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Regulation of pentose utilisation by AraR, but not XlnR, differs in *Aspergillus nidulans* and *Aspergillus niger*

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Abstract Filamentous fungi are important producers of plant polysaccharide degrading enzymes that are used in many industrial applications. These enzymes are produced by the fungus to liberate monomeric sugars that are used as carbon source. Two of the main components of plant polysaccharides are L-arabinose and D-xylose, which are metabolized through the pentose catabolic pathway (PCP) in these fungi. In *Aspergillus niger*, the regulation of pentose release from polysaccharides and the PCP involves the transcriptional activators AraR and XlnR, which are also present in other Aspergilli such as *Aspergillus nidulans*. The comparative analysis revealed that the regulation of the PCP by AraR differs in *A. nidulans* and *A. niger*, whereas the regulation of the PCP by XlnR was similar in both species. This was demonstrated by the growth differences on L-arabinose between disruptant strains for *araR* and *xlnR* in *A. nidulans* and *A. niger*. In addition, the expression profiles of genes encoding L-arabinose reductase (*larA*), L-arabitol dehydrogenase (*ladA*) and xylitol dehydrogenase (*xdhA*) differed in these strains. This data suggests evolutionary changes in these two species that affect pentose utilisation. This study also implies that manipulating regulatory systems to improve the production of polysaccharide degrading enzymes, may give different results in different industrial fungi.

Keywords *Aspergillus nidulans* · *Aspergillus niger* · Regulation · Pentose catabolic pathway

Introduction

Plant polysaccharide degradation by fungi has been a topic of study for many decades due to the relevance of these enzymes for various industrial applications (food and feed,

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paper and pulp, detergents, beverages, bio-fuel, etc.). A detailed understanding of the regulatory systems that control the production of these enzymes in relevant fungi is essential to improve their production. For saprobic fungi, these systems are mainly aimed at liberating digestible carbon sources to support growth, and therefore, the hydrolysis of the polysaccharides is often linked to the metabolic conversion of the monomeric component. This is controlled at the transcriptional level by specific transcriptional activators and repressors, where the monomeric components of the polysaccharides are often the inducers of the transcriptional activators (de Vries and Visser 2001). Understanding the utilisation of the monomeric sugars will therefore also provide insight in the mechanism behind polysaccharide hydrolysis.

L-Arabinose and D-xylose are important components of the plant cell wall polysaccharides arabinan, arabinogalactan (substructures of pectin), xyloglucan and xylan. Aspergilli are able to release these pentoses from polysaccharides using a broad enzymatic system (reviewed by de Vries and Visser 2001). In Aspergilli, L-arabinose and D-xylose are catabolised through the pentose catabolic pathway (PCP; Witteveen et al. 1989). All corresponding genes and their regulation have been studied in *Aspergillus niger* (de Groot et al. 2007; Hasper et al. 2000; Mojzita et al. 2010a; Mojzita et al. 2010b; vanKuyk et al. 2001). Two regulatory systems that act in antagonistic fashion are involved in the PCP of *A. niger*, responding to D-xylose (xylanolytic regulator, XlnR; van Peij et al. 1998) and L-arabinose/L-arabitol (arabinolytic regulator, AraR; Battaglia et al. 2011).

XlnR regulates the first step (D-xylose reductase, *xyrA*) of the D-xylose pathway (Hasper et al. 2000). AraR predominantly regulates the L-arabinose pathway specific reactions; the L-arabitol dehydrogenase encoding gene (*ladA*) is under the control of AraR and indications that the L-arabinose reductase and L-xylulose reductase encoding genes (*larA* and *lxrA*) are under regulation of the arabinolytic system have been reported (Battaglia et al. 2011; de Groot 2005). AraR and XlnR both regulate the common steps of L-arabinose and D-xylose catabolism (*xdhA*, *xkiA*; Battaglia et al. 2011; de Groot et al. 2007).

The presence of the genes of the PCP as well as *xlnR* and *araR* is conserved in *Aspergillus* species (Battaglia et al. 2011; Flipphi et al. 2009; de Vries et al. 1994), but it is not clear if their expression and mechanism are identical in these fungi. In this study, we characterized AraR from *A. nidulans* and analysed its regulatory role, together with XlnR, in the PCP. In addition, we compared these results to those previously reported for *A. niger* to demonstrate the differences between these two species.

Materials and methods

Strains, media and growth conditions

The *A. nidulans* and *A. niger* strains used in this study are listed in Table 1. The *A. nidulans* AN031 strain was obtained from Prof. C. Scazzocchio (Institut de Genetique et Microbiologie, Orsay, France). *A. niger* and *A. nidulans* were grown in minimal medium (MM) or complete medium (CM; de Vries et al. 2004). For the growth of auxotrophic strains, the medium was supplemented with 0.2 g/l arginine, 0.2 g/l leucine, 0.2 g/l uridine and/or 1 mg/l nicotinamide. Agar was added at 1.5% (*w*/*v*) for solid medium. Liquid cultures were inoculated with 5×10^6 spores/ml and incubated on a rotary shaker at 220 rpm. Solid medium plates were inoculated with 1×10^3 spores. *A. nidulans* was cultivated at 37 °C and *A. niger*, at 30 °C.

