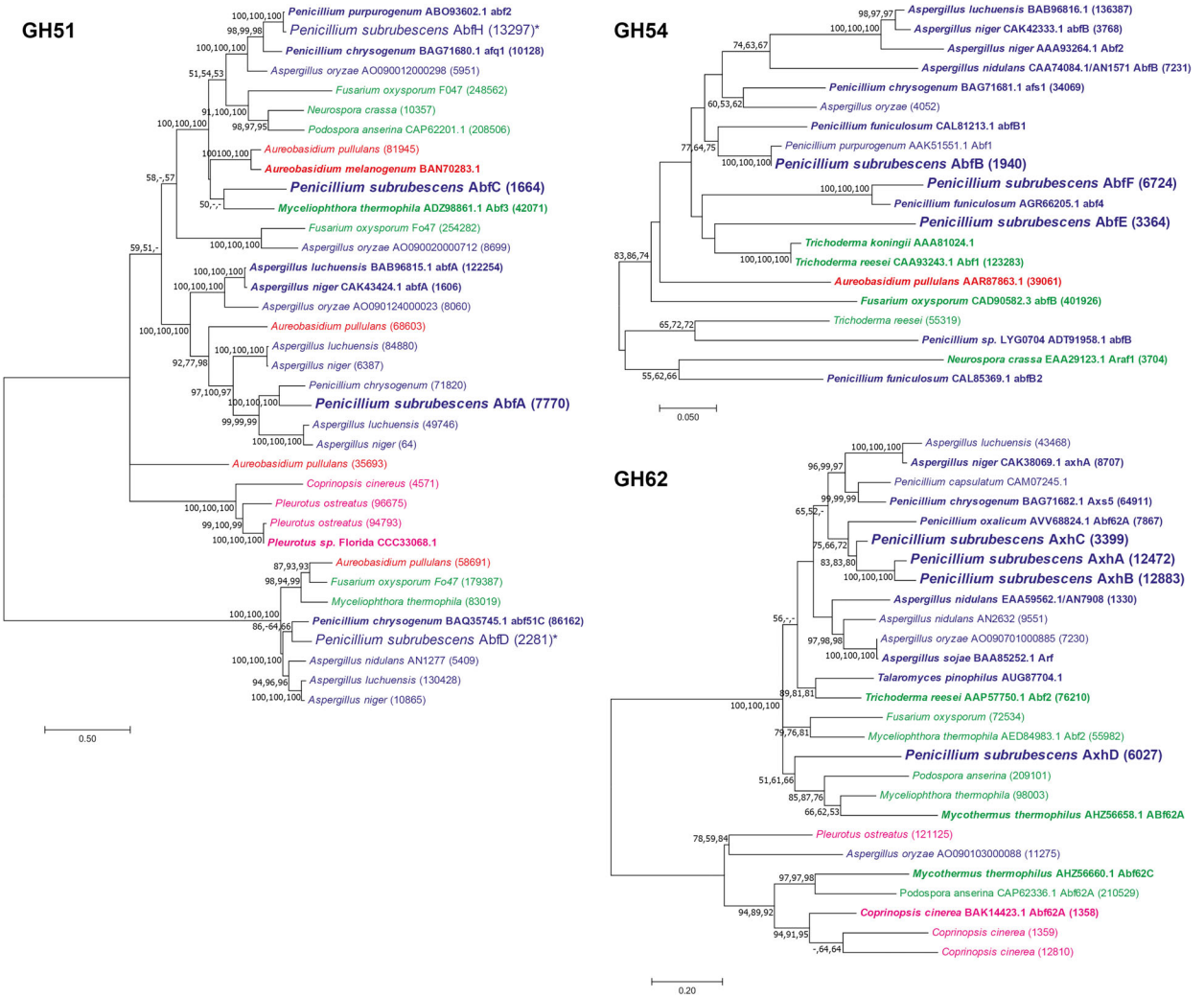

Fig. 1. Analysis of phylogenetic relationships among the (putative) fungal α-arabinofuranosidases from *Penicillium subrubescens* and selected fungal species (selected based on the presence of characterized enzymes and taxonomic distance to *P. subrubescens*) from GH51, GH54 and GH62. The phylogram was inferred using the ML method and the optimal tree is shown. Values over 50% bootstrap support (500 replicates) are shown next to the branch nodes from ML (first position), NJ (second position) and minimal evolution (ME, third position) tree values from the same data set. All enzymes with biochemical characterization are in boldface. The putative *P. subrubescens* α-arabinofuranosidases that we did not manage to produce and were therefore not biochemically characterized are indicated with an asterisk.

