Molecular Regulation of Arabinan and L-Arabinose Metabolism in 
*Hypocrea jecorina* (Trichoderma reesei)*\(^{\text{\dag}}\)

Eda Akel, Benjamin Metz, Bernhard Seiboth,\(^{*}\) and Christian P. Kubicek

*Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria*

Received 6 June 2009/Accepted 25 September 2009

*Hypocrea jecorina* (anamorph: *Trichoderma reesei*) can grow on plant arabinans by the aid of secreted arabinan-degrading enzymes. This growth on arabinan and its degradation product L-arabinose requires the operation of the aldose reductase XYL1 and the L-arabinitol dehydrogenase LAD1. Growth on arabinan and L-arabinose is also severely affected in a strain deficient in the general cellulase and hemicellulase regulator XYR1, but this impairment can be overcome by constitutive expression of *xyr1* encoding the aldose reductase. An inspection of the genome of *H. jecorina* reveals four genes capable of degrading arabinan, i.e., the \(\alpha\)-L-arabinofuranosidase encoding genes *abf1*, *abf2*, and *abf3* and also *bxl1*, which encodes a \(\beta\)-xyllosidase with a separate \(\alpha\)-L-arabinofuranosidase domain and activity but no endo-arabinanase. Transcriptional analysis reveals that in the parent strain QM9414 the expression of all of these genes is induced by L-arabinose and to a lesser extent by L-arabinitol and absent on D-glucose. Induction by L-arabinitol, however, is strongly enhanced in a \(\Delta lad1\) strain lacking L-arabinitol dehydrogenase activity and severely impaired in an aldose reductase (\(\Delta xyrl\)) strain, suggesting a cross talk between L-arabinitol and the aldose reductase XYL1 in an \(\alpha\)-L-arabinofuranosidase gene expression. Strains bearing a knockout in the cellulase regulator *xyr1* do not show any induction of *abf2* and *bxl1*, and this phenotype cannot be reverted by constitutive expression of *xyr1*. The loss of function of *xyr1* has also a slight effect on the expression of *abf1* and *abf3*. We conclude that the expression of the four \(\alpha\)-L-arabinofuranosidases of *H. jecorina* for growth on arabinan requires an early pathway intermediate (L-arabinitol or L-arabinose), the first enzyme of the pathway XYL1, and in the case of *abf2* and *bxl1* also the function of the cellulase regulator XYR1.

The recently reinitiated interest in second-generation biofuel production (i.e., from renewable plant material whose use does not compete with use for food and feed production) also led to a renaissance in cellulase and hemicellulase production by the ascomycete *Hypocrea jecorina* (anamorph *Trichoderma reesei*), the current best producer of these enzymes (27). The efficiency of use of these enzymes for plant biomass hydrolysis, however, is still limited by several factors, including incomplete knowledge of regulation of production of these enzymes.

The L-arabinose polymer arabinan is another polysaccharide found in plant cell wall heteropolysaccharides as a side chain of pectin (9), and L-arabinose is particularly abundant in the form of glucuronarabinoxylans in monocotyledon primary cell walls of *Commmelinoid* flowery plants and as arabinoxylans in cereal grains, where it can mount up to 25 to 30% (4, 11). *H. jecorina* is also able to degrade arabinan to L-arabinose by an arabinanolytic system. To date, two arabinofuranosidases have been characterized: an \(\alpha\)-L-arabinofuranosidase (ABF1) (26) and a \(\beta\)-xyllosidase which has a separate \(\alpha\)-L-arabinofuranosidase domain and activity (23). Two further \(\alpha\)-L-arabinofuranosidases (ABF2 and ABF3) have been found during genome sequence analysis (14, 27), and a recent analysis of the secretome of two high-producer strains (22).