For transfer experiments, *A. nidulans* was grown for 16 h at 37 °C in CM with 1% D-fructose, after which mycelia

Table 1 *Aspergillus* strains used in this study

Strain	CBS number	species	Genotype	Reference
AN031	129193	*A. nidulans*	*pyrG89*, *argB2*	Prof. C. Scazzocchio
Reference	129194	*A. nidulans*	*pyrG89*, *argB2*, *pCDA21*(*pyrG*+)	This study
Δ*araR*	129195	*A. nidulans*	*pyrG89*, *argB2*, Δ*araR::pyrG*	This study
Δ*xlnR*	129196	*A. nidulans*	*pyrG89*, *argB2*, Δ*xlnR::argB*	This study
Δ*araR*/Δ*xlnR*	129197	*A. nidulans*	*pyrG89*, *argB2*, Δ*araR::pyrG*, Δ*xlnR::argB*	This study
Reference UU-A049.1		*A. niger*	*cspA1*, *pyrA6*, *nicA1*, *leuA1*, Δ*argB::pIM2101*(*argB*+)	Battaglia et al. (2011)
Δ*araR* UU-A033.21		*A. niger*	*cspA1*, *pyrA6*, *nicA1*, *leuA1*, Δ*argB:: pIM2101*(*argB*+), Δ*araR*	Battaglia et al. (2011)
Δ*xlnR* UU-A062.10		*A. niger*	*cspA1*, Δ*argB*, *nicA1*, *leuA*, *pyrA6:: A. oryzae pyrA*, Δ*xlnR*	Battaglia et al. (2011)
Δ*araR*/Δ*xlnR* UU-A063.22		*A. niger*	*cspA1*, *nicA1*, *leuA1*, Δ*argB:: pIM2101*(*argB*+), Δ*araR*, *pyrA6::A. oryzae pyrA*, Δ*xlnR*	Battaglia et al. (2011)

aliquots were transferred to MM containing either 25 mM D-fructose, L-arabitol, D-xylose, xylitol or 25 mM L-arabinose. After 2 and/or 4 h, the mycelium was harvested, frozen in liquid nitrogen and stored at −80 °C. For chromosomal DNA isolation, the strains were grown overnight in CM containing 2% D-glucose and the necessary supplements. The mycelium was harvested and frozen in liquid nitrogen.

Molecular biology methods

General methods were performed according to standard procedures (Sambrook and Russell 2001), unless stated otherwise. For chromosomal DNA isolation, a standard phenol–chloroform extraction method was performed. For PCR and Southern blot analysis, 50–100 ng and 5–10 μg of chromosomal DNA were used, respectively. DNA fragments were amplified using an Expand High Fidelity PCR Amplification System (Roche). The 50 μl reaction mixture contained 5 μl of 10× Expand buffer, 3.5 units of the Expand DNA polymerase mixture, 200 μM dNTP mixture, 50 ng of a DNA template and 40 pmol of each primer. The ligations were performed using a Rapid Ligation Kit (Roche). The ligation mixtures were used to transform *Escherichia coli* One Shot® Top 10 competent cells. The transformants were selected on LB plates with 50 μg/ml of kanamycin. The plasmids and the primers used for DNA cloning are listed in Supplemental Table 1.

For the construction of plasmid pSFH1, *araR* was amplified with primers R1.7 T40 and R1.7 B3795. The PCR product was cloned into the pCR®-Blunt II-TOPO® (Invitrogen). To generate the deletion of Δ*araR*, the *araR* 5′ flanking region was amplified with primers R1.7 *Spe*I T603 and R1.7 *Nde*I B4008. The PCR product was digested with *Spe*I/*Nde*I and used to replace the 546 bp *Spe*I/*Nde*I fragment of pSFH1. This construct was subsequently digested with *Nde*I/*Bst*Z17I, and both ends were made blunt by Klenow DNA polymerase I fragment (Roche). The *Aspergillus fumigatus pyrG* gene (1.9 kb), digested by *Eco*RV from the pCDA21 vector (Chaveroche et al. 2000), was ligated in the *Nde*I/*Bst*Z17I blunted vector, generating vector pSFH3. In the deletion construct, the *pyrG* gene replaces 214 bp of the 5′ flanking sequence upstream of the ATG codon and 301 bp of the coding part including the Zn_2Cys_6 binuclear cluster.

The deletion of *xlnR* coding region was performed by insertion of the *argB* gene between the flanking regions of *xlnR*. The 5′ and 3′ flanking regions (1.8 and 2 kb, respectively) were amplified by PCR with the primers *xlnR* T245 *Xho*I, *xlnR* B2067 *Kpn*I, *xlnR* T3501 *Bam*HI and *xlnR* B5510 *Not*I. The PCR product of the 5′ flanking region was digested with *Xho*I/*Kpn*I, and subsequently ligated into pUC21 (Vieira and Messing 1991). The construct was digested with *Bam*HI/*Not*I and used for the insertion of the 3′ *xlnR* PCR fragment. The resulting vector was digested with *Kpn*I/*Bam*HI and used for insertion of the *A. nidulans argB* gene (2.4 kb). This gene was amplified by PCR using primers *argB* T1 *Kpn*I and *argB* B2690 *Bam*HI and plasmid pIC20-*arg* as template DNA. The resulting plasmid pSFH4 was used to transform *A. nidulans*.

The primers used to generate probes for Northern analysis are listed in Supplemental Table 1. The probes were DIG-labelled using the PCR DIG Probe Syntheses Kit (Roche Applied Science) according to the supplier's instructions.

Total RNA was isolated from powdered mycelium using a standard RNA isolation method with the TRIzol Reagent (Invitrogen). For Northern analysis, 3 μg total RNA was transferred to a Hybond-N^+ membrane (Amersham Biosciences). Equal loading was determined by staining the blot for 5 min with 0.04% methylene blue, 0.5 M acetate pH 5.2 solution. The hybridization of the DIG-labelled probes was performed according to the DIG user's manual (www.roche-applied-science.com). All blots were incubated overnight at 50 °C. The blots were exposed for 25 min up to 3 h to a Lumi-Film Chemiluminescent Detection Film (Roche Applied Science).