as it lacks a secretory signal sequence. In contrast, the GH54 members from *P. subrubescens* were positioned more closely in their phylogenetic tree, with AbfE and AbfF located in the same branch (Fig. 1). They are similar to other Eurotiomycete enzymes, although AbfE also had similarity to enzymes from *Trichoderma*. In GH62, three of the four *P. subrubescens* members are clustered very closely which is likely due to recent gene duplications (Fig. 1). The fourth member (AxhD) is in a branch with enzymes from Sordariomycetes, suggesting that this could also have originated from horizontal gene transfer.

Functional diversity of *P. subrubescens* ABFs

Using PCR and a cDNA pool of *P. subrubescens* as a template, sequences encoding the mature polypeptide of nine ABFs (AbfA, AbfB, AbfC, AbfE, AbfF, AxhA, AxhB, AxhC, AxhD) were obtained and cloned in-frame with a C-terminal His-tag in *P. pastoris* expression vectors. Despite several attempts, we did not succeed in obtaining a cDNA fragment for AbfH, so this enzyme was not included in the further comparison. The recombinant enzymes were produced in *P. pastoris* and purified (Fig. S1), and their physical

properties and specific activity towards *p*NPαAra were compared (Table 1). The molecular mass of the enzymes (after deglycosylation) matched the calculated values. Only for AxhD, a larger molecular mass was observed on gel (Fig. S1).

All ABFs had slightly acidic pIs, varying from 4.62 to 6.39 (Table 1) as well as an acidic pH optimum, varying between pH 4 and 5, with the GH62 enzymes having a slightly higher pH optimum (Fig. 2A). In contrast, most GH51 and GH54 ABFs have a higher temperature optimum (50 °C) than the GH62 ABFs (40 °C; Fig. 2B). Most enzymes are stable between pH 3 and pH 7, but AxhA and AxhB maintain a high stability at pH 8, while AxhD and AbfF retain more than 50% of their activity at pH 2 (Fig. 2C). AbfA and AbfE have the highest temperature stability and maintain 100% of their activity up to 50 °C, while the other enzymes maintain this until 40 °C (Fig. 2D). The similar physical properties are likely related to the natural habitat of *P. subrubescens*, which is a mesophilic species commonly found in soil [24]. Similar values have also been observed for ABFs from other mesophilic species (Table S1), such as *Penicillium purpurogenum* [25], *Penicillium chrysogenum* [26–28], *A. niger* [21,29] and *Aureobasidium pullulans* [30].

Differences were observed between the *P. subrubescens* ABFs in their activity on the model substrate *p*NPαAra, for which AbfB and AbfC had the highest activity, while very low activity was determined for AbfA, AxhA and AxhC (Table 1). Previously, it was shown that GH62 ABFs have very low or no activity on this substrate [23], supporting our results. The large differences within GH51 and GH54 are noteworthy as this may provide insight into the efficiency of different enzymes of these families. When compared to other characterized ABFs, the *P. subrubescens* enzymes have similar physical properties.

Although all enzymes have affinities around or below 1 mM, based on their K_m-values, the enzymes display significant differences in their kinetics (Table 2). AbfB has the highest and AbfA the lowest affinity for *p*NPαAra. AbfB also has the highest catalytic efficiency for this substrate and AxhC the lowest. The values for AbfB are in the same range as those observed for its ortholog from *Penicillium purpurogenum* [31]. In general, GH62 ABFs had a lower catalytic efficiency for *p*NPαAra than most of the GH51 and GH54 ABFs (Table 2), which confirms earlier reports regarding GH62 ABFs. This confirms previous reports that demonstrated that GH62 ABFs belong to type B, which are more active on polymeric arabinoxylan than on unbranched arabinan and *p*NPαAra [27,32].