The L-arabinose formed as a consequence of the attack by \(\alpha\)-L-arabinofuranosidases is metabolized via the fungal specific L-arabinose catabolic pathway (Fig. 1), which converts L-arabinose to D-xylulose 5-phosphate. The latter is then metabolized via the nonoxidative pentose phosphate pathway. Three genes involved in this pathway have been cloned from *H. jecorina* for D-xylulose aldolase reductase XYL1, which is not only responsible for D-xylulose reduction but also responsible for the reduction of L-arabinose to L-arabinitol (37); an L-arabinitol dehydrogenase LAD1 (30, 33), which converts L-arabinitol to L-xylulose; and a xylitol dehydrogenase XDH1, which oxidizes xylitol to D-xylulose (38). LAD1 can partly replace the function of XDH1 in a \(\Delta xdh1\) strain (38). A putative L-xylulose reductase has also been cloned (34), but recent investigations showed that its knockout does not affect L-arabinose utilization and that the enzyme is in fact a D-mannitol dehydrogenase (28). The L-xylulose reductase, which is functional in vivo, has not been cloned yet.

Little is thus far known about the regulation of the arabinanase system in *H. jecorina*. In *Aspergillus niger*, arabinanolytic genes are specifically and coordinately induced when *A. niger* is grown on arabinan-containing substrates or the monomeric compounds L-arabinose and L-arabinitol (13, 16, 17, 32, 44, 45). Since the accumulation of intracellular L-arabinitol correlates with higher production of the enzymes involved in arabinan breakdown in *A. nidulans*, de Vries and Visser (9) hypothesized that L-arabinitol could be the true inducer of this system.

\(^{*}\) Corresponding author. Mailing address: Molecular Biotechnology Group, Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9, 166-5, A-1060 Vienna, Austria. Phone: 43-1-58801-17210. Fax: 43-1-58801-17299. E-mail: bseiboth@mail.tuwien.ac.at.

\(^{\text{\dag}}\) Published ahead of print on 2 October 2009.
de Groot et al. (8) identified two loci (araA and araB) in A. niger, which seem to contain positive-acting genes involved in the expression of arabinanase-encoding genes and the genes encoding the enzymes of the L-arabinose catabolic pathway. However, the genes corresponding to these two loci have not been cloned yet.

The Zn(II)2-Cys6 binuclear cluster protein XYR1/XlnR has been shown to be a transcriptional activator of cellulase and xylanase gene transcription in H. jecorina and different Aspergillus spp. (for a review, see reference 43). However, XlnR has been reported not to regulate arabain and L-arabinose metabolism in the Aspergilli (8). In analogy, Stricker et al. (42) reported that a xylI knockout in H. jecorina did not affect growth of the fungus on L-arabinose and also L-arabinose reductase activities. However, expression of the H. jecorina D-xylolose/aldox reductase XYL1 is controlled by XYR1 during growth on D-xylolose (41) and a xylI knockout results in strongly impaired growth on L-arabinose due to the major involvement of XYL1 in the total L-arabinose reductase activity in this strain (37).

These potentially conflicting data prompted us to perform a more detailed investigation on the possibility of regulation of arabain and L-arabinose metabolism by XYR1 and the involvement of XYL1 in arabain and L-arabinose catabolism. We present here clear evidence that XYR1 is essential for the catabolism of arabain and L-arabinose by directly regulating XYL1 and two α-L-arabinofuranosidases (ABF2 and BXL1), and by indirectly regulating also ABF1 and ABF3 via XYL1. In addition, we show that L-arabinose/L-arabinose and XYL1 are also essential for induction to occur.

**MATERIALS AND METHODS**

Strains and culture conditions. The fungal strains used in the present study were all derived from H. jecorina (T. reesei) QM9414, a moderate cellulase producing mutant strains, and are listed in Table 1. They were maintained on malt extract agar and supplemented with uridine (10 mM) when necessary. Strains were grown in 1-liter flasks on a rotary shaker (250 rpm) at 28°C in 250 ml of the medium described by Mandels and Andreozzi (25) with the respective carbon source at a final concentration of 1% (wt/vol). Sugar beet arabain (AraGalRha:GalUA [88:3:2:7]) was purchased from Megazyme (Wicklow, Ireland).