Transformation procedure and sexual crossing

The *A. nidulans pyrG89 argB2* recipient (AN031) strain was used for deletion of *araR* and *xlnR*. A standard method of sorbitol/PEG transformation was applied (Tilburn et al. 1983). Transformants were screened for impaired growth on L-arabinose or a loss of β-D-xylosidase activity in a plate test with 4-methylumbelliferyl-β-D-xyloside for Δ*araR* and Δ*xlnR* deletions, respectively. The sexual cross of the strains Δ*araR* (*argB2*) and Δ*xlnR* (*pyrG89*) was performed as described previously (Todd et al. 2007). Prototrophs were isolated as Δ*araR*Δ*xlnR* double deletions for further analysis.

Genomic cluster comparison

The conservation of the genomic region flanking *araR* across ten *Aspergillus* genomes was visualized using Sybil, a web-based software for comparative genomics (Crabtree et al. 2007). The genomic sequence data and the Sybil comparative visualization tool were accessed through the *Aspergillus* genome database (AspGD; http://www.aspgd.org). The *Aspergillus* genomes used in the comparison comprised *A. nidulans* FGSC A4, *A. niger* CBS 513.88, *A. niger* ATCC 1015, *Aspergillus terreus* NIH2624, *Aspergillus oryzae* RIB40/ATCC 42149, *Aspergillus flavus* NRRL 3357, *Aspergillus clavatus* NRRL 1, *Neosartorya fischeri* NRRL 181, *A. fumigatus* A1163 and *A. fumigatus* Af293.

Enzymatic assays

The cell-free extract was prepared by adding 1 ml extraction buffer (50 mM K_2HPO_4, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.5 mM EDTA) to the powdered mycelium. The mixtures were centrifuged for 10 min at 12,000 rpm at 4 °C. Intracellular enzyme measurements and activity calculations were performed as described previously (Battaglia et al. 2011). L-Xylulose reductase (Lxr) activity was determined using 50 mM Tris buffer (pH 7.8), 0.2 mM NADPH and 4.5 mM L-xylulose. The absorbance changes were measured at 340 nm in a Spectronic Unicam UV1 spectrophotometer.

Absorbance measurements were performed in triplicate. One unit of activity equals the conversion of 1 mmol of substrate per minute. Total protein concentrations were determined using a BCA protein assay kit (Pierce).

Results

Identification of *A. nidulans* AraR

BlastP analysis of *A. niger* AraR (An04g08600) revealed a close ortholog (AN0388.4) in *A. nidulans*. AN0388.4 is found on contig 7, which is located on chromosome VIII. BLAST alignment of *A. niger* and *A. nidulans* AraR revealed 70% DNA and 77% amino acid identity.

The genomic region surrounding this gene is conserved in the eight Aspergilli that were analysed (Fig. 1). The alignment of AraR proteins from eight Aspergilli showed highly conserved regions, particularly in the DNA-binding domain and C-terminal region (Fig. 2). The region between the second and third cysteine is nearly identical between AraR from *A. niger* ($C_2HARRVRC_3$) and *A. nidulans* ($C_2HSRRVRC_3$; Fig. 2) and differs significantly from this