P. subrubescens ABFs display highly diverse activities on natural substrates

The activity of the ABFs was also tested on four polymeric plant biomass substrates that have been previously used to characterize other fungal ABFs (Table S1): wheat arabinoxylan, sugar beet pectin, arabinan and debranched arabinan. All enzymes, except AbfA, released the highest amount of L-arabinose from arabinan and this reduced strongly from debranched arabinan (Fig. 3). AbfB showed the highest activity on all substrates. Compared to AbfB, AbfA had the lowest activity on all substrates, which matches its low activity on *p*NPαAra (Table 1). It was unusual in that it had a higher L-arabinose release from debranched arabinan than from (branched) arabinan. AbfC had similar activity to AbfB on arabinan

Table 1. Comparison of physical properties and specific activity towards *p*-nitrophenylα-L-arabinofuranoside of nine recombinant *Penicillium subrubescens* ABFs. The specific activity was based on the determined molecular mass.

Enzyme name	Protein id (JGI)	CAZy Family	pI	Molecular mass			Specific activity (U·mg^{-1}[a])
				Calculated	Before EndoH	After EndoH	
AbfA	7770	GH51	4.77	67.6	90	68	3.3 ± 0.7
AbfC	1664	GH51	5.18	68.7	90	69	448.5 ± 101.6
AbfB	1940	GH54	5.17	49.4	55	55	507.9 ± 64.1
AbfE	3364	GH54	4.97	50.0	55	55	43.9 ± 7.4
AbfF	6724	GH54	5.84	50.1	55	55	61.2 ± 8.8
AxhA	12472	GH62	4.63	32.8	33	33	4.1 ± 0.5
AxhB	12883	GH62	4.62	33.1	33	33	14.6 ± 2.1
AxhC	3399	GH62	6.39	32.7	33	33	3.8 ± 0.6
AxhD	6027	GH62	5.33	38.8	50	49	10.9 ± 2.8

[a]One unit of ABF activity is defined as the amount of protein required to release one μmol of *p*-nitrophenol per minute.