Escherichia coli strain JM109 (Promega, Madison, WI) was used for plasmid propagation.

Strain constructions. Construction of strains constitutively expressing the D-xylol reductase gene xylI in a xylI-negative background was performed as follows: 740 bp of the promoter region the H. jecorina tef1 (encoding translation elongation factor 1) were amplified by PCR using the oligonucleotides tef1Xhofw and tef1ClaSalrv. The XhoI/ClaI-restricted tef1 fragment was then cloned in the corresponding sites of pLH1hph (20), which contains the E.coli hygromycin B phosphotransferase expression cassette as fungal selection marker. A 1,590-bp xylI PCR fragment including the coding and terminator region was amplified with the oligonucleotides xylIClafw and xylIHindIII and inserted downstream of the tef1 promoter region by ClaI/HindIII, resulting in Ptef1-xylI. Transformation of the Δxyr1 strain with Ptef1-xylI was done as described previously (18) by using hygromycin B (50 μg/ml) as a selection agent. The strains were purified twice for mitotic stability, and integration of the Ptef1-xylI expression cassette was verified by PCR analysis.

Nucleic acid isolation and hybridization. Fungal mycelia were harvested by filtration, washed with sterile tap water, shock frozen in liquid nitrogen, and ground to a fine powder. DNA and RNA extraction were done as described previously (39). Standard methods (36) were used for DNA electrophoresis, blotting, and hybridization of RNA. Probes were amplified by PCR (Table 2) and labeled with [α-32P]dCTP by random priming.

Determination of fungal growth. To determine hyphal growth on agar plates, plates were inoculated with a small piece of agar in the center of an 11-cm plate, and the increase in colony diameter was measured daily twice. To measure growth in submerged cultures, the increase in dry biomass was recorded. To this end, mycelia were harvested after appropriate times, washed extensively with distilled water, and dried to constant weight in an oven at 80°C. The data shown are the average of the three separate biological experiments, which deviated by not more than ±15%.

High-pressure liquid chromatography analysis of intracellular L-arabinose. The high-pressure liquid chromatography analysis was performed essentially as described previously (28), but with 70% 5 mM sulfuric acid and 30% acetonitrile at 65°C in the eluent to improve the separation of the L-arabinose and L-arabinitol. For estimation of the intracellular concentration, an intracellular high-pressure liquid chromatography analysis was performed essentially as described previously (28), but with 70% 5 mM sulfuric acid and 30% acetonitrile at 65°C in the eluent to improve the separation of the L-arabinose and L-arabinitol. For estimation of the intracellular concentration, an intracellular volume of 2.4 ml/g of dry biomass was used (41).

Phylogenetic analysis. DNA and protein sequences were visually aligned by using Genedoc 2.6 (29). Phylogenetic trees were constructed by the neighbor-joining method (35), using the computer program MEGA, version 4.0 (24). Unalignable N- and C-terminal regions in the amino acid sequences were omitted from the analyses, and gaps and missing data were pairwise deleted.

| Strain     | Description          | Source or reference |
|------------|----------------------|---------------------|
| QM9414     | Parent strain        | ATCC 26921          |
| TU-6       | pyr4 auxotroph       | 18                  |
| ΔxylI      | xylI deleted         | 41                  |
| Δxyr1      | xyrl deleted         | 37                  |
| Ptef1xylIΔxyr1 | xylI under tef1 promoter in a Δtef1 background | This study |
| Δladl     | ladl deleted         | 38                  |
| Δxdhl     | xdhl deleted         | 38                  |
RESULTS