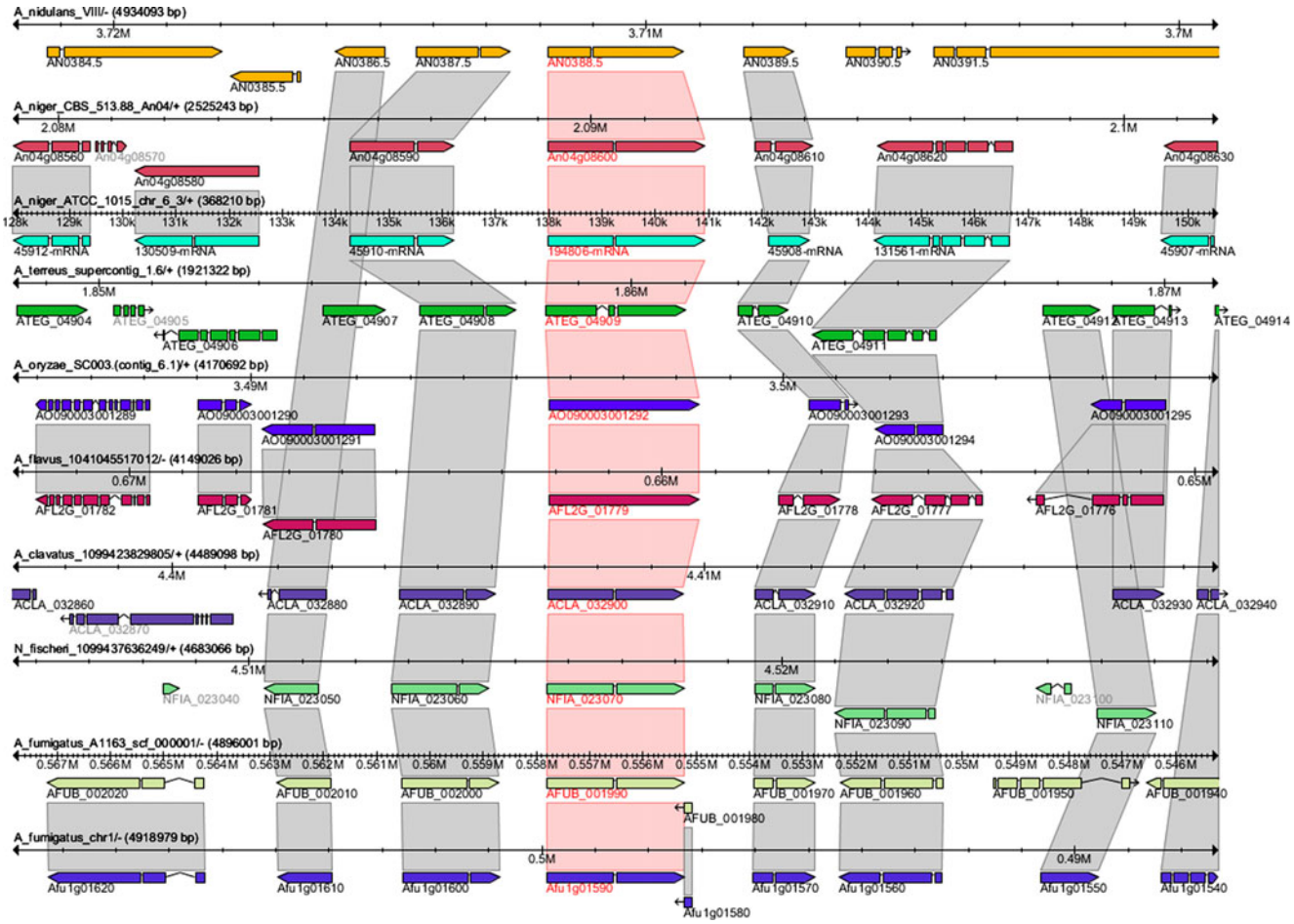


Fig. 1 Orthologous gene cluster of AraR. Graphic presentation of the genomic region of *A. nidulans araR* and comparison to other Aspergilli (Crabtree et al. 2007). *AN0384* conserved serine rich protein, *AN0385* putative branched-chain amino acid transaminase, *AN0386* conserved hypothetical protein, *AN0387* DNA photolyase, *AN0388* AraR, *AN0389* and *AN0390* conserved hypothetical proteins, *AN0391* NACHT domain-containing protein. The *araR* genes are connected by *pink-shaded boxes*. Nearby genes (in the 10-kb region on each side of *araR*) are shown, and those that are conserved are connected by *grey-shaded boxes*

region in XlnR in both species ($C_2NQLRTKC_3$). This region was demonstrated to determine DNA-binding specificity in several zinc cluster proteins (Marmorstein et al. 1992; Marmorstein and Harrison 1994; Swaminathan et al. 1997; Walters et al. 1997).

Phenotypic comparison of *A. niger* and *A. nidulans* Δ*araR* and Δ*araR*/Δ*xlnR*

We constructed *araR*, *xlnR* and double (*araR*/*xlnR*) disruptant strains and verified them by Southern analysis (Suppl. Fig. 1). The growth was analysed in these strains on D-glucose, L-arabinose, L-arabitol, D-xylose and xylitol. The deletion of *xlnR* in both *A. nidulans* and *A. niger* did not affect growth on the tested carbon sources. The growth of the other deletion strains revealed significant differences between *A. niger* and *A. nidulans* (Fig. 3). The growth of the *A. nidulans* Δ*araR* strain was nearly impaired on L-arabinose, but only a small growth reduction was observed on L-arabitol. In contrast, the *A. niger* Δ*araR* strain showed only a small growth decrease on L-arabinose, while the growth on L-arabitol was strongly reduced. Both *A. nidulans* and *A. niger* Δ*araR*/Δ*xlnR* strains showed strongly impaired growth on D-xylose. Surprisingly, the growth of the *A. nidulans* Δ*araR*/Δ*xlnR* strain was only slightly affected on L-arabinose, whereas the growth was absent on this carbon source in the *A. niger* Δ*araR*/Δ*xlnR* strain. Furthermore, the absence of AraR and/or XlnR in *A. nidulans* did not affect the growth on xylitol, whereas the growth was reduced on this carbon source in the *A. niger* Δ*araR*/Δ*xlnR* strain.