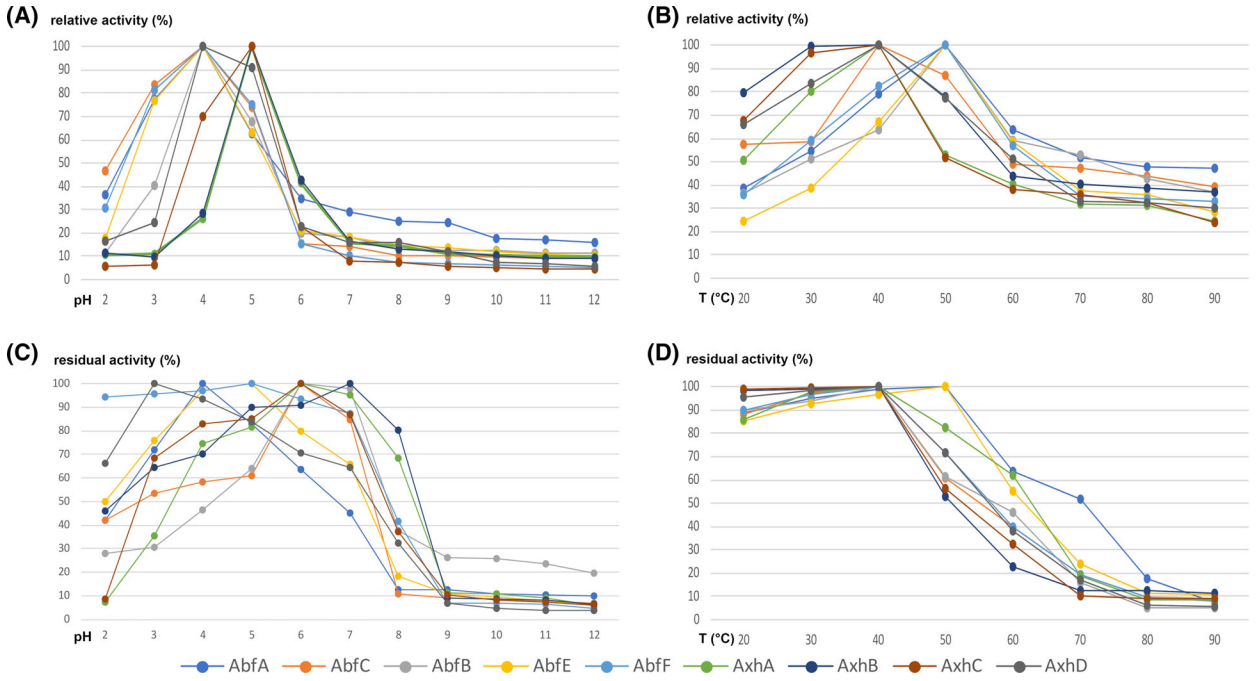

Fig. 2. pH and temperature profiles of the recombinant α-arabinofuranosidases produced in *Pichia pastoris*. Effect of pH (A) and temperature (B) on the activity and stability of recombinant α-arabinofuranosidases using *p*NPαAra as substrate. The pH and temperature dependence for activity was evaluated at 30 °C in 40 mM Britton-Robinson buffer, pH 2.0–12.0, or in 50 mM sodium acetate, pH 5.0, at 20–90 °C, respectively. The pH and temperature stability (C, D) was deduced from the residual activity after 1 h incubation. All assays were carried out in triplicate.

Table 2. Kinetic parameters for hydrolysis of *p*-nitrophenyl-α-L-arabinofuranoside (*p*NPαAra) catalysed by recombinant ABFs from *Penicillium subrubescens*. Parameters were calculated from the initial velocities of *p*-nitrophenol released from *p*NPαAra at different substrate concentrations.

			*p*NPαAra		
Enzyme name	Protein id (JGI)	CAZy Family	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}·s^{-1}$)
AbfA	7770	GH51	1.469	692.3	471.3
AbfC	1664	GH51	0.099	925.3	9348.1
AbfB	1940	GH54	0.080	1169.1	14599.6
AbfE	3364	GH54	0.681	506.4	744.1
AbfF	6724	GH54	0.620	600.0	968.1
AxhA	12472	GH62	0.930	426.1	458.2
AxhB	12883	GH62	0.662	271.4	410.3
AxhC	3399	GH62	1.088	339.7	312.2
AxhD	6027	GH62	0.720	490.8	681.7

and debranched arabinan, but lower activity on wheat arabinoxylan and sugar beet pectin (Fig. 3), indicating a higher specificity for arabinan, which matches previous reports on GH51 and GH54 ABFs [26]. AbfE and AbfF are members of the same branch of the phylogenetic tree and have highly similar activities on the polysaccharide substrates, with a clear preference for pectin-related substrates. The four GH62 enzymes can be divided into two groups with respect to their activity on wheat arabinoxylan and sugar beet pectin. While AxhA and AxhC are clearly more active on wheat arabinoxylan, AxhB and AxhD have similar activity on wheat arabinoxylan and sugar beet pectin (Fig. 3). Activity on pectin-related arabinan has also been reported for related enzymes, such as Abf62A from *Penicillium oxalicum* [33] and Abf62A and Abf62C from *Mycothermus thermophilus* [34]. It has been suggested that this activity is particularly related to the α-1,3-L-arabinose decorations of the α-1,5-L-arabinose backbone or arabinan [32]. However, as all tested GH62 ABFs in our study also hydrolyzed unbranched arabinan, they also have activity on α-1,5-linked L-arabinose.

A previous study described the transcriptome response of *P. subrubescens* to two plant biomass substrates: wheat bran (rich in arabinoxylan) and sugar beet pulp (rich in pectin that contains arabinan) [35]. We therefore analysed the expression of the ABF-encoding genes from *P. subrubescens* this data set (Fig. S2). This revealed that the highest expressed genes were *abfB* and *axhD* (Fig. S2), while no expression was observed for *abfG*, and very low expression