Growth of *H. jecorina* on arabinan and L-arabinose is dependent on the function of the D-xylose reductase XYL1 and the L-arabinitol dehydrogenase LAD1. *H. jecorina* is able to grow on sugar beet arabinan or its major constituent L-arabinose, both on plates and in submerged cultures (Fig. 2). In order to assess that this growth is in fact due to catabolism of the L-arabinose content of arabinan and to assess the involvement of the thus-far-characterized enzymes of the L-arabinose catabolic pathway (see Fig. 1), we compared growth on arabinan with that on L-arabinose in strains deleted in the first two steps of the metabolic pathway: an XYL1-deficient strain (*H9004 xyl1*), which is strongly impaired in its ability to convert L-arabinose to L-arabinitol (37), and a strain deficient in the L-arabinitol dehydrogenase LAD1 (*H9004 lad1*), which is impaired in its ability to convert L-arabinitol to L-xylulose (30). Growth in submerged cultures of these strains on D-glucose was indistinguishable from that of the parent strain (Fig. 3a). Growth on L-arabinose, however, was different: growth of the *H9004 xyl1* and the *H9004 lad1* strains was strongly reduced (Fig. 3b). Biomass formation in submerged cultures on L-arabinitol was only affected in the *Δlad1* strain and occurred normally in the *Δxyl1* strain (Fig. 3c). The *Δlad1* and *Δxyl1* strains were almost unable to grow on arabinan at all (Fig. 3d). These findings are consistent with the assumption that growth on sugar beet arabinan is mainly due to the catabolism of L-arabinose via L-arabinitol and L-xylulose and involves XYL1 and LAD1.

Growth of *H. jecorina* on arabinan and L-arabinose but not L-arabinitol is dependent on the function of the cellulase regulator XYR1. Deletion of *xyr1* encoding the Zn(II)2Cys6 reg-

---

TABLE 2. Oligonucleotides used in this study

| Gene    | Primer | Sequence (5’–3’)* | Size (bp) |
|---------|--------|-------------------|-----------|
| *bxd1* | bXYLfw  | GACACTTGCCACGCTCACAC | 1,922     |
|         | bXYLrv  | CGAAGGTAAGACGGGAATC |           |
| *abf1* | aBF1fw  | AGCCTCCTGGA CGTAAATG | 1,310     |
|         | ABF1fw  | AGGCTCTCTGTGTCGTCG |           |
| *abf2* | aBF2fw  | TGCCGTTGCTGCTAGCTTG | 925       |
|         | aBF2rv  | TGGTCGATAGGGCAAGAGGC |          |
| *abf3* | aBF3fw  | GTGACATCTACGCATCTGG | 1,346     |
|         | aBF3rv  | GTGGAATGACGCAGCACTAG |          |
| *18S rRNA* | 18sRF | GGTGGAGTATTTGGCTTG | 300       |
|         | 18sRF   | CTTACTAGGGATTCCTCG |           |
| *tef1* | Tet1Xhofw | GCCCTGAGGGACAGAATGAC | 740       |
|         | Tet1ClaSalrv | TGTGCAATGATGACGGGTTGATGATGATGCGTG |          |
| *xyl1* | xyl1Clafw | TAATCGATGGCGCTCTCCACGCTCAAG | 1,540     |
|         | xyl1HindIII | TGAAGCTGCAGACAGATGACGCCACAAC | |  

a Restriction sites are underlined.

---

FIG. 2. Radial growth of *H. jecorina* on various carbon sources on plates (a) and biomass production in submerged cultures (b). Carbon sources are indicated as follows: D-glucose (○), L-arabinose (▲), L-arabinitol (◇), and arabinan (●), all at 1% (wt/vol).

FIG. 3. Growth of *H. jecorina* QM9414 and strains deleted in different steps of the L-arabinose catabolic pathway on D-glucose (a), L-arabinose (b), L-arabinitol (c), and arabinan (d). Strains are indicated as follows: *H. jecorina* QM9414 (●), *Δxyl1* (■), and *Δlad1* (▲).
enzymes with L-arabinose and L-arabinobiose as the sole carbon source.