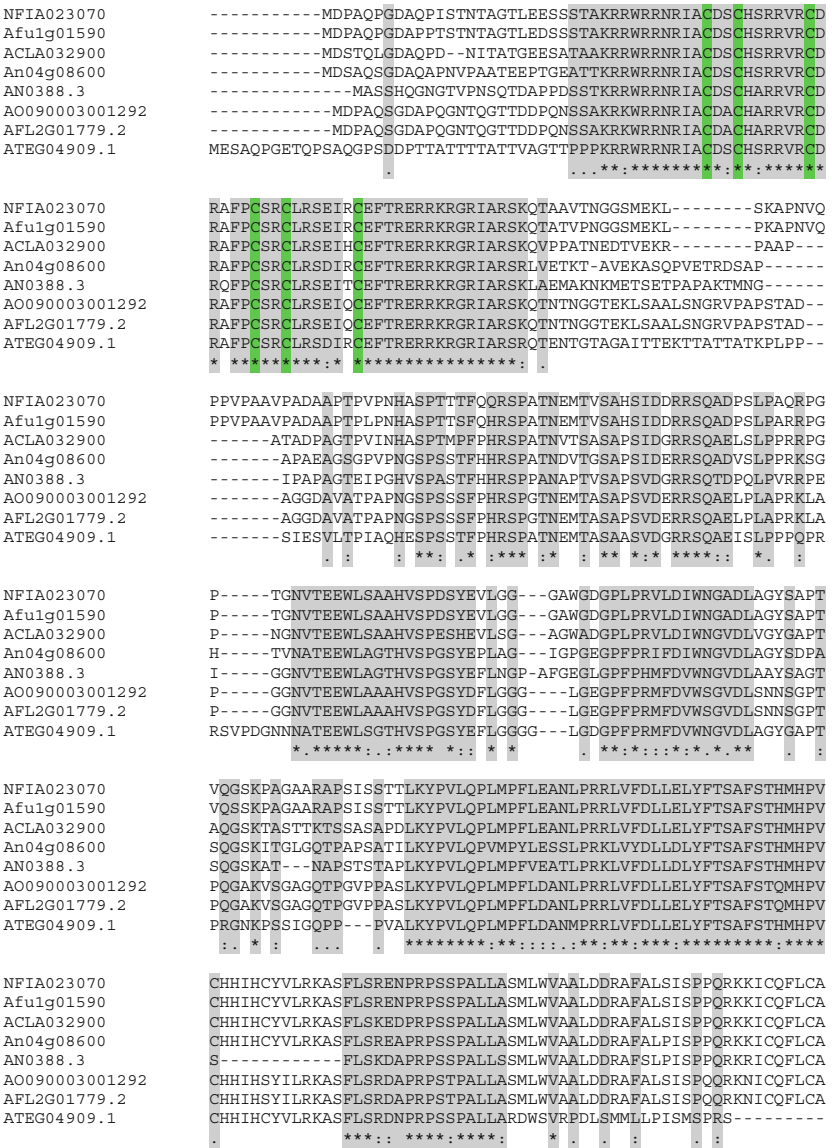
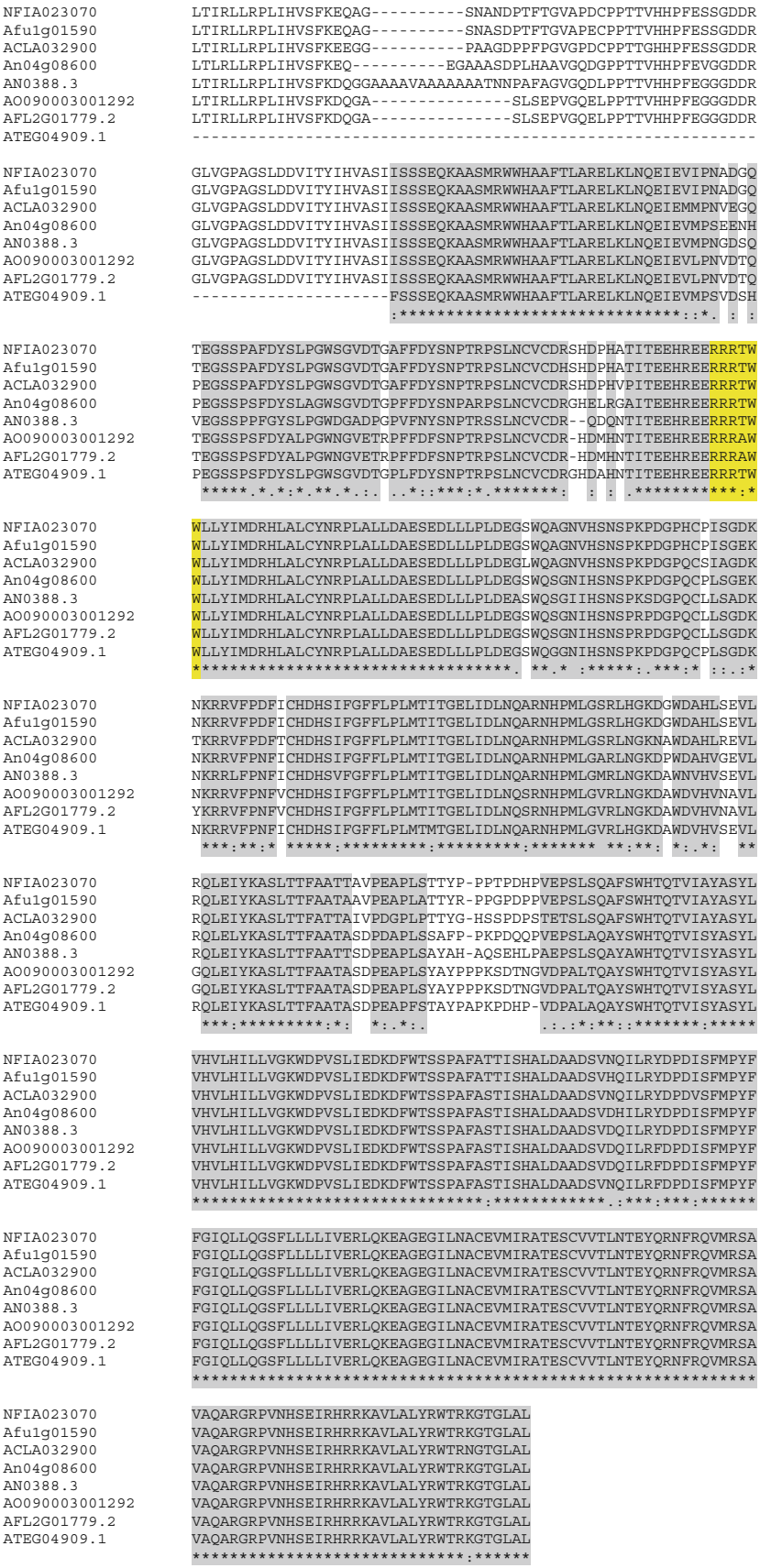


Fig. 2 Multiple sequence alignment of AraR. The following amino acid sequences are aligned by ClustalW (Chenna et al. 2003): *Neosartorya fischeri* (NFIA023070), *Aspergillus fumigatus* (Afu1g01590), *Aspergillus clavatus* (ACLA032900), *Aspergillus niger* (An04g08600), *Aspergillus nidulans* (AN0388.3), *Aspergillus oryzae* (AO090003001292), *Aspergillus flavus* (AFL2G01779.2) and *Aspergillus terreus* (ATEG04909.1). Conserved amino acid residues are shown in *grey*. *Single asterisk* indicates identical residues. *Semi-colon* indicates conservative substitutions. *Period* indicate less conservative substitutions. Cysteine residues are indicated in *green*. Fungal-specific transcription-factor domain of unknown function (Suarez et al. 1995) is shown in *yellow*

Fig. 2 (continued)