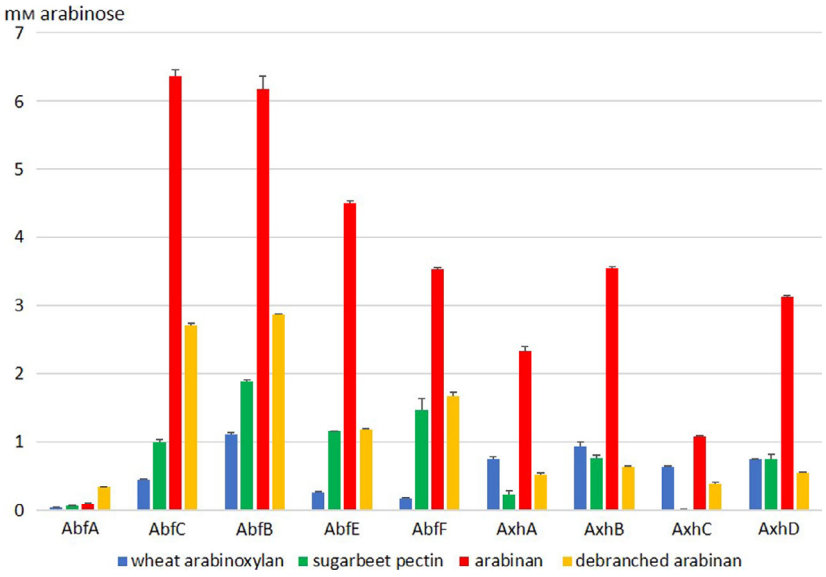

Fig. 3. Arabinose-containing lignocellulosic substrate hydrolysis by recombinant α-arabinofuranosidases. Blue bar, wheat arabinoxylan; green bar, sugar beet pectin; red bar, arabinan; yellow bar, debranched arabinan. Substrates (1% w/v) were incubated with 3 $\mu g \cdot mL^{-1}$ of recombinant enzyme at 30 °C for 24 h. The relative arabinose released was calculated as a percentage of the highest hydrolysis reached for each treatment, which was set to 100%. Values are represented as mean values ± SD ($n = 2$).

for *axhA* and *axhB*. For most genes, expression was higher on sugar beet pulp than on wheat bran. In contrast, highly similar expression levels on both substrates were observed for *axhD*. This reveals that the substrate profile of the enzymes and expression profile of the corresponding genes do not clearly correlate. AbfB and AbfC are the most active enzymes (Table 1; Fig. 3), but while *abfB* is the highest expressed gene on sugar beet pulp, *abfC* is only lowly expressed on both biomass substrates (Fig. 2). The four GH62 ABFs are all very active on wheat arabinoxylan (Fig. 3), but no preferred expression on wheat bran could be observed (Fig. 2). Only *axhD* had similar expression levels on both substrates, while *axhC* was higher expressed on sugar beet pulp, and *axhA* and *axhB* had very low expression on both substrates. A possible explanation for this could be that these substrates are still less complex and diverse, and our results therefore do not reflect the natural response of *P. subrubescens*. Regulation of the expression of genes encoding plant biomass degrading enzymes is highly complex in filamentous fungi. Previous studies in *A. niger* [36] and *P. oxalicum* [37] revealed the complex interplay between several regulators, while it has also been shown that orthologous regulators can have significantly different effects in different species [38]. A better understanding of the regulatory network governing plant biomass conversion in *P. subrubescens* will be needed to shed more light on these expression and activity profiles.

Conclusion

In this study, we demonstrated that nine ABF-encoding genes from *P. subrubescens* encode functional enzymes with diverse expression patterns and substrate specificity. In contrast, their physical properties are highly similar, likely driven by the native habitat of *P. subrubescens*. We have now analysed two expanded enzyme classes (ABFs and AGLs [19]) in this fungus, both of which demonstrate to consist of active proteins with diverse functionalities, suggesting that the expansion in gene numbers is accompanied by a diversification of enzyme function. This indicates that the expansion of these and other enzyme classes in the *P. subrubescens* genome reflects an evolutionary adaptation towards a more diverse and flexible enzymatic toolbox for the degradation of hemicellulose and pectin. This makes this species and others with similar expansions highly interesting candidates for biotechnological applications.

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Author contributions

NCL: Investigation, Formal analysis, Writing - review & editing. XL: Investigation, Data curation, Formal analysis, Writing - review & editing. AD: Supervision,

Writing - review & editing. RPdV: Conceptualization, Supervision, Funding acquisition, Project administration, Resources, Writing – original draft, review & editing.

Data accessibility

Data of this study that is not included in the manuscript is available in the supplemental files. Previously published data used in this study are referred to in the text.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. SDS/PAGE analysis of the purified α-L-arabinofuranosidases from *Penicillium subrubescens* before and after deglycosylation with Endo H.

Fig S2. Expression of *Penicillium subrubescens* ABF-encoding genes on wheat bran and sugar beet pulp [1].

Table S1. Comparison of the physical properties of fungal α-L-arabinofuranosidases. The enzymes are organized based on their position in the phylogenetic tree (Fig. 1).