In order to test which of these effects is direct (i.e., not caused by the regulation of XYR1), we compared their formation in the L-arabinose and L-arabinitol strains (to rule out indirect effects via XYL1). As shown in Fig. 6c, induction of abf2 and bxl1 by L-arabinose was completely impaired, whereas a very weak expression of abf1 and abf3 still occurred. Transcript formation in the presence of L-arabinose displayed essentially the same picture, with the exception that the transcripts were still present at an earlier time point (20 h) (Fig. 6b).

In the Δxyl1 strain, in contrast, all four transcripts were significantly more abundant than in the parent strain QM9414 and were highest when induced by L-arabinose (Fig. 6b).

**Regulation of α-l-arabinofuranosidase gene expression by XYR1.** To test whether the xylanase and cellulase regulator XYR1 would be involved in the regulation of α-l-arabinofuranosidase gene expression in *H. jecorina*, we compared their transcript levels during growth on L-arabinose and L-arabinitol. In contrast, during growth on arabinoxylan, the Δxyl1 strain with the constitutively expressed *xyl1* gene was not able to reach the growth of the parent strain, which points to a regulation of the arabinoxylanolytic system by XYR1.

α-l-Arabinofuranosidase gene expression in *H. jecorina.*

The genome of *H. jecorina* contains four genes that encode enzymes with α-l-arabinofuranosidase activity, i.e., *abf1, abf2,* and *abf3* and the β-xilosidase-encoding gene *bxl1*, which has a separate α-l-arabinofuranosidase domain (23, 27). Interestingly, we were not able to detect any orthologue of an endo-arabinoxylanase gene as described for *Aspergillus niger* (12) in the *H. jecorina* genome (http://genome.jgi-psf.org/Trire2/Trire2.home.html). ABF1 and ABF3 are members of glycosyl hydrolase family 54 and contain a carbohydrate-binding module of family 42. They seem to be the result of a gene duplication event which took place early in the evolution of the *Hypocreaceae*, but the ABF3 orthologues have subsequently been lost from other genera, such as *Gibberella/Parasitella* (Fig. 5).

Transcription of all four genes occurred during growth on L-arabinose and L-arabinitol and was absent during growth on glucose (Fig. 6a). This is consistent with the findings that we could not detect any α-l-arabinofuranosidase activity in the culture filtrate or associated with cell walls during growth on glucose (unpublished data). Maximal abundance of transcripts was similar on L-arabinose and L-arabinitol, although initial transcript levels were higher on L-arabinose.

Metabolic modulation of α-l-arabinofuranosidase gene expression in *H. jecorina.* In order to learn whether changes in the metabolism of L-arabinose would influence the transcript levels of *abf1, abf2, abf3,* and *bxl1*, we compared their formation in the Δxyl1 and the Δlad1 strains. In the Δxyl1 strain, the expression of *abf2* and *bxl1* in the presence of L-arabinose was completely abolished, whereas a very weak expression of *abf1* and *abf3* still occurred. Transcript formation in the presence of L-arabinose displayed essentially the same picture, with the exception that the transcripts were still present at an earlier time point (20 h) (Fig. 6b).

In the Δlad1 strain, in contrast, all four transcripts were significantly more abundant than in the parent strain QM9414 and were highest when induced by L-arabinose (Fig. 6b).