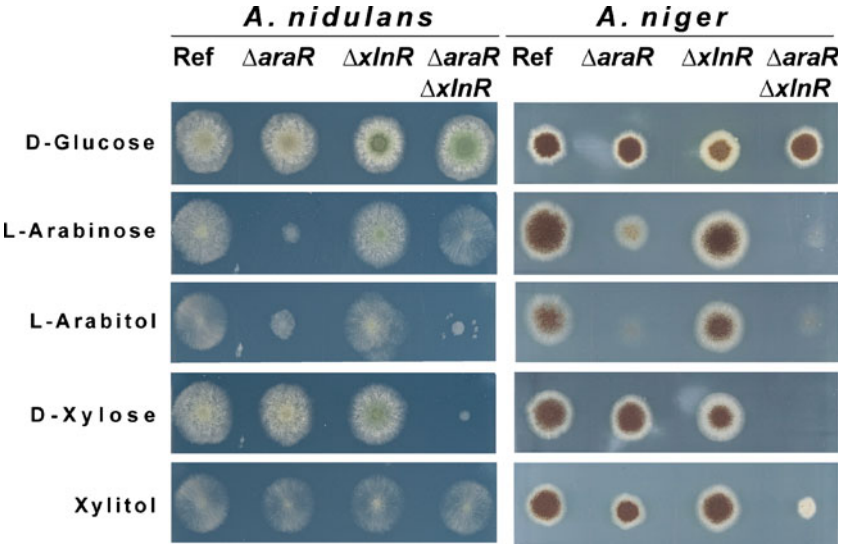


Fig. 3 Growth of the *A. niger* and *A. nidulans* reference, Δ*araR*, Δ*xlnR* and Δ*araR*/Δ*xlnR* strains on 25 mM D-glucose, L-arabinose, L-arabitol, D-xylose and xylitol. The *A. niger* growth data was represented from our previous study (Battaglia et al. 2011) with permission from the publisher

Transcriptional analysis of the pentose catabolic pathway genes

To study the regulation of the pentose catabolic pathway in *A. nidulans*, a transcriptional profile of the PCP genes was compared in the *A. nidulans* reference, Δ*araR*, Δ*xlnR* and Δ*araR*/Δ*xlnR* strains. To this end, a transfer experiment was performed (see the "Materials and methods" section) using the strains and the carbon sources indicated in Fig. 4. In the presence of xylitol, no or low induction was detected for any of the genes in any of the strains tested. In the reference strain, the expression of the L-arabinose specific PCP genes (*ladA*, *larA*, *lxrA*) was induced on L-arabinose and L-arabitol, but not on D-xylose (Fig. 4). The expression of *xyrA* (D-xylose specific) was only observed on D-xylose, whereas the genes encoding the last two steps common in both pathways (*xdhA*, *xkiA*) were induced by both L-arabinose and D-xylose.

No *xyrA* expression was observed on D-xylose in the Δ*xlnR* strain, while genes of the L-arabinose pathway (*ladA*, *larA* and *lxrA*) were induced. A reduced level of *xdhA* was observed on both L-arabinose and D-xylose in the Δ*xlnR* strain, while a higher expression level of both *xdhA* and *xkiA* was detected on L-arabitol.

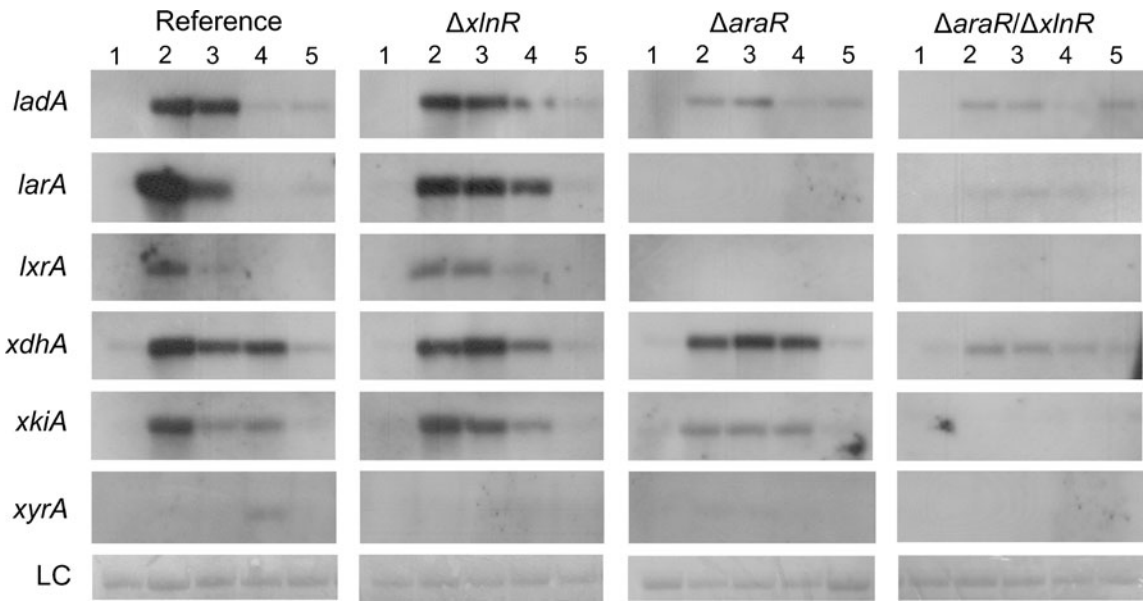


Fig. 4 Expression analysis of the *A. nidulans* pentose catabolic pathway genes. All *A. nidulans* strains were transferred to 25 mM of the indicated carbon source for 2 h of growth. *ladA* L-arabitol dehydrogenase, *larA* L-arabinose reductase, *xdhA* xylitol dehydrogenase, *xkiA* xylulose kinase, *xyrA* D-xylose reductase, *LC* loading control. *1* D-fructose, *2* L-arabinose, *3* L-arabitol, *4* D-xylose, *5* xylitol

A reduced level of *ladA* expression was observed on L-arabinose and L-arabitol in the Δ*araR* strain, and no expression of *larA* and *lxrA* was detected. For *xdhA* and *xkiA*, expression on L-arabinose was decreased in the absence of AraR. An increase of *xdhA* expression was observed on L-arabitol, while both *xdhA* and *xkiA* expression levels remained unaffected on D-xylose.

Reduced, but detectable expression levels of *ladA*, *larA* and *xdhA* were observed in the Δ*araR*Δ*xlnR* strain on L-arabinose and L-arabitol, while *xdhA* was also expressed on D-xylose in the Δ*araR*Δ*xlnR* strain. No *xkiA* specific transcript was detected in a double-knockout strain under all the conditions tested.

Analysis of the activity of pentose catabolic pathway enzymes

The enzymatic activities for L-arabitol dehydrogenase (Lad), xylitol dehydrogenase (Xdh), L-arabinose reductase (Lar), D-xylose reductase (Xyr) and L-xylulose reductase (Lxr) were measured in the mycelium samples of a transfer experiment to L-arabinose and D-fructose (see the "Materials and methods" section) using the strains indicated in Fig. 5.

After 2 h of growth on L-arabinose, the reduced activities of Lad, Xdh, Lar, Xyr and Lxr were detected in the *A. nidulans* Δ*araR* strain. The results showed no Lad and Xdh activity in *A. nidulans* Δ*araR*Δ*xlnR* (Fig. 5). After 4 h of growth on L-arabinose, increased activities of Xdh, Lar and Xyr were detected in the *A. nidulans* Δ*araR* strain, while Lxr activity was still lower than the reference strain. Only Lxr activity was decreased in the *A. nidulans* Δ*araR*Δ*xlnR* strain after 4 h on L-arabinose, while the other activities were similar to the reference strain (Fig. 5). Very low activities of Lad, Xdh, Lar, Xyr and Lxr were observed on D-fructose in any of the strains (data not shown).

Discussion

In this study, we analysed the function of the arabinolytic regulator (AraR) in *A. nidulans* and compared it to the recently reported *A. niger* AraR (Battaglia et al. 2011). Plate growth tests revealed reduced growth of the *A. nidulans* Δ*araR* strain on L-arabinose and L-arabitol, confirming a role for AraR in L-arabinose catabolism in this species. In addition, the expression analysis confirmed that AraR predominantly controls the *A. nidulans* PCP genes, including the recently identified genes encoding L-arabinose reductase (*larA*; Mojzita et al. 2010a) and L-xylulose reductase (*lxrA*; Mojzita et al. 2010b). However, the data presented in this paper reveals that the functional role of AraR in the PCP differs significantly in *A. nidulans* and *A. niger*.

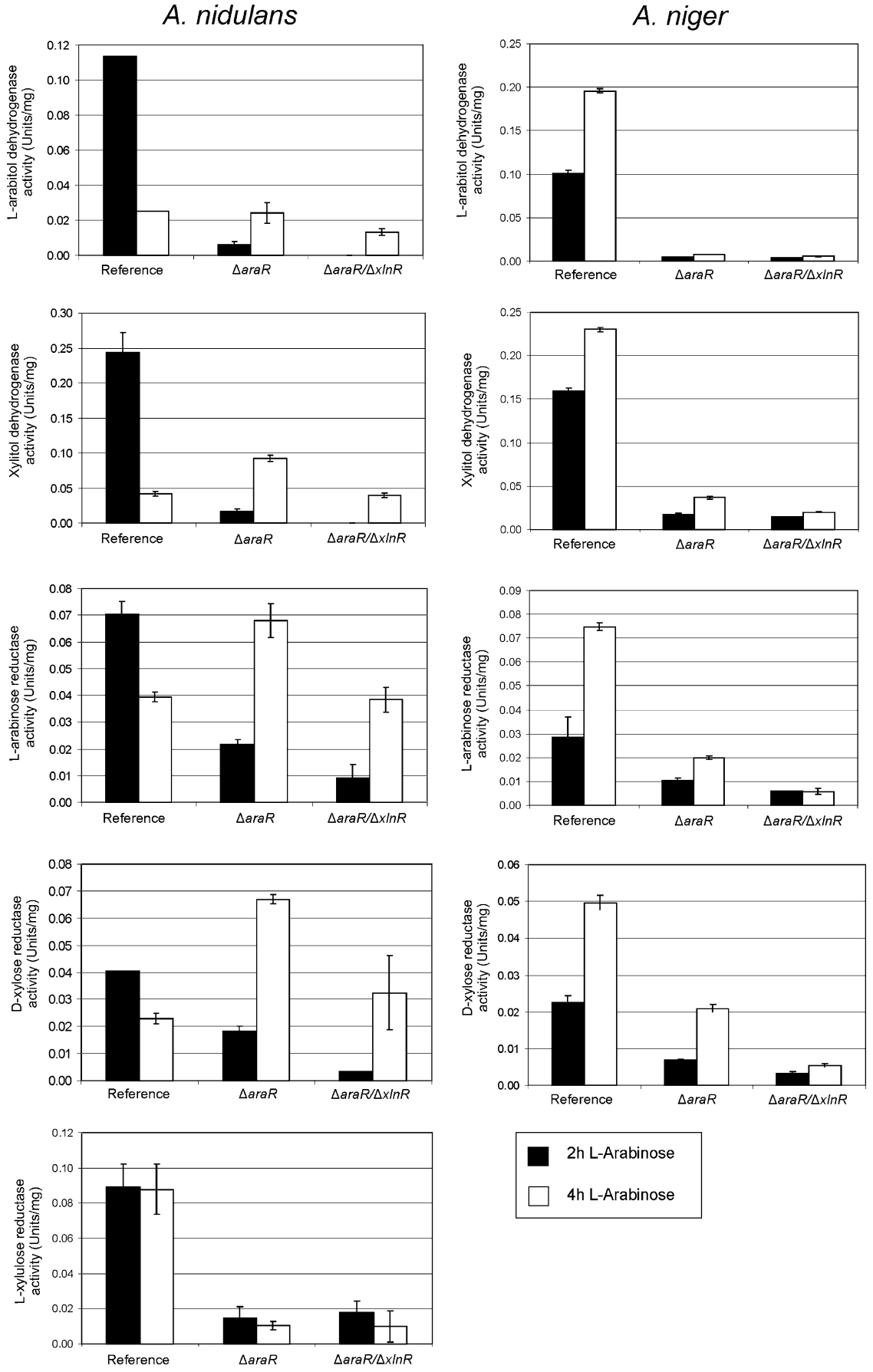
Fig. 5 Comparison of intracellular enzyme activities in *A. nidulans* and *A. niger*. L-arabitol dehydrogenase (Lad), xylitol dehydrogenase (Xdh), L-arabinose reductase (Lar), D-xylose reductase (Xyr) and L-2-keto-3-deoxyarabonatexylulose reductase (Lxr) activity were measured in the *A. nidulans* and *A. niger* reference, Δ*araR* and Δ*araR*/Δ*xlnR* strains. The *A. niger* enzyme activity data was represented from our previous study (Battaglia et al. 2011) with permission of the publisher. The enzyme activities are given in units per milligramme of the total protein