**FIG. 4.** Effect of the loss of XYR1 on growth of *H. jecorina* on L-arabinose and L-arabinobiose. (a) Growth of *H. jecorina* QM9414 (solid symbols) and Δxyl1 (open symbols) on L-arabinose (triangles), L-arabinitol (squares), and arabinan (diamonds) as the carbon source. (b) Growth of *H. jecorina* QM9414 (Δ, ·, ○) and *P. tef1::xyl1/DΔxyl1 (C, ×) strains on L-arabinose and arabinan, respectively. (c) Growth of *H. jecorina* QM9414, Δxyr1, and *P. tef1::xyl1/DΔxyl1* strains on plates containing L-arabinose as the sole carbon source.
ently directly regulated by XYR1 prompted us to examine whether we could also detect the respective XYR1 binding sites \([5'-\text{GGC(A/T)(3-4)}-3']\) (15, 40) in the 5' upstream regions of these two, but not the other two (\(\text{abf1}\) and \(\text{abf3}\)) genes (Fig. 7). \(\text{abf2}\) and \(\text{bxl1}\) differed in two respects. First, the two exhibited a higher number of consensus binding motifs in the first 1,000 bp upstream of the start ATG (9 and 7 for \(\text{abf2}\) and \(\text{bxl1}\) versus 5 for \(\text{abf1}\) and 2 for \(\text{abf3}\), respectively). Second, however, only \(\text{abf2}\) and \(\text{bxl1}\) contained the consensus binding sites as a divergent (\(\text{abf2}\)) and tandem (\(\text{bxl1}\)) repeat, separated by 3 and 4 nucleotides.

**DISCUSSION**

As a saprobe specialized to feed on decaying plant material, \(H.\ jecorina\) must contain genes whose products enable a syn-

---

**FIG. 5.** Phylogenetic analysis of \(H.\ jecorina\) ABF1 and ABF3. The other sequences used were identified as those showing highest similarity in BLAST. Neighbor joining was used, and gaps were not included. Numbers over the branches represent bootstrap coefficients from 1,000 replicas.
ergistic and efficient usage of the available polymers, i.e., cellulose and hemicelluloses. To degrade arabian, a component belonging to the latter, *H. jecorina* has three α-L-arabinofuranosidases (ABF1, ABF2, and ABF3) and a single β-xylanase (BXL1) with a separate α-L-arabinofuranosidase domain. The lack of endo-arabinanase-encoding genes indicates that, in contrast to other fungi such as *Aspergillus* spp. (9), *H. jecorina* is likely specialized on the degradation of the single and short l-arabinose side chains present in different hemicelluloses but seems to have problems with longer side chains as present in pectins. The presence of cellulose-binding domains in ABF1 and ABF3 suggests a probable link between cellulose and arabino-oligosaccharide degradation: cellulose microfibrils are known to be tethered through noncovalent interactions with matrix polysaccharides that are involved in the primary cell wall assembly (21). This binding is brought about by the arabinan, xylan, and galactan oligosaccharide side chains of the pectins and, particularly, the xyloglucan contains terminal L-arabinose residues (47). The fact that *H. jecorina* can only slowly grow on arabian polymers (e.g., slower than on cellulose or xylan), as shown here, is consistent with this view and illustrates that an exclusive exo-attack is poorly efficient in the degradation of a polymer. It is possible that hemicelluloses with terminal l-arabinose residues are the preferred carbon sources for *H. jecorina* in its natural habitat.

Although the inducible formation of ABF1, ABF2, and BXL1 by sophorose and lactose has already been reported earlier (14), ABF3 has only recently received attention (22). Interestingly, ABF3 appears to be a relict of an early clade in the evolution of the GH54 α-L-arabinofuranosidases, only present also in *Penicillium marneffei* and *Talaromyces stipitatus*. However, the phylogenetic origin of ABF3 is not in accordance with the species phylogeny. This may be due to either a history of horizontal gene transfer or operation of a birth and death mechanism. At this stage, no clear answer can be offered. We note, however, that among the sordariomycetes *H. jecorina* is the only fungus that contains two genes of the GH54 family.

The l-arabinose, resulting from the operation of ABF1-3 and BXL1, is taken up into the hyphae and metabolized via the reductive pentose catabolic pathway (Fig. 1). In *A. niger*, de Groot et al. (7) reported on a separate l-arabinose reductase with little activity on d-xylose. Similarly, Stricker et al. (42) provided evidence for the presence of a specific l-arabinose reductase in *H. jecorina*, albeit using activity assays in cell extracts only. We have here provided clear evidence that the XYL1 aldose reductase, which is primarily responsible for catalyzing the first reductive reaction on D-xylose, L-arabinose, and D-galactose catabolism (37), is also responsible for arabinan degradation. Thus, in contrast to the aspergilii, *H. jecorina* contains a single enzyme to catalyze the first step in the degradation of different hemicellulose sugars. This appears to be beneficial for the performance of *H. jecorina* in its habitat, where l-arabinose is not available in isolation but always accompanied by d-xylose.