Comparative analysis clearly shows differences between growth on L-arabinose and the PCP in the *A. nidulans* and *A. niger* Δ*araR* and Δ*araR*/Δ*xlnR* strains. The results observed in these *A. nidulans* strains can be explained by either an additional regulatory system or an alternative pathway for L-arabinose catabolism.

As *A. nidulans* appears to have AraR/XlnR independent and L-arabinose-specific induction of several PCP genes (*larA*, *ladA* and *xdhA*), this suggests the involvement of an as-yet-unknown regulator. That this effect is not caused by a low constitutive expression level of these genes is demonstrated by the absence of expression on D-fructose. This unknown regulator may be activated by L-arabinose or its intermediate product, but appears to be dependent on the intracellular accumulation of this inducer. A similar effect was observed for XlnR (Battaglia et al, unpublished results). In the *A. nidulans* Δ*araR* strain on L-arabinose, XlnR activates the expression of xylanase genes, which does not occur in a wild-type strain. This suggests that XlnR responds to the presence of accumulated concentrations of L-arabinose or a conversion product. Such a sophisticated mode of regulation, where a pathway-specific regulator can trigger the transcription of target genes in the presence of a threshold level of an inducing molecule not directly linked to this metabolic pathway may be used by *A. nidulans* as a rescue strategy and give an additional advantage to the fungus.

BlastP analysis of AraR and XlnR to the *A. nidulans* genome gave three significant hits, AraR and XlnR itself and a third unknown regulator (data not shown). The function of this regulator is currently under investigation.

No expression was detected of the L-xylulose reductase encoding gene (*lxrA*) and xylulose kinase encoding gene (*xkiA*), suggesting a partially active PCP in Δ*araR*/Δ*xlnR* strain on L-arabinose. For the utilisation of this substrate, other enzymes that can compensate for the loss of LxrA and XkiA activity must be involved. We propose that these unknown genes are not induced in the presence of XlnR because of the impaired growth of the Δ*araR* strain on L-arabinose. It is currently unknown if isoenzymes exist that compensate for the reactions of the PCP in the *A. nidulans*. Putative paralogs of several PCP genes are present in the *A. nidulans* genome (Flipphi et al. 2009), but their biochemical function is not determined. We observed similar activities for 4 of the tested enzymes (Lar, Xyr, Lad, Xdh) in the *A. nidulans* Δ*araR*/Δ*xlnR* and the reference strain after 4 h on


A. nidulans
A. niger
L-arabitol dehydrogenase activity (Units/mg)
Xylitol dehydrogenase activity (Units/mg)
L-arabinose reductase activity (Units/mg)
D-xylose reductase activity (Units/mg)
L-xylulose reductase activity (Units/mg)
Reference
ΔaraR
ΔaraR/ΔxlnR
2h L-Arabinose
4h L-Arabinose

L-arabinose, while expression of these genes was significantly reduced in the Δ*araR*/Δ*xlnR* strain. This suggests that other enzymes can in part compensate for the absence of the PCP-specific enzymes. However, this was not the case for Lxr, suggesting that growth on L-arabinose in the *A. nidulans* Δ*araR*/Δ*xlnR* strain is not likely caused by isoenzymes generating a similar pathway as the normal PCP. It is therefore more likely that an alternative pathway can convert L-arabinose that may in part use similar activities as the PCP.

There is currently no evidence for the existence of an alternative fungal L-arabinose pathway and the data of our study suggests that should such a pathway exists, it is not dependent on AraR and is only activated in the absence of XlnR. Alternative pathways for carbon utilisation have been shown to exist in fungi and bacteria. A second pathway for D-galactose catabolism (the oxido-reductive pathway) was previously found in *A. nidulans* (Fekete et al. 2004). In bacteria, two L-arabinose pathways are known: the arabinose (*araBAD*) operon (Lee et al. 1986) and an alternative pathway through L-2-keto-3-deoxyarabonate (Watanabe et al. 2006a; Watanabe et al. 2006b).

The comparison of growth, expression and activity profiles demonstrates that the role of XlnR in the regulation of the PCP is similar in *A. nidulans* and *A. niger*. In both *Aspergillus* species, it has been demonstrated that XlnR also share the same functional regulatory role with respect to extracellular D-xylose releasing enzymes (Tamayo et al. 2008; van Peij et al. 1998).

In summary, this study demonstrates that the regulatory role of AraR in the PCP differs between two Aspergilli, while this is not the case for XlnR. This may indicate that the two species optimized their carbon source utilisation by subtle, but significant variations in the catabolic pathway itself and its regulatory mechanism. It demonstrates that the influence of homologous regulatory systems in different (even related) fungi can differ significantly. This also implies that manipulating regulatory systems to improve the production of polysaccharide degrading enzymes, may give different results in different industrial fungi.

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