No α-L-arabinofuranosidase activity was detected during growth on d-glucose (unpublished data), and the respective transcripts of *abf1, abf2, abf3,* and *ball* were also absent. We have not investigated whether this is a consequence of carbon catabolite repression or lack of the inducer (or both), but we note that all genes were transcribed during growth on l-arabinose and (less) on L-arabinitol. However, in a Δlad1 strain, which lacks the l-arabinitol dehydrogenase needed for the conversion of l-arabinitol to l-xylulose, the formation by l-
arabinose and L-arabinose was greatly increased. This is consistent with the hypothesis proposed for A. niger (9, 44) that L-arabinositol may be the true intracellular inducer. We note, however, that a possible action of L-arabinose cannot be rejected from our data, which is supported by our findings that L-arabinose can be formed by the reverse reaction of XYL1 during growth on L-arabinositol. Under these conditions, the parent strain QM9414 accumulated an intracellular concentration of 0.18 to 0.4 mM L-arabinose, and this value was decreased to 0.19 to 0.20 mM in the Δxyr1 strain and elevated to 0.8 to 0.95 mM in the Δabf1Δbxl1 strain (data not shown). Interestingly, the transcription during growth on L-arabinose (or L-arabinositol) was almost completely abolished in the absence of the aldose reductase XYL1 after the initial phase of growth. Since xyl1 is dispensable for growth on L-arabinositol, this effect must therefore be a regulatory one. De Groot et al. (8) reported that the induction of arabinoan-degrading enzymes by L-arabinositol in A. niger depends on the function of the products of the araA and araB genes, which positively regulate their expression and also that of the genes encoding the L-arabinose catabolic pathway. Unfortunately, the respective genes have not yet been identified. Compared to the findings of the present study, we cannot rule out that one of them (or both) would indeed encode an L-arabinose reductase, since strongly decreased L-arabinose reductase activities were found in the araA and araB mutants (8). Alternatively, if these genes indeed encode a positive regulator of the expression of an L-arabinose reductase gene, this would result in a deficiency of L-arabinose reductase activity and, when applying our findings of a cross talk by L-arabinositol/L-arabinose and XYL1 to A. niger, this would also, albeit indirectly, block α-L-arabinofuranosidase formation. Although the mechanism of the cross talk of XYL1 and the inducer in H. jecorina still needs to be investigated, the function of metabolic enzymes as transcriptional (co)regulators would not be without precedent (3, 5, 19), and regulatory roles of aldose reductases have been described in mammalian cells (1, 31), albeit without explaining the underlying mechanism. To the best of our knowledge, no similar finding has been made in a filamentous fungus, thus rendering H. jecorina and L-arabinose reductase an attractive subject for further research in this field.

The Zn(II)2Cys6-type transcription factor XYR1 has previously been shown to be centrally involved in the expression of the cellulolytic and hemicellulolytic enzymes of H. jecorina (42). Although XYR1 is an orthologue of A. niger XlnR, the main transcriptional activator of cellobiohydrolase- and xylanase-encoding genes (16, 46), the molecular mechanisms of transcriptional activation in H. jecorina are different and generally more differentiated (see, for example, reference 43). In A. niger, growth on araban and L-arabinose are not regulated by XlnR (9). Growth of H. jecorina on L-arabinose has recently been reported to be unaffected in a Δxyr1 strain of H. jecorina (2). In contrast, we show here that biomass accumulation of H. jecorina on arabanin and L-arabinose is clearly affected in the Δxyr1 strain, although the radial growth is similar. However, this appeared to be due to the positive regulation of the aldose reductase by XYR1 (37, 42) because a strain in which the formation of XYL1 was constitutive and uncoupled from xy1 regulation no longer showed this reduction on growth. The reason why this effect escaped the detection by Stricker et al. (42) is probably due to the use of agar plates for quantification of growth, which can lead to erroneous interpretations due to overlapping the different density of the mycelial mat formed on the plates (cf. Fig. 2a and 4c).

Expression of two of the α-L-arabinofuranosidase genes (abf1 and abf3) was only slightly affected in a Δxyr1 strain. This indicates that the triggering of their expression by L-arabinositol/L-arabinose and XYL1 requires another transcriptional regulator. In contrast, however, abf2 and bxl1 clearly were under the control of XYR1, and this control even overruled the positive action of L-arabinositol/L-arabinose and XYL1, thus showing that the two require xyrl for expression. The identification of regulation of these two genes by XYR1 is also consistent with the detection of a higher number of copies of the XYR1-binding consensus GGC(T/A)₄ (15, 40) in their promoters than in abf1 and abf3. In addition, only abf2 and bxl1 contained this motif in the form of a divergent and tandem repeat, respectively. Furukawa et al. (15) concluded that both single and double motifs are functional in H. jecorina. Our findings of the regulation by the general cellulase and hemicellulase regulator XYR1 are also supported by the data of Foreman et al. (14), who found elevated transcripts for bxl1 and abf2 (although the latter only very weakly) on sorphorose and lactose in H. jecorina QM6a. Thus, in contrast to aspergilli, which have developed more differentiated mechanisms to adapt their regulation of polysaccharide hydrolases to the presence of available substrates (9), H. jecorina also induces at least part of the arabanin-degrading enzymes simultaneously with the other enzymes required for cellulose and hemicellulose degradation. The simplest model which can be designed would therefore postulate a complex of L-arabinositol or L-arabinose, XYL1, and XYR1 to function in the regulation of abf2 and bxl1 gene expression. Further, our data are consistent with the speculation that, for abf1 and abf3, the part played by XYR1 is overtaken by a different transcriptional factor that is as yet unknown.

ACKNOWLEDGMENTS

This study was supported by grants from the Austrian Science Foundation (P19690) to C.P.K. and (P19421) to B.S. We are grateful to R. L. Mach for providing the Δxyr1 strain for these studies.

REFERENCES

1. Alexiou, P., K. Pegkildou, M. Chatzopoulou, I. Nicolau, and V. J. Demopoulos. 2009. Aldose reductase enzyme and its implication to major health problems of the 21(6)th century. Curr. Med. Chem. 16:734–752.
2. Ausubel, F. M., B. Roger, R. E. Kingston, D. D. Moore, J. G. Seidman, J. G. G. J. Ruijter, and J. Visser. 2001. Structure and biogenesis of the cell walls of grasses. Annu. Rev. Physiol. Plant Mol. Biol. 54:477–498.
3. Cho, Y. H., S. D. Yoo, and J. Sheen, J. 2006. Regulatory functions of nuclear hexokinase complex in glucose signaling. Cell 127:579–589.
4. Reference deleted.
5. de Groot, M. J., W. Prathumpai, J. Visser, and G. J. Ruiter. 2005. Metabolic control analysis of Aspergillus niger L-arabinose catabolism. Biotechnol. Prog. 21:1610–1616.
6. de Groot, M. J., P. J. van de Vondervoort, R. P. de Vries, P. A. van Kuyk, G. J. Ruiter, and J. Visser. 2003. Isolation and characterization of two specific regulatory Aspergillus niger mutants shows antagonistic regulation of arabinan and xylan metabolism. Microbiology 149:1183–1191.
7. de Vries, R. P., and J. Visser. 2001. Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. Microbiol. Mol. Biol. Rev. 64:897–952